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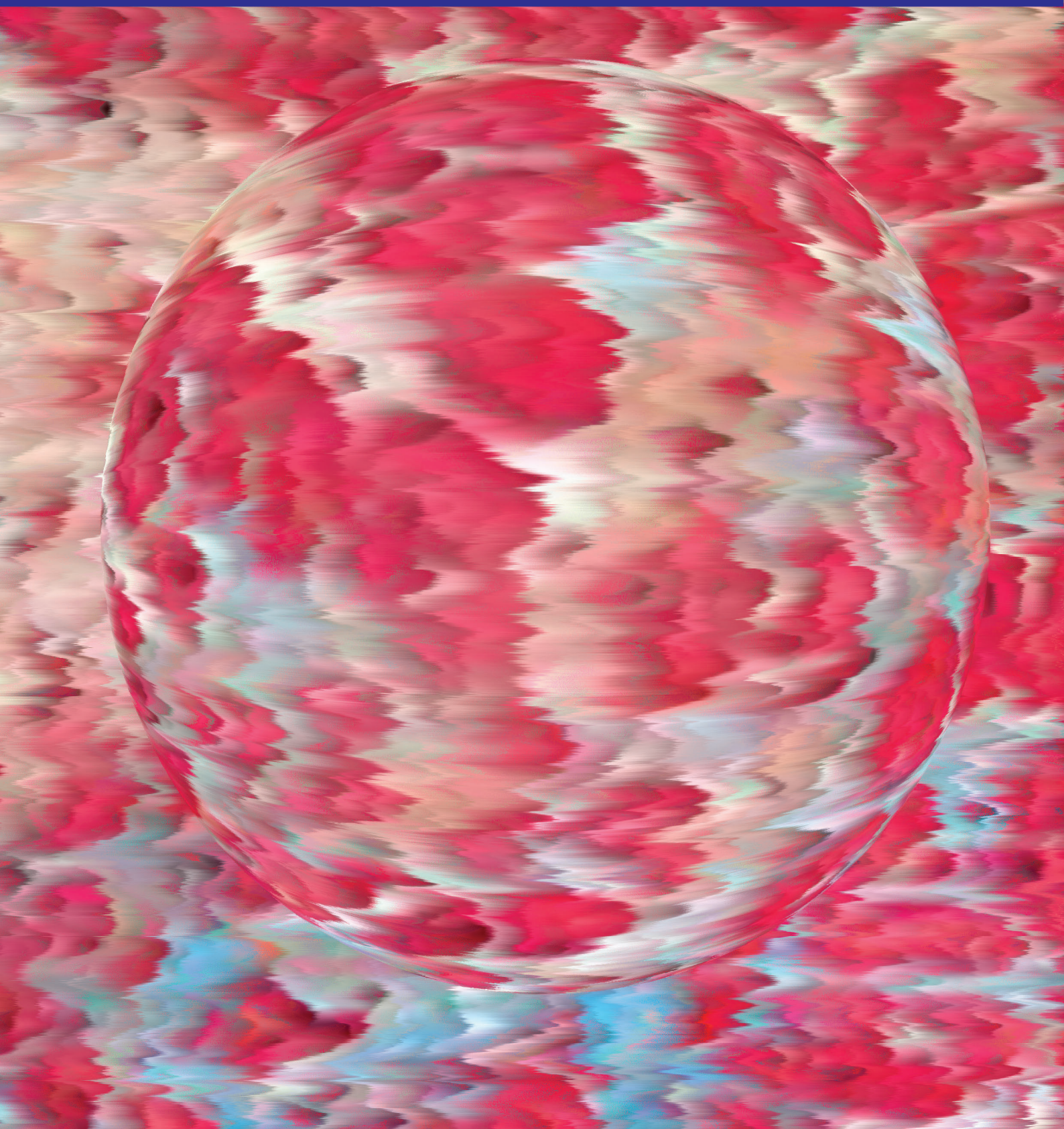

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Identification of altered platelet proteins by proteomic study in stress oxidative

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Abstract

Background: Activated platelets and oxidized low density lipoprotein LDL (ox-LDL) play a key role in the Atherosclerotic plaque formation. Proteomics, according the vast analytical power offered by mass spectrometry and two-dimensional gel electrophoresis (2-DE) ,is the best tool to study of nucleated platelets proteome changes. In this study we aimed to identify novel platelet signaling proteins induced by ox LDL.

Material and methods: This is a differential proteomics study on human platelets. All whole blood samples were obtained from healthy donors who referred to Tabriz blood transfusion center (East Azerbaijan. Iran). The isolated Platelets, divided in two groups, resting and cu2+ oxLDL activated platelets. 2DE was used to separate platelets lysate proteins. Scanned gels of two groups were compared by Progenesis SameSpots statistical software. MALDI-TOF and profound bioinformatics tool were used to identify proteins.

Results: 241 spots were found to be significantly different. Of these, 16 spots were randomly chosen and 14 spots (proteins) were successfully identified and correspond to 12 known platelet proteins and 2non-platelet protein. We found some candidate signaling proteins such as kinesin-like protein KIF21A variant swiss-prot; Q7Z4S6 and leucine-rich PPR-motif, containing protein and c-mer proto-oncogene tyrosine kinase swiss-prot; Q08828 and etc.

Conclusion: Our findings showed that may be some of these signaling proteins involved in cu2+ oxLDL platelets activation pathway(s). Additionally presentation of altered proteins, can thus add to our basic knowledge of platelets and to the recognition of the platelet pathways and development of novel antiplatelet drugs.

Key words: Atherosclerosis, Platelets, Proteome, Two-dimensional gel electrophoresis, oxidized low density lipoprotein (ox-LDL).

Introduction

It is believed that reactive oxygen species (ROS) damage to vary cells developing of various diseases such as cancer, [1] metastasis,[2] Alzheimer[3] and vascular diseases.[4] Accumulated ROS modulate several intracellular signals, thus altering cellular events in some cells such as platelets.[5] Oxidized low density lipoprotein (ox-LDL) as an inducer for oxidative modifications induces several proinflammatory effects[6], including, cytokine releasing endothelial activation and smooth muscle proliferation. Platelet proteins may be first targets of ROS/reactive nitrogen species (RNS).[7, 8] In platelets, massive changes in structure, protein expression profile and post translational modification are happened upon Exposure to oxidative stress.[9, 10] Platelets are ideal samples for a proteomics studies not only because they can be obtained as a pure cell population in high yield but also, they are anucleated and has the less complexity for proteomics studies .[11] Over the last few years, proteomics has been used to the research around the general proteome and signaling pathways in human platelets. Indeed, it can be applied to provide a novel view towards the biochemical happenings underlying platelet activation and help to candidate new drug targets and therapeutic agents to treat thrombotic disease.[12, 13] Garcia et al had already accomplished a differential proteome analysis in platelets activated by thrombin-receptor-activated peptide (TRAP). Extracted proteins of Resting and TRAP-activated platelet were separated by 2D gels (18 × 18 cm) leading to the detection of 62 different protein features. From these features, 41 were identified by LC-MS/MS. Most

of them were signaling proteins, such as pleckstrin, raf kinase inhibitory protein (RKIP), integrin-linked protein kinase 2 (ILK-2), etc.[14]

In a first study on phosphorylation of the regulator of G-protein signaling was illustrates how the application of proteomics to the study of signaling pathways in platelets.[15] Indeed, in progress, scientists carried out a similar comparative study by considering the proteome of collagen-related-peptide (CRP)-activated platelets.[16, 17] Glycoprotein VI (GPVI) was recognized as the major activation-inducing receptor for collagen with a hundred differentially regulated proteins in platelets. In summary, proteomics become a key tool for the study of the platelet biology. As we mentioned previously ox-LDL is the potent stimulator of platelets, and there was not any reported comprehensive study on this agonist effect on platelets we decided to carried out a differential proteomics approach on resting and ox-LDL activated platelets to identify related signaling proteins.

Materials and methods

Materials

Immobilized pH gradient IPG strips (pH 3-10), acrylamide, bisacrylamide and SDS, TEMED were acquired from BioRad. Trypsin Gold, Mass Spectrometry Grade,Urea, CHAPS, Prostaglandin E1, ammonium bicarbonate, and ammonium Sulfate and thiourea were obtained from Fluka Chemie (Buchs,Switzerland). All other chemicals were purchased from Promega

Platelet isolation and activation

Human whole blood was collected from healthy donors who had referred to Tabriz Blood Transfusion Center (East Azarbayjan,Iran). Any of them have no medication for the previous 20 days. Major exclusion criteria was plasma P-selectin < 40ng/ml. All samples were obtained in compliance with the Ethical Committee regulations of the Tabriz University of Medical Sciences. The platelets were isolated from each of these samples that described briefly, the human whole blood was mixed immediately with 1/9th volume of acid-citrate-dextrose solution (ACD, 75 mM trisodium citrate, 124 mM dextrose, and 38 mM citric acid) Then centrifuged at 200×g for10 min at room tempreture to remove red blood cells (RBC) and leukocytes to obtain platelet rich plasma (RPR).The platelets were pelleted by room temperature centrifugation of this suspension at 1500×g. The platelet pellet was gently re-suspended in citrate wash buffer (11 mM glucose, 2.4 mM citric acid 7.5 mM Na2HPO4, 128 mM NaCl, 4.3 mM NaH2PO4, 4.8 mM sodium citrate, pH 6.5) and re-centrifuged at 1200×g for 10 min at room temperature to isolate the pure platelets as pellets. The platelet pellet was resuspended in a Ca2+ free Tyrode's buffer (140 mM NaCl, 2 mM MgCl2, 5.6 mM glucose; 3 mM KCl, 0.4 mM NaH2PO4, 12 mM NaHCO3, pH 6.2) and centrifuged at 700 × g at 37°C for 10min. Collected pellet was then resuspended in a Tyrode's buffer (3 mM KCl, 140 mM NaCl, 1 mM MgCl2, 12 mM NaHCO3, 2 mM CaCl2,5 .6 mM glucose, 0.4 mM NaH2PO4, pH 7.4) to a final concentration of 10^9mL (standard platelet suspension), subsequently incubation was performed at 37°C for 30 min . Onyx Coulter Counter blood counter (Beckman Coulter, Brea, CA, USA) was used for counting the platelets.

Oxidation of LDL

The commercial LDL was dialyzed against PBS (120 mM NaCl, 10 mM NaH2PO4, 2.7 mM KCl, pH 7.8) to eliminate EDTA. The LDLs were Oxidized (1 mg/ml) by 10 μM CuSO4 in PBS for 24 h at 37 °C. Malondialdehyde (MDA) was measured as a lipid peroxidation index by

Thiobarbituric acid assay

Standard platelet suspension (SPS) was divided into two aliquots. Using an aggregometer (Bio/Data) the amounts of required ox-LDL was optimized. Finally the platelets were treated with 75μg/ml cu2+-oxidized LDL /SPS/ml in polypropylene tubes at 37°C. Four times of the sample volume of cold acetone (-20° C), was added to each SPS for 4 hr, and subsequently centrifuged at 20,000 × g at 4°C for 15 min.

Two-dimensional gel electrophoresis

The precipitated proteins were dissolved in 2-D GE buffer (4% CHAPS, 0.5% DTT, 8 M urea, , IPG buffer, pH 3–10,18 cm).150 micrograms of protein were applied to isoelectrofocusing (IEF) using 18-cm linear IPG dry strips with a pH range of 3–10 (GE Healthcare, Uppsala, Sweden). IEF was carried out in an IPGphor (GE Healthcare, Uppsala, Sweden) using the following protocol: rehydration,

10 h; 50 V, 3 h; 1,500 V, 1 h; 7,000 V, 10 h. following IEF, equilibration of strips were done twice ,the first in 6 M urea , 20% SDS, 50 mM Tris–HCl, pH 8.8, 30% glycerol with 1% DTT for 10 min, and the second in the same buffer containing 4% iodoacetamide as a replacement for DTT. Equilibrated strips were located on top of 10% SDS PAGEs and were sealed with 0.5% agarose in 62.5 mM Tris–HCl, pH 6.8, 0.1% SDS. SDS-PAGE was done in a DALTsix in recommended conditions (constant power 50 W, for 7 h). Gels were fixed in 10% acetic acid and 20% methanol for 10–12 h. silver staining was used to visualize Proteins in gels. We generated two 2D gels for each condition.

Image analysis

Scanned images of gels (tiff format, Grey scale, Bit Depth 16, Resolution300 dpi) analyzed with calculation of volumes of spots by the SameSpots software (Nonlinear Dynamics, Newcastle upon Tyne, UK) .The main applied filtering were pvalue ≤ 0.5 and fold ≥ 2.

Protein identification

Chosen Protein spots were cut, destained and subjected to in-gel digestion with trypsin (modified sequence-grade porcine; Promega, Madison, WI, USA). Trypsin digested peptides were absorbed on a microC18 ZipTip (Millipore Billerica, MA, USA) and eluted with 65% acetonitrile including matrix (α-cyano-4-hydroxycinnamic acid) Then applied gently onto the MALDI target and analyzed on Bruker Ultraflex MALDI TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Trypsin autolytic peptides were used as internal calibrator. The ProFound (http: //65.219.84.5/service/prowl/profound.html), search engine was used to protein identification by using NCBI data source . Iodoacetamide and Methionine oxidation were chosen for Complete and partial Modification respectively. Z score and sequence coverage were considered to evaluateThe probability of identification. The experimental pI and Mw values of proteins with their theoretical values were compared aswell.

Results

Four gels were prepared in the experiment and divided into two individual groups resting and ox-LDL activated platelets following comparison of two groups scanned gels using SameSpot software. More than 684 spots detected. 463 spots kept on the analysis following pre-filtering (minimal spot area, area exclusion or edge). using statistical significance (Anova $p < 0.05$, fold ≥ 2) excluded other 242 spots. In Figure 1, Locations of 221 significantly differed spots are presented in 2D gel scanned image of resting platelets. Figure 2 presents image of resting platelets gel without annotation for a better clearness.

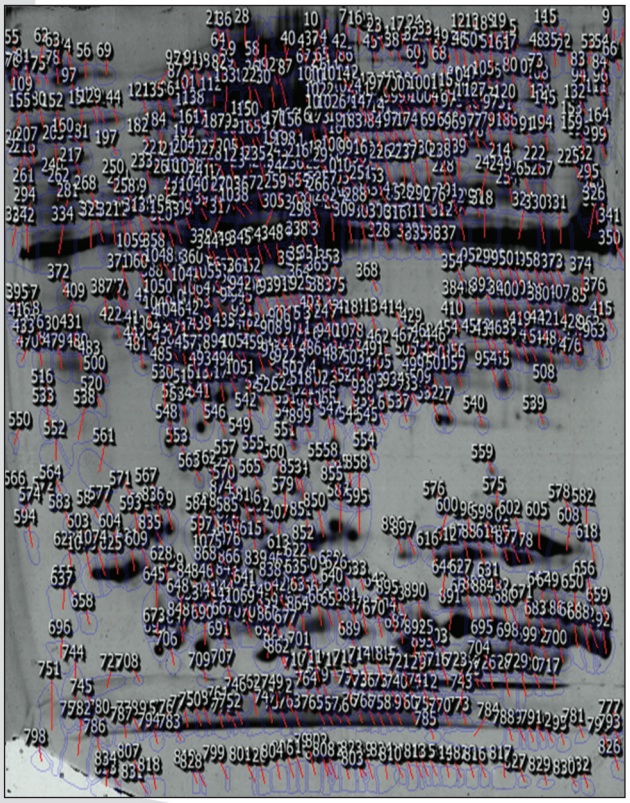

Figure 1. Locations of significantly differed spots on a silver stained 2D gel. Locations of all spots that were found to significantly differ in 2D gels of resting and ox LDL activated when compared by SameSpots software. The 2D gel of resting platelets was used as an illustrative gel to display spot positions

16 spots among 221 spots were randomly chosen for identification and 14spots successfully identified by LC-MS. All of them were platelets protein excepting two proteins. At least, two distinctive peptides were considered to identify proteins. Table 1, presents the spots (proteins) parameters including, Protein swiss-prot accession number, both calculated and experimental values of pI and MW Anova p-value, fold and Z score.

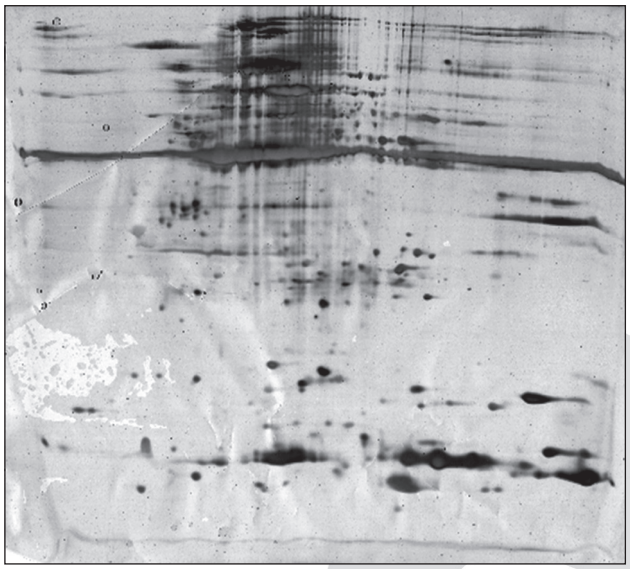

Figure 2. Scanned image of silver stained 2DE gel without annotation. An illustrative 2D SDS-PAGE gel image of resting platelets without displayed positions of significantly differed spots

Figure 3 presents six examples of spots including their statistical p value and location and fold. Two human but non-platelets identified proteins were AT-rich interactive domain-containing protein swiss-prot; CAD97814 and Thioredoxin Reductase 3 swiss-prot; Q86VQ6. Platelets interacting proteins related to identified proteins has presented in table 2 based on Platelet Web database. As well as Being platelets protein were confirmed using the Platelet Web which is database including the platelet pathways, transcriptome, and interactome, published studies about platelets and etc.). We used "www.uniprot.org" to achieve informations about subcellular location of proteins, ontology and other characters of proteins.

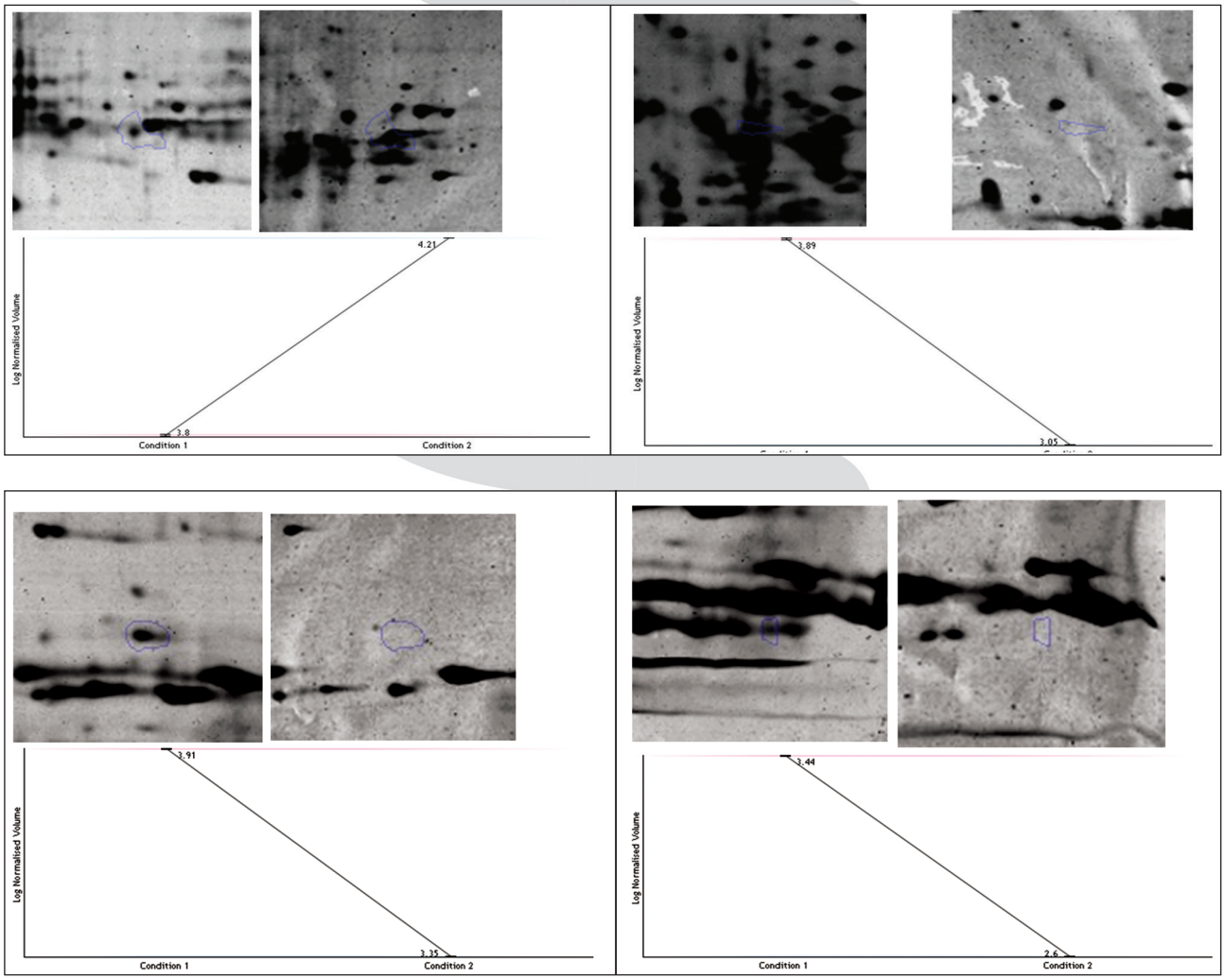

Figure 3. Four selected spots. Expression profiles with the logarithms of spot expression volumes for each group (resting platelets and by ox-LDLactivated platelets)

Table 1. The List of spots that significantly differ in ox-LDL activated platelets

ID	AN	Name	Location	Calculated		Experimental		Z score	Fold	Pvalue
				PI	Mw kDa	PI	Mw kDa			
702	gi264668574 D1M744	gamma-aminobutyric acidAreceptor gamma 2	mitocondrial membrane	9.6	19	9.1	29	0.10	6.9	7.378e-012
659	gi31873426 CAD97814	AT-rich interactive domain-containing protein	Non-platelets	9.8	56	9.8	42	0.78	3.5	2.493e-006
692	gi11611843 P29084	general transcription factor, IIE, polypeptide 2, beta 34kDa	cytoplasm	9.6	33	9.7	33	0.27	3.7	1.148e-010
240	gi256818774 Q96MT7	WD repeat domain 52, isoform CRA_a	membrane	5.3	208	3.8	205	0.07	5.5	2.397e-011
505	gi|801893 P42704	leucine-rich PPR-motif, containing protein	mitochondrione	5.5	146	7.5	120	0.27	2.6	8.286e-012
575	gi458119 Q15067	acyl-CoA oxidase	RE membrane	8.8	74	8.5	99	0.26	3.7	2.493e-006
869	gi220675603 B1APN9	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4	membrane	5.9	124	5.5	86	0.07	6.9	4.885e-011
612	23764066 Q86VQ6	Thioredoxin Reductase 3	Non-platelet	5.3	23.7	7.5	64	0.15	2.1	1.802e-008
511	O15020	spectrin, beta, non-erythrocytic 2	Sub-memmbrane	5.7	269	5.5	117	0.94	3.4	2.004e-012
304	gi|120587025 Q9Y566	SH3 and multiple ankyrin repeat domains protein 1	Sub-memmbrane	8.6	225	9.6	170	1.56	13	8.664e-012
388	gi83582518 Q7Z4S6	kinesin-like protein KIF21A variant	submembrane	6,05	181	4.77	144	0.38	2.3	6.924e-010
465	gi|40538728 Q9Y4G2	Pleckstrin homology domain-containing family	cytoplasm	5.7	105	5.2	128	0.07	4.5	3.731e-006
55	gi|188672| P11717	Cation-independent mannose-6-phosphate receptor	lysosomal membrane	5.7	261	3	225	0.24	6.3	8.446e-012
699	gi149242798 Q12866	c-mer proto-oncogene tyrosine kinase	mambrane	6.6	36	8.2	50	0.86	5.6	1.293e-011

Table 2. The list of identified proteins with related platelet interacting proteins

Protein name	Platelets interacting proteins
gamma-aminobutyric acid A receptor 'gamma 2	ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited), protein phosphatase 3, catalytic subunit, alpha isozyme, protein kinase C, beta, trafficking protein, kinesin binding 2
general transcription factor IIE, polypeptide 2, beta 34kDa	dynamin 2, heat shock protein 90kDa alpha (cytosolic), class B member 1, ubiquitin protein ligase E3 component n-recognin 5 and etc.*
WD repeat domain 52, isoform CRA_a	unknown
leucine-rich PPR-motif containing protein	GABA(A) receptor-associated protein-like 2, glutathione S-transferase kappa 1, pleckstrin homology-like domain, family A, member 3 and ect.*
liprin	Calpastatin, G protein-coupled receptor kinase interacting ArfGAP 1
spectrin, beta, non-erythrocytic 2	actin, alpha 1, skeletal muscle, dynactin 1 and ect*
acyl-CoA oxidase	sterol carrier protein 2
SH3 and multiple ankyrin repeat domains protein 1	ATP-binding cassette, sub-family A (ABC1), member 1, ribosomal protein S6 kinase, 90kDa, polypeptide 3, Rho guanine nucleotide exchange factor (GEF) 7and etc.*
Bcr-abl1,e19a2 chimeric protein	actin, alpha 1, skeletal muscle, annexin A1, caspase 9, apoptosis-related cysteine peptidase and etc.*

**.see ddditional proteins in platelets web*

Discussion

This is a simple comparative proteomics study that was focused on human platelets at two situations, oxidative stress and quiescence to identify novel signaling or adaptor proteins related to platelet activation in oxidative stress. In this study we presented 14 proteins (randomly selected within 241 proteins) that differed between these mentioned groups. In spite of the limits in identification, we have found good evidence about stress oxidative effects on platelets, for example three proteins, PTPRFswiss-prot; B1APN9, WD repeat domain 52, isoform CRA_a swiss-prot; Q96MT7, kinesin-like protein KIF21A variantswiss-prot; Q7Z4S6 have been never reported in activated platelets by other agonist such as Thrombin or Arashidonic acid.[18] WD repeat domain 1, isoform CRA_a reported in Pavel Májek et al as a candidate protein in Thrombin activated platelets, It means at least, there are clear differences between theses agonist in pathway(s) by which platelets are activated. In this study, we identified proteins from various intracellular compartments such as Cation-independent mannose-6-phosphate receptor/ IGFII swiss-prot; p11717 and leucine-rich PPR-motif, containing protein swiss-prot; P42704in the lysosomal membrane and mitochondrion respectively. Three of them c-mer proto-oncogene tyrosine kinase, WD repeat domain 52 and Cation-independent mannose-6-phosphate receptor were signaling proteins.[18,19]

Phosphorylation/dephosphorylation as one of the most important protein posttranslational modifications involved in platelet activation.[20] We can observe pI shift(s) in gels due to Phosphorylated/ dephos-phorylated protein (also called spot trains)[21] Spot trains may be produce by alterations other than (de) phosphorylation. Probably, It is reason for difference in measured and calculated pI of c-mer proto-oncogene tyrosine kinase swiss-prot; Q08828 (see table1). We observed a significant difference in estimated and calculated Mw in kinesin-like protein KIF21A variant swiss-prot; Q7Z4S6 and leucine-rich PPR-motif,containing protein which may be happened by nonspecific protein modifications not by(de) phosphorylation. Platelet activation by ox-LDL is recognized to produce large quantities of reactive oxygen and nitrogen species (RONS).[22] Although Thrombin and collagen can yield RONS throughout platelet activation[23] but the assembly of reactive species is inseparable from ox-LDL production . From the 2D PAGE view, RONS activity can lead not only protein pI changes, but also can change protein molecular weight by the linking of proteins together.[24] We focused on c-mer proto-oncogene tyrosine kinase swiss-prot; Q12866 that in-

teracts with several ligands including Tubby protein homolog (TUB) Ligand, Galectin S3 (LGALS3), adaptor proteins between the extracellular matrix and the cytoplasm (Table 2). It regulates many physiological processes as well as migration, differentiation, cell survival, and efferocytosis.[25] Additionally it is able to bind a ligand at the external domain and induce autophosphorylation of MERTK on its intracellular domain.[26] After stimulation by ligand and interaction with 1-phosphatidyl inositol 4, 5-bisphosphate phosphodiesterase gamma-2(PLCG2) or Growth factor receptor-bound protein 2 (GRB2) induces phosphorylation of Mitogen-activated protein kinase 1 (MAPK1), MAPK2, Tyrosine-protein kinase Mer (MERTK). MERTK signaling has an central role in several processes like efferocytosis [27] cytoskeleton rearrangement, platelet aggregation. It has also an important role in suppress of Toll-like receptors (TLRs)-mediated innate immune response by activating STAT1, which induces inhibitors of cytokine signaling. Aside from its role in platelet aggregation, inflammatory pathways , and modulatory of innate immune response a reason for importance of MerTK is a recent report indicating that this receptor protein is sensitive to oxidative stress induced by H2O2which similarly suppressed the phagocytic power of ARPE-19 cells. The mechanism of relieve was recognized to suppression of focal adhesion kinase (FAK) and MerTK signaling.[28]

Acyl-CoA oxidase swiss-prot; Q15067is another identified protein. That Widely expressed with highest levels of isoform 1 and isoform 2 detected in testis. It catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs.this protein intract with sterol carrier protein 2.Practically there is no evidence of important role related to this proteinin platelet activation.[29, 30]

Platelets intracting proteins related to Cation-independent mannose-6-phosphate /IGF-II receptor are listed in Table 2. This protein has important role in transport of phosphorylated lysosomal enzymes originated from the Golgi complex (31). This receptor also binds IGF2 and acts as a activator of T-cell, by binding Dipeptidyl peptidase 4 DPP4.[32]

Pleckstrin homology domain-containing family so called Kindlin3 is one of the well-known Kindlin family that are reported as co-activators with talin of integrins, but there is evidences based on kindlins role in mediation of integrin outside-in signaling. In kindlin-3 knockout mice Platelets adhered to fibrinogen in the presence of Mn2+, but they failed to form lamellipodia because,kindlin-3 is required for outside-in signaling of integrins αLβ2 and αIIbβ3. Electric cell-substrate impedance-sensing measurements showed that kindlin-3 knockout cells were defective in spreading. Whether kindlin-3, like its paralogs kindlin-1 and kindlin-2, is involved in mediating integrin αLβ2- or αIIbβ3-derived RhoGTPase signaling remains to be determined. [33]

Kinesin-like protein KIF21A variant Microtubule-binding motor protein may be participate in neuronal axonal transport. In vitro, has a plus-end directed motor activity.[34]

Protein polybromo-1 has well known role in transcription and repression of select genes by remodelation of DNA-nucleosome topology and acts as an inhibitor of cell proliferation.[35]

The last identified protein was protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4swiss-prot; B1APN9 interact with several platelets proteins such as Calpastatin, G protein-coupled receptor kinase interacting ArfGAP 1. Liprin-alpha/SYD-2 is a family of multidomain proteins with four known isoforms. One of the reported functions of liprin-alpha is to regulate the development of presynaptic active zones, but the underlying mechanism is poorly understood. Evidence shows, liprin-alpha directly interacts with the ERC (ELKS-Rab6-interacting protein-CAST) family of proteins.[36]

Conclusion

Our study showed considerable changes in proteomes of platelets activated by ox-LDL.We were able to present 241 differentially expressed spots (proteins) and to identify 12 proteins Most of the identified proteins related to signaling or submembrane /skeletal proteins such as c-mer proto-oncogene tyrosine kinase and SH3 and multiple ankyrin repeat domains protein 1. Some of the most important signaling cascades in platelets especially wnt pathway and T cell activation were recognized to involve in platelets activation followig oxidative stress.

Finally our results, illustrating the differences in proteins in cu2+ ox-LDL activated and resting platelets, add to the basic information about platelets and to development of novel anti-platelet drugs.

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Ethical issues

The protocol for the research project has been approved by the ethic committee at TUMS (Tabriz University of Medical Sciences) which is in compliance with the Helsinki Declaration.

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Health behaviours, perceived self-efficacy and health locus of control tendency in vocational school of health students

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Abstract

Aim: To determine the health behaviours, perception status of health self-efficacy and health locus of control tendencies in first and last class students of vocational school of health.

Methods: The descriptive study was performed on students of two vocational schools of health. First and last class students were included in the study to evaluate effects of vocational education. Data were obtained with a questionnaire and two scales (perceived health competence scale, multidimensional health locus of control scale).

Results: Of the 175 students participated in the study, 53% was of first class, 47% was of last class, and 69% was female. Average score of perceived health competence scale was 26.9±7.4 over 40. Powerful others locus of control score of Multidimensional Health Locus of Control Scale was lower among students of fourth class ($p<0.001$).

Conclusions: Students whose both self-efficacy and locus of control scores were high were more inclined to have positive health behaviours. Contrary to the expectations, however, formal vocational education did not increase the levels of self-efficacy and locus of control. Attempts to improve self-responsibility might be useful in formal vocational education of health.

Key words: Health self-efficacy, locus of control, vocational school of health, student.

Introduction

Desire to know, believe and perform what is necessary to protect and improve health is important for acquiring healthy life behaviours. Adolescence, independently, is an important period in which the habits for acquiring and maintaining healthy life behaviours are obtained. During this period, young people's thoughts about their own health may not agree with the external measurement data about young. Adolescent's approach to his/her own health may be significant for his/her health behaviour development. Adolescent's knowledge about his/her own physical and mental health may contribute to self-assessment of their health (1). Concepts like self-efficacy and health locus of control draw attention in literature about how measurements should be done to evaluate health (2). In this context, an individual should be aware of his self-responsibility with regard to his/her health to evaluate his own health.

Self-efficacy belief is individual's belief in his power to affect events that have an impact on his life by displaying a certain level of performance. Self-efficacy belief determines how an individual feels, thinks, motivates himself, and behaves (3). According to Rotter locus of control concept puts forward the differences between individuals' acts and results in terms of personal perceptions. Individuals can have internal or external locus of control, according to Rotter's social learning theory (4). Locus of control reflects the degree of personal expectations about results of personal behaviours (internal locus of control) against chance/fate/powerful others/unknown powers (external locus of control). Although not as frequent as it is done in psychology and other social sciences, studies about locus of control are performed in public health and politic sciences and translated into many languages for testing individual differences in locus of control (5).

Due to their occupational responsibilities and their lifestyles shaped by their social roles, medical staffs are role models, and they have an influence on the group they serve (6). For this reason, medical staff candidates who will provide health service in the future are expected to be aware of what is necessary for protecting their own health. As students of vocational school of health receive education about health in their adolescence and in addition to providing health service, they will educate the population about health; it is important for them to develop a sound locus of control and health belief.

This study was performed on students of vocational school of health to determine the perception status of health self-efficacy at the beginning and end of their education, and their tendency in constituting locus of control in health.

Material and Methods

This descriptive study was conducted in Nigde (Turkey) in 2012. The universe of the study was the students of two vocational schools of health in Nigde (Turkey). The schools had two occupational branches: nursing and emergency medical technicians (EMT). The sample consisted of first and fourth year students to evaluate the effect of occupational education. In addition to approval of research ethics committee and written institutional consents from Provincial Directorate of National Education, oral consents of participants were also taken. A questionnaire and two scales (perceived health competence scale, multidimensional health locus of control scale) were used to obtain data.

The questionnaire consisted of 16 questions about the students' demographic information, smoking, brushing teeth, cause and time of last visit to doctor, and the things they cared in nutrition etc. Income levels of families were recorded according to statement of students.

Perceived Health Competence Scale (PHCS)

The Perceived Health Competence Scale (PHCS) is a measure of self-efficacy regarding general health related behaviour. The scale developed by Smith et al (7) consists of 8 items (6 point Likert) 4 of which were reversed and the total score was between 0-40. Turkish version of the scale (8, 9) had internal consistency of 0.75 and test-retest reliability of 0, 71.

Multidimensional Health Locus of Control Scale (MHLC)

The A form of MHLC developed by Wallston et al (10) and used in this study consisted of 18 items, and it was a Likert type scale that had three subscales (11, 12). The scale was adapted to Turkish with 4 subscales (8, 9). Internal consistency factor of the scale was 0, 63, and this score varied between 0.39 and 0.68 among subscales. High scores in the scale indicate external control (CHLC-PHLC-FHLC) while low scores indicate internal control (IHLC) (13).

Data collection

All students who agreed to fill the questionnaire and the scales were included in the study. The data were obtained by having the questionnaires and scale forms filled by the participants under supervision of researchers. Participants were briefly instructed in the classrooms before they filled the questionnaire and the scale forms.

Demographic variables were independent, and health self-efficacy and perception status of health locus of control were dependent variables. Each subfield of health locus of control scale included the same number of questions. The Turkish version included different number of questions, so the average scores were calculated instead of total points in order to make the subscales comparable according to the same unit. Presentation and comparisons were made with these average scores. Percentages, averages, and standard deviation were used in description.

While categorical data were analyzed with chi-square, numeric data were analyzed with Kruskal-Wallis and Mann-Whitney U tests or independent samples t-test, one-way ANOVA and Post Hoc Tukey HSD with respect to the result of normality analysis (Kolmogorov Smirnov test). $p<0.05$ was considered to be significant for the results of Post Hoc Tukey test.

Results

Of the 175 students participated in the study, 53% were in the first class, 47% were in the fourth class, 31% were male, 56% were in nursing department, 44% were in emergency medical technician department, 81% stayed with their family, 65% sta-

ted that their income level was average. Students who lived with their friends reported a lower family income (p=0.004). Average age of first class students was 15±1 and last class was 18 ±1 years.

9 % of the students stated that they had chronic diseases, and all of them stayed with their family (p=0.044). The diseases were hypercholesterolemia, sinusitis, asthma, heart diseases, iron deficiency anaemia, migraine, ulcer, and diabetes. 21% did regular exercise, 37% brushed their teeth regularly. They were interested in football, volleyball and basketball. Male students participated more in sport activities (p<0.001) while female students had a higher ratio of tooth brushing (p<0.001). 65% stated that they were careful about their nutrition; on the other hand, only 37% stated that they kept a balanced diet. 56% of the students stated that they went to a physician in the thirty days before the study. Most common causes for visit were 37% common cold /sore throat and 22% eye/teeth/allergy/orthopedics related problems. The ratio of smoking was 4% among first classes, while it was 17% among fourth classes (p=0.006), and it was higher in male students (p=0.001). The ratio of visiting a physician was higher in smokers (78% versus 53 %) (p=0.049). For a healthy life, 66% of the students suggested doing regular exercise and a balanced diet, and female students who suggested it were more (p=0.029).

Average score of perceived health competence scale was 26.9±7.4 over 40. Internal consistency coefficient of the scale for all of the items was 0.78.

Table 1. Distribution of health competence score according to demographic features, mean (SD)

Variables		n (%)	Health competence score
Gender	Male	54(30.9)	28.4(7.0)
	Female	121(69.1)	26.3(7.5)*
Department	Nursing	98(56.0)	26.3(7.7)
	EMT	77(44.0)	27.7(7.0)
Class	1st Class	92(52.6)	26.8(7.5)
	4th Class	83(47.4)	27.5(6.7)
Living with	Family	141(80.6)	26.7(7.8)
	Friends	34(19.4)	28.1(5.8)
Family income level	Good	44(25.1)	26.4(7.1)
	Moderate	114(65.2)	27.2(7.2)
	Low	17(9.7)	26.5(9.5)

**p=0.046*

Table 2. Distribution of health competence score according to health behaviors, mean (SD)

Variables		n (%)	Health competence score
Exercise/sports	Not doing	138 (78.9)	26.6 (7.4)
	Doing	37 (21.1)	28.3 (7.3)
Chronic disease	Doesn't have	159 (90.9)	27.5 (6.7)
	Have	16 (9.1)	20.8 (10.8)**
Have been ill in the last month	No	77 (44.0)	27.8 (7.4)
	Yes	98 (56.0)	26.3 (7.4)
Nutrition state	Careless	62 (35.4)	26.0 (7.9)
	Low calorie	48 (27.4)	26.3 (7.1)
	Balanced	65 (37.1)	28.2 (7.1)
Smoking	Non smoking	157 (89.7)	26.9 (7.3)
	Smoking	18 (10.3)	26.9 (7.4)
Tooth brushing	Irregular	110 (62.9)	26.1 (7.5)
	Regular	65 (37.1)	28.3 (7.0)*
Suggestion for healthy life	Sports-balanced diet	116 (66.3)	26.6 (7.3)
	No/other	59 (33.7)	27.6 (7.6)

*p=0.062, **p<0.001*

There was no difference in health self-efficacy scale total score with respect to class, department, family income perception, people staying with them, nutrition care and regular exercising (p>0.05). There was only a limited increase in those who kept a balanced diet (p=0.065). In contrast, total score of health self-efficacy scale was lower than the others in female (p=0.046) and chronically ill (p<0.001) students, and there was a borderline significant level increase in students who brushed their teeth regularly (p=0.062) (Table 1, Table 2).

Internal consistency coefficient of multidimensional health locus of control scale for all of the items was 0.68, varying between 0.31 and 0.68 among subscales. The average score of sub dimensions in the scale was 4.5±0.9 for internal control, 2.5±1.1for chance, 3.4±1.0 for powerful others, 3.9±1.0 for fate (F=125.2, p<0.001). Of the dimensions of multidimensional health locus of control, powerful others locus of control average score was lower in fourth classes than first classes (p<0.001). Students who kept a balanced diet had higher internal control score (p=0.042), and students who had a low calorie diet had higher powerful others score (p=0.044). Students who did not have a suggestion for a healthy life had a higher fate score than those who suggested exercise and healthy nutrition (p=0.032) (Table 3, Table 4).

To determine which locus of control was relatively dominant in which category of independent variables, average scores of subscales of multidimensional health locus of control were separately compared with each other with respect to independent variables. Fate score and powerful others control score were similar in males, first classes, nurses, students staying with their friends, and students regularly exercising. On the other hand, fate score was higher than powerful others control score in emergency medical technicians, students staying with their families and students who didn't exercise regularly (p<0.05). Powerful others, chance and fate scores were similar in students whose perceived family income was low (p>0.05), while they were different in students whose perceived family income was high (p<0.05). Among students with chronic diseases, internal control score dropped to the level of fate score and powerful others control score dropped to the level of chance score (p>0.05) while those scores were different among students without chronic diseases (p<0.05). Powerful others control score was the same with the fate score among students who had been ill in the last month (p>0.05) while fate score was higher than powerful others control score in the remaining group (p>0.05). Internal control score dropped to the level of fate score among students who did not care about their nutrition (p>0.05), while internal control score was higher than fate score among students who cared about their nutrition (p<0.05). Powerful others locus score was as low as chance among smokers (p>0.05), while powerful others locus score was

Table 3. Distribution of health locus of control scores according to demographic features, mean (SD)

Variables		Loci of control				
		Internal	Powerful others	Chance	Fate	p
Gender	Male	4.4 (1.0)[a]	3.4 (0.9)[b]	2.5 (1.0)[c]	3.8 (1.0)[b]	<0.001
	Female	4.5 (0.9)[a]	3.4 (1.0)[b]	2.4 (1.1)[c]	3.9 (1.0)[d]	<0.001
Department	Nursing	4.5 (0.9)[a]	3.4 (1.0)[b]	2.5 (1.1)[c]	3.8 (1.1)[b]	<0.001
	EMT	4.4 (1.0)[a]	3.3 (0.9)[b]	2.4 (1.1)[c]	3.9 (0.9)[d]	<0.001
Class	1st	4.5 (0.9)[a]	3.6 (1.0)[b]	2.5 (1.1)[c]	3.9 (1.0)[b]	<0.001
	4th	4.4 (1.0)[a]	3.1 (0.9)[#b]	2.4 (1.0)[c]	3.9 (1.0)[d]	<0.001
Living with	Family	4.4 (1.0)[a]	3.4 (1.0)[b]	2.5 (1.1)[c]	3.9 (1.0)[d]	<0.001
	Friends	4.6 (0.7)[a]	3.4 (0.9)[b]	2.4 (0.9)[c]	3.8 (1.0)[b]	<0.001
Family income level	Good	4.5 (1.0)[a]	3.4 (0.8)[b]	2.7 (1.2)[c]	3.9 (1.1)[d]	<0.001
	Moderate	4.4 (0.9)[a]	3.4 (1.0)[b]	2.3 (1.0)[c]	3.9 (1.0)[d]	<0.001
	Low	4.5 (1.0)[a]	3.2 (1.3)[b]	2.6 (1.2)[b]	3.8 (1.0)[a]	<0.001

[†] *Different letters show statistical differences (Tukey HSD, p<0.05).*
[*] *According to. Class 1 p=0.023,* [#] *According to. Class 1 p<0.001*

Table 4. Distribution of health locus of control scores according to health behaviors, mean (SD)

Variables		Loci of control†				
		Internal	Powerful others	Chance	Fate	p
Exercise/sports	Not doing	4.5(0.9)[a]	3.4(1.0)[b]	2.4(1.1)[c]	3.9(1.0)[d]	<0.001
	Doing	4.5(0.9)[a]	3.5(0.8)[b]	2.7(1.1)[c]	3.8(1.1)[b]	<0.001
Chronic disease	Doesn't have	4.5(1.0)[a]	3.4(1.0)[b]	2.5(1.1)[c]	3.8(1.0)[d]	<0.001
	Have	4.3(0.8)[a]	3.1(1.1)[b]	2.3(1.2)[b]	4.2(0.9)[a]	<0.001
Have been ill in the last month	No	4.4(1.1)[a]	3.3(1.0)[b]	2.6(1.2)[c]	4.0(1.1)[a]	<0.001
	Yes	4.5(0.8)[a]	3.5(1.0)[b]	2.4(1.0)[c]	3.8(1.0)[b]	<0.001
Nutrition state	Careless	4.3(0.9)[a]	3.2(1.0)[b]	2.6(1.1)[c]	4.0(1.1)[a]	<0.001
	Low calorie	4.4(0.9)[a]	3.6(0.9)[*b]	2.5(0.9)[c]	3.7(0.9)[d]	<0.001
	Balanced	4.7(0.9)[*a]	3.5(1.0)[b]	2.3(1.2)[c]	3.9(1.0)[d]	<0.001
Smoking	Non smoking	4.5(1.0)[a]	3.4(1.0)[b]	2.5(1.1)[c]	3.9(1.0)[d]	<0.001
	Smoking	4.5(0.6)[a]	3.0(0.9)[b]	2.5(0.9)[b]	3.9(1.1)[d]	<0.001
Tooth brushing	Irregular	4.4(0.9)[a]	3.4(0.9)[b]	2.5(1.0)[c]	3.8(1.1)[d]	<0.001
	Regular	4.5(1.0)[a]	3.4(1.0)[b]	2.5(1.1)[c]	4.0(0.9)[d]	<0.001
Suggestion for healthy life	Sports-balanced diet	4.5(0.9)[a]	3.4(0.9)[b]	2.5(1.1)[c]	3.8(1.0)[d]	<0.001
	No/other	4.5(0.9)[a]	3.4(1.2)[b]	2.5(1.0)[c]	4.1(0.9)[#d]	<0.001

† *Different letters show statistical differences (Tukey HSD, p<0.05).*
* *With respect to those don't care p<0.05*
\# *With respect to those who suggest sports and balanced diet p=0.032*

higher than chance among non-smokers (p<0.05) (Table 3, Table 4).

When the sub scale scores of health locus of control scale were separated as high and low according to median, self-efficacy score was higher among students with high internal control (p=0.006), while it was lower among students whose fate locus of control score was higher (p=0.019). There was no relationship between self-efficacy score and chance-powerful others locus of control level (p>0.05).

Discussion

In this study, which was performed to determine the students' level of health self-efficacy perception and ability to create health locus of control at the beginning and the end of their education in vocational school of health, the fact that all of the students who constituted the target population were included may be considered as a strong point of the study. On the other hand, answers to the questionnaire and scales were limited with the students of two vocational schools of health. Internal consistency coefficients of both scales were similar to validity-reliability studies, which might indicate the validity of the results. In the original MHLC there were three subscales, and in some countries, such as Japan (14) reliability and validity study was done on these three subscales. On the other hand, it was adapted to Turkish as four subscales (9) similar to some other studies (15, 16). This situation results in some difficulties in comparisons. Scale scores were given as total points in some studies, as average scores in some other studies, which is another source of difficulty for comparisons.

When it is evaluated in general, it was found that the levels of health behaviours like exercise/sports, tooth brushing, and healthy diet were lower than expected among students of vocational school of health (Table 2). Students' score of health perceived health competence was intermediate and in accordance with other studies in Turkey (17). It can be said that students are homogenous in terms of health competence score. Health competence score is the highest among university students who receive occupational education about health, which may be an evidence for a general relationship between healthy behaviour and self-efficacy. In general, when students' health locus of control tendencies were examined, all fields had different scores and internal locus of control was

higher than others, which complied with the Type 1 in typology of multidimensional health locus of control (18). This type of locus of control structure is the most common type described as pure where one locus is dominant in three locus structures. Although there were four locus structure in our study, it was compatible with the mentioned typology of Wallston and Wallston (18) because the internal locus of control score was over other loci ($p<0.05$). The issue that the level of fate control tendency advanced to second place with respect to some variables will be discussed below.

The fact that the percentages of exercise/sports and smoking were higher in males, and percentage of tooth brushing was higher in females, was compatible with the expectations in society. Perceived self-efficacy score was higher among males in the study, which might be a result of the traditional image of self-sufficient male imposed on them during early ages. Similarly, when tendencies of health locus of control were examined, females were found to be more fatalist than males (Table 3). When it is considered in Turkey that girls are raised more dependent to their families, their needs are generally satisfied by their families and they are not totally independent in their decisions; it is easily understood why they are submissive/fatalistic. In a study carried out on university students (17), it was reported that Self-efficacy score did not vary with regard to gender. On the other hand, Mutlu (19) found that score of perceived responsibility about health was higher in female students. However this finding cannot be compared with this study directly because of the different scale they used.

Self-efficacy scores of first and last class students at vocational school of health were similar, while powerful others locus of control tendency decreased in last classes as in the study of Partlak-Gunusen and Ustun (20). This suggests that students are more dependent to others before taking occupational courses about health. Influence of family and environment may be stronger in early ages. This influence may be expected to diminish with the increasing age and occupational health education. Although there was no increase in internal control score, the decrease in the external control score in the last classes might indicate that they would be more independent in their decisions. That result was also supported by the fact that the internal control had the highest score. In a study (21) adolescents whose internal control locus of control tendencies was high were found to be more willing to take responsibilities and be more active about their health. In conclusion, even at high school level, occupational health education can be said to decrease external control and increase the internal control. This result is parallel to the study hypothesis. However, fate locus of control score of the students at the EMT department is higher than powerful others locus of control with respect to nurses. The fact that EMT's social status was lower than nurses might incite some uncertainty in them.

Most of the students cohabited with their families. It is also understandable why all students with chronic diseases live with their families. It is very likely that families prefer keeping their ill children with them. Students staying with their families have more fatalist tendencies than those staying with their friends. This shows that students staying with friends are used to taking more responsibilities about their lives, so they incline to more tangible loci of control rather than fate. Fate control tendency of the majority staying with family is similar to the general tendencies in the community. It is a fact that fatalism is more dominant in Asian countries than western societies. The fact that students living with friends reported their family income lower might be a perception or a fact. It is impossible to validate this information in this study's frame. However, external control score of the students who have perceived low-income is the lowest, which is in accordance with the refrainment of students living with friends from fatalism.

The level of leading healthy life behaviours of students was generally low in the study. In a study performed in a nursing college (6), it was similarly found that students apply health improvement behaviours in low level. Adolescence is a special period for developing and preserving healthy lifestyle behaviours. It is necessary to improve health behaviours of health staff candidates with different techniques like peer education which involves interactive participation, because health behaviours obtained in adolescence may be important in individual's general health behaviours.

As far as we have found in this study, few students participate in regular exercise/sports. There is no difference between the students who regularly

exercise and who do not with respect to the scores of health self-efficacy and health locus of control. Similarly in a study (21) performed on university students, people who took exercise regularly and people who did not were not found significantly different with respect to health locus of control tendencies, whereas it was stated in another study (19) that students who take exercise regularly perceive responsibility better than others in all levels of education. However, it was found in this study that the students who did not take exercise had a higher score of fate locus of control than powerful others locus of control, which might indicate that they tend to take less responsibility about their health. The students who exercise incline to powerful others rather than fatalism.

Very few of the students had chronic diseases. This is what is expected in this age group. Students without chronic diseases had higher health self-efficacy score than students with chronic diseases, which is thought-provoking. It is probable that students with chronic diseases think that they are not self-sufficient in terms of health. Students without diseases may perceive their self-efficacy higher because they do not struggle with health problems and may think that they can handle those problems better. Among the students with chronic diseases, internal control score decreased to the level of fate score and powerful others control score decreased to the level of chance score. This may be caused by inevitability of disease and submission to it or explaining the disease with luck. Also decrease of both internal and external loci of control and the increase of fate locus of control score may be result of a feeling of uncertainty in the young about their health.

Half of the students at vocational school of health consulted physicians for acute causes in the last month, which is a surprisingly high ratio. This consulting frequency equals approximately 6 visits a year, which is too much for young people. This redundancy may be caused by easy access to healthcare service due to their education and internship in health institutions. Their vocational education about health may have increased their consultation frequency for protection and early diagnosis. Young people are advised to utilize recently designed adolescent friendly primary healthcare services like counselling services about sexual health and substance dependence (22), especially in metropolises. Centres of Counselling and Healthcare Services for the Young are being opened in Turkey in this concept, although not in adequate numbers. Another subject is the high consultation frequency in smokers. However, the ratio of smoking is low (10 %), so we believe that it does not affect the explanations above. While there was not any difference in self-efficacy scores, fate locus of control tendency decreased and powerful others locus of control tendency increased in the students who recently had a disease, which may be connected to the intervention of a physician because of the disease.

The ratio of students who stated that they took balanced and sufficient nutrition was about one over three. There was a limited relationship between care about diet and self-efficacy. In a study performed on university students, the ratio of caring about diet was higher (43 %), and these people had higher self-efficacy level (17). Acikgoz accepted statistical significance level to be 0.10 in that study, so it is similar to the findings in this study. Self-efficacy can be said to be related to healthy diet according to these findings. Students who keep a healthy diet have a high tendency towards internal control locus, which reinforces the argument about self-efficacy. Also powerful others locus of control is higher in students who have a low calorie diet and fate locus of control is higher in students who are not careful about their diet, which indicates a hierarchical relationship between healthy diet and health locus of control (Table 4). Education can improve adolescents' health behaviours towards nutrition (23), so both self-efficacy level and internal locus of control tendency can be increased by educating young people on this issue.

Although the number of smokers seems to be low (Table 2), there is a negative situation when their education about health and age are considered. Also higher ratio of smoking among last classes arouses questions about benefits of vocational health education to student's own health in this aspect. In Acikgoz's study performed on university students, 31% of them were found to be smokers. This shows an increase in the ratio of smoking with the increasing age. Although there was not any difference between smokers and non-smokers with respect to self-efficacy level in our study, Mutlu (19) fo-

und the responsibility level of smokers lower in his study. Acikgoz (17) also found competence score lower in the students who smoked. Non-existence of such a difference in this study may be due to the low number of smokers. On the other hand, relatively lower tendency of powerful others locus of control among smokers may be interpreted as indifference of them about external powers and their incline to be introverted, so protective measures should be taken to prevent smoking in early ages with more convincing methods.

Less than half of the students brush their teeth regularly. When it is considered that the habit of tooth brushing should be acquired in early ages, the reasons for the lack of application in this subject should also be investigated. The increase in the health self-efficacy scores of students who regularly brushed their teeth (Table 2) strengthens the relationship between positive health behaviours and self-efficacy. Higher ratio of regular tooth brushing (57.1 % boys–78.1 % girls) habit among high school students (24) than vocational school of health students is something unexpected. The negative level of smoking and tooth brushing health profession candidates who are expected to be model for health behaviours need to be revised of the education and environment in these schools. Female students have a higher ratio of tooth brushing something unrelated to self-efficacy level, which suggests that tooth brushing is performed with aesthetic concerns rather than health. It is probably because of this contradiction that there is no connection between tooth brushing and health locus of control tendency (Table 4).

Fate locus of control tendency is higher among those who do not have any suggestions about leading a healthy life. This may either be due to unconcernedness of the adolescents or their ignorance what to do in real terms. Sanberk (13) stated that there was not any differentiation in life quality with regard to health locus of control level. In a study carried out on 6th and 7th grade students (25) it was found that there was a positive correlation between perceived body health and self-realization, exercise, diet, stress management and healthy life style behaviours. Education during adolescence may be a good opportunity to teach the need for perceiving and taking responsibility for one's own health to develop healthy life behaviours.

Conclusion

As a result it was found that a small percentage of students had healthy life behaviours. Health self-efficacy and multidimensional health locus of control tendencies showed homogenous character in general. Students who had high self-efficacy score and low fate locus of control score were more inclined to have positive health behaviours. Formal vocational education at high school level did not affect perceived self-efficacy or health locus of control significantly, so attempts to improve responsibility might be useful in formal vocational education of health. It was also found that preparing physical and social environments where adolescents could practice their information and applications about health might facilitate their acquisition of positive health behaviours. An increase in the number of studies about self-efficacy and health locus of control in adolescence might contribute to increasing awareness about health self-responsibility and directing informing about this subject.

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Bone mineral density and body composition in pediatric patients with celiac disease and inflammatory bowel disease

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Abstract

Background: Celiac disease (CD) and inflammatory bowel disease (IBD) are frequently associated with reduced bone mineral density (BMD). In the present study we investigated bone mineral density in pediatric patients with either CD or IBD in stable remission and compared them with their peers. Furthermore, we investigated the applicability of bioimpedance analysis (BIA) as a diagnostic tool for lowered BMD.

Methods: Altogether, 104 children aged between 10 and 18 years (49 boys, 55 girls) were selected and followed-up. Lumbar and left hip BMD were measured using dual-energy X-ray absorptiometry (DXA), and results expressed as Z scores for a specific chronological age. CD and IBD were diagnosed previously in each test group and children/adolescents were treated accordingly. Additionally, levels of vitamin D were determined in all the groups including control. Percentage of body fat (BF %) and lean body mass (LBM) were measured using BIA method.

Results: BMD did not differ significantly between CD, IBD or control group. In CD and IBD group vitamin D levels were significantly lower than in the control group ($p < 0.01$).

Conclusion: Results of the present study showed that pediatric patients with CD and IBD in a stable remission have good BMD. As vitamin D levels in both test groups were significantly lower than in the control, supplementation of vitamin D should be considered in order to prevent possible long term deteriorations of bone mineral mass. Our results showed that BIA is not a reliable method in determining lowered BMD.

Key words: Bioimpedance analysis, bone mineral density, celiac disease, children, inflammatory bowel disease.

Introduction

Chronic inflammatory gastrointestinal diseases like celiac disease (CD) and inflammatory bowel disease (IBD) are known to cause alterations in bone metabolism.[1–6] CD is an immune mediated disease that occurs in genetically susceptible individuals after exposure to certain peptides such as gliadin present in wheat, and similar prolamins in rye and barley.[1] Several risk groups are known to have an increased risk for developing CD among which first-degree relatives certainly represent an important group.[7] Without therapy and strict gluten-free diet (GFD), CD is associated with malabsorption of calcium and vitamin D, which reduces serum calcium and stimulates the release of parathormone, thereby exacerbating bone reabsorption from the mobilization of bone calcium.[8,9] This progressively leads to a decrease in BMD that, in turn, can lead to osteopenia or even osteoporosis.[10] Over 75 % of untreated patients with CD have low BMD.[11] Children with osteopenia have a higher risk of fractures not only in childhood, but also later in adulthood due to not reaching their optimal peak bone mass.[3] However, a GFD improves bone mineralization, already within the first year of treatment.[12] Early diagnosis and treatment of CD during childhood will prevent the development of osteoporosis later in life.[12,13] Lower BMD in patients with CD may also be associated with systemic inflammation characterized with increased concentrations of proinflammatory cytokines, such as: interleukin 1 (IL-1), tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6), which fall shortly after the treatment.[14,15] IBD is characterized by severe inflammation of the small bowel and/or colon leading to recurrent diarrhea and persistent abdominal pain. Crohn's disease and ulcerative colitis are two main clinico-

pathological subtypes of IBD. Patients who cannot be classified as neither of those two subtypes are diagnosed as indeterminate colitis. IBD can be very disabling, due to a fatigue associated with inflammatory symptoms, but also due to chronic pain suffered by patients.[16] Children with IBD gain bone mass, but the rate of bone mineral accrual is slower than in their peers, at least during the first 2 years after the diagnosis.[9] It is reasonable to hypothesize that decreases in the rate of bone accrual may lead to suboptimal peak bone mass which is known to be reached in the third decade of life.[17,18] Catch-up growth may not occur in children with IBD, and inadequate linear growth may stunt the normal acquisition of bone minerals.[9] For that reason, these young adults could be in a disadvantageous position regarding their bone health in later life.[17] Children with Crohn's disease have a higher risk of developing osteopenia than children with ulcerative colitis.[3] There are several possible mechanisms of IBD-related reduction of BMD, including malabsorption and malnutrition, prolonged corticosteroid therapy, restriction diets that are low in vitamin D and calcium, and also immobilization.[4,19] In recent years, the role of inflammation is becoming more and more important in the pathogenesis of reduced BMD.[4,11,15] In IBD, the immune response, mediated by T lymphocytes and other inflammatory cells like macrophages, leads to a production of various proinflammatory cytokines such as interleukin (IL)-2 and TNF- α.[5] In addition, elevated mucosal and serum levels of proinflammatory IL-1α, TNF-α, and IL-6 have been reported in Crohn's disease, ulcerative colitis and CD.[15] Within mononuclear cells, the key nuclear transcription factor is nuclear factor-kappa B (NFκB), which regulates the transcription of IL-1, IL-6, IL-8, and other peptides that are central to inflammatory response.[20] The mucosal density of colonic $CD68^+$ macrophages is significantly higher in children with Crohn's disease than in those with ulcerative colitis. In addition, the number of activated $CD40^+$ macrophages is significantly elevated in both histologically inflamed and uninflamed colon of IBD children.[21] A new TNF family pathway involved in bone metabolism, known as the RANK-RANKL-OPG pathway, has recently been described.[22] RANKL (receptor-activator of NFκB ligand) is expressed on the surface of osteoblasts, synovial stromal cells and activated T cells. RANKL binds to either osteoclast precursors expressing the RANKL receptor (receptor-activator of NFκB, RANK) or a soluble decoy receptor osteoprotegerin (OPG), which is produced by osteoblasts. If RANKL and RANK interact, osteoclasts differentiate and mature, resulting in increased bone loss. OPG blocks this interaction, thereby inhibiting osteoclast production. Compounds such as parathyroid hormone, $1\alpha,25\text{-}(OH)_2D_3$, prostaglandin E2 and dexamethasone stimulate RANKL expression and inhibit OPG production, thereby causing increased osteoclastogenesis, whereas 17β-estradiol increases OPG and decreases RANKL, reducing osteoclastogenesis.[15,22] The small bowel of patients with CD contains a marked increase in intraepithelial and lamina propria lymphocytes, potentially releasing cytokines that alter the balance of the RANK-RANKL paradigm toward osteoclastogenesis.[23,24] Recent clinical studies in patients with IBD have revealed that serum OPG levels may be elevated and that inflamed intestinal tissue secretes increased amounts of OPG. It is suspected that OPG levels are elevated as a counterregulatory response to low BMD, as serum OPG levels in IBD have been found to be inversely associated with BMD.[25]

Patients with CD and IBD are also at higher risk for vitamin D deficiency.[26-28] Decreased exposure to sunlight, decreased dietary intake, malabsorption and gastrointestinal loss may all well contribute to lowered serum levels of vitamin D in IBD patients.[17,29] The loss of albumin as the binding protein for vitamin D is well documented in patients with IBD, and may also be a risk factor that contributes to the pathology of disease.[31] To avoid complications in the future, like impaired growth, suboptimal peak bone mass or risk of fractures, it is important to diagnose and treat complications of chronic inflammatory gastrointestinal diseases.

The aim of the present study was to determine the differences in BMD and vitamin D between CD and IBD patients, compared to control group. Furthermore, we investigated the usefulness of BIA in detecting lowered BMD. To our best knowledge, no other study analyzed the relation between BF % and BMD in children and adolescents with CD and IBD using BIA.

Patients and methods

In this prospective case controlled study, which was conducted between March 2011 and April 2012, a total of 104 children aged between 10 and 18 years were selected and followed-up. They were divided into three different groups: children with CD (n = 35; 14 boys, 21 girls), children with IBD (n = 35; 19 boys, 16 girls) and control group (n = 34; 16 boys, 18 girls). Even though all of the CD patients declared to be on a strict GFD during the period of at least 6 months before the study, their serological markers (antibodies against tissue transglutaminase - t-TG) indicated that there were significant differences in the strictness of the diet. All patients with CD were previously diagnosed according to the diagnostic criteria of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), which includes histological demonstration of villous atrophy in duodenal mucosa and positive serological markers.[32] IBD group consisted of 19 patients with Crohn's disease (11 boys, 8 girls), 13 patients with ulcerative colitis (6 boys, 7 girls) and 3 patients with indeterminate colitis (2 boys, 1 girl). IBD patients were receiving appropriate treatment and were all in stable remission for at least 6 months prior to the study. IBD patients were also given calcium and vitamin D supplements and were advised to take them regularly. They did not receive any corticosteroid treatment during the period of the study. After initial diagnosis was established and written consent obtained from parents or children if they were old enough, children and adolescents were included in the study. None of our patients had skeletal symptoms.

The control group consisted of children and adolescents who showed no symptoms of CD or IBD and who came to the Pediatric Clinic for their check-ups following their hospitalization due to a particular health condition such as pneumonia, urinary tract infection, otitis media, diarrhoea, etc. During their visit, parent(s) and child/adolescent received information regarding the study. After obtaining the written consent, participants were included in the study and all necessary measurements taken by trained medical personnel.

Patients with CD and IBD were invited to participate during their regular follow-up visits. Two participants with CD, however, did not reach inclusion criteria and were consequently excluded form the study. The reasons for exclusion were, hypertension (1 adolescent) and chronic kidney disease (1 adolescent). In addition, 3 adolescents with IBD and 5 children and adolescents from the control group refused to participate. All participants included in the study were Caucasians residing in North-Eastern Slovenia.

Lumbar spine (L1-L4) and left hip BMD were measured in 97 patients using dual-energy X-ray absorptiometry (Hologic Explorer QDR). The device and the system were calibrated daily. Results were expressed as Z scores that were determined from local reference data.

Laboratory studies were conducted at the Department of laboratory diagnostics, University Medical Centre Maribor, Slovenia. Serum vitamin D levels were determined in all the patients and controls with standard methods. Normal range of serum vitamin D is between 47.7 and 144 nmol/L.

Antibodies against tissue transglutaminase were measured by Department of laboratory diagnostics using established methods (Luminex, AtheNA Multi-Lyte® Celiac Plus Assay, The Zeus Scientific).

BIA is a fast, non-invasive and painless method. It (QuadScan 4000, Bodystat Ltd., Douglas, Isle of Man) was used in our study to determine percentage of body fat (BF %) and lean body mass (LBM, measured in kg). Measurements were conducted at Gastroenterology Unit, Department of Pediatrics at University Medical Centre in Maribor. All our subjets were fasting and were appropriately hidrated. Children's and adolescent's anthropometric measurements, body height (cm), waist circumference (cm) and body weight (kg) were collected by medical personnel using standardized procedures. Measurements were conducted with our subjects lying in supine position on a flat, nonconductive bed. Bodystat QuadScan 4000 has four electrodes. Two electrodes were placed on the right wrist with one just proximal to the third metacarpophalangeal joint (positive) and one on the wrist next to the ulnar head (negative). The other two electrodes were placed on the right ankle with one just proximal to the third metatarsophalangeal joint (positive) and one between the medial and lateral malleoli (negative). Multifrequency (5, 50, 100, and 200 kHz) currents were introduced from the positive leads and traveled throughout the

body to the negative leads. BF % was calculated by using the manufacturer's software.

Statistical methods

ANOVA test was used to determine statistical significance. Statistical analysis was performed with the SPSS 16.0 statistical package for Windows. All statistical values were considered significant at the P level of 0.01.

Results

When comparing the mean lumbar spine BMD Z scores for two test groups we found that the CD patients had mean Z values of BMD of – 0.2, which is similar to control group (- 0.3) (Table 1). Patients with IBD had mean lumbar spine BMD Z score of - 0.4 (Figure 1). BMD Z scores measured at lumbar spine in patients with CD, patients with IBD and control group did not differ significantly (p = 0.583).

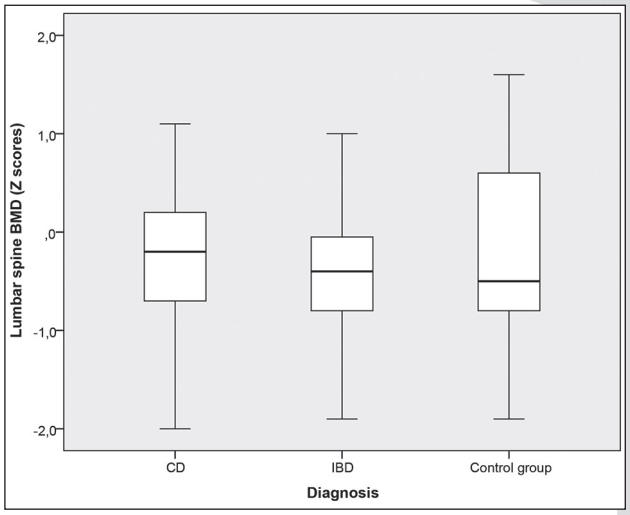

Figure 1. Lumbar spine BMD in different groups Figure shows comparison of lumbar spine BMD Z scores between different groups. BMD Z scores measured at lumbar spine in patients with CD, patients with IBD and control group do not differ significantly (p = 0.583).

After analyzing the mean left hip BMD Z scores for two test groups we observed a similar distribution of measured data. Mean left hip BMD Z score of CD patients was - 0.4, compared to – 0.8 in control group. IBD patients had mean Z scores of - 0.6 (Figure 2). The mean BMD Z scores were calculated in all patients in CD group, regardless of the strictness of their GFD. However, differences in left hip BMD Z scores between groups did not show statistical significance (p = 0.215).

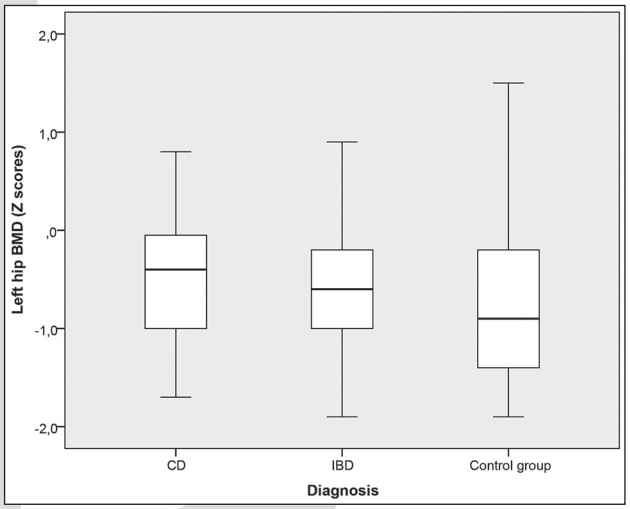

Figure 2. Left hip BMD in different groups Figure shows comparison of left hip BMD Z scores between different groups. BMD Z scores measured at left hip in patients with CD, patients with IBD and control group do not differ significantly (p = 0.215).

When measuring serum vitamin D levels, the mean vitamin D levels of 32.5 nmol/L in CD patients was observed. Mean vitamin D levels of IBD patients was similar with 32.6 nmol/L. Control group had the highest measured levels of vitamin D (49.9 nmol/L). Reference values used at our University Medical Centre range from 47.7 to 144 nmol/L. Thus our control group vitamin D levels are positioned slightly above the lower lim-

Table 1. Mean values of lumbar Spine BMD, left hip BMD and vitamin D concentration in different groups and their statistical significance (p)

Diagnosis	Lumbar Spine BMD [Z score]	Left Hip BMD [Z score]	Vitamin D [nmol/L] Ref. val. (47.7 - 144)
Celiac disease	- 0.2	- 0.4	32.5
Inflammatory bowel disease	- 0.4	- 0.6	32.6
Control group	- 0.3	- 0.8	49.9
p	0.583	0.215	‹ 0.01

it, and vitamin D levels of CD and IBD patients below reference values (Figure 3). Significant difference in vitamin D levels was found in CD and IBD group when compared to the control. In those two groups levels of vitamin D were significantly lower than in the control group ($p < 0.01$).

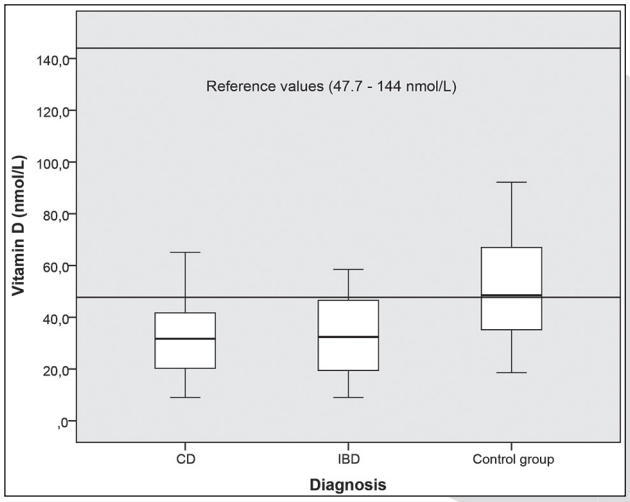

Figure 3. Mean serum vitamin D in different groups Significant difference ($p < 0.01$) in mean vitamin D levels was found in patients with CD and IBD when compared to the control group. Mean vitamin D level in control group was within the reference values with 49.9 nmol/L. The serum vitamin D levels were decreased with mean value of 32.5 nmol/L for CD patients and 32.6 nmol/L for IBD patients.

Within groups children were divided by sex, because their BF % differs anthropometrically. Boys with CD had mean BF % of 13.8 (n=12), boys with IBD had mean BF % of 20.7 (n=14) and boys in control group had mean BF % of 18.0 (n = 18) (Figure 4). Girls had mean BF % values higher than boys. Mean BF % of CD girls was 22.1 (n = 17), mean BF % of IBD girls was 26.9 (n = 12), and mean BF % of girls in control group was 26.5 (n = 19). BF % did not differ significantly between the groups ($p = 0.201$ and $p = 0.134$ for boys and girls, respectively).

The mean LBM in boys with CD was 51.4 (n = 12), boys with IBD 49.6 (n = 14), boys in control group had mean LBM of 44.5 (n = 18) (Figure 5). Girls with CD had mean LBM of 34.2 (n = 15), girls with IBD 47.1 (n = 12) and girls in control group had mean LBM of 38.3 (n = 19). All measurements are expressed in kilograms. LBM did not differ significantly between the groups of boys ($p = 0.262$) but we found statistical significant difference between groups of girls ($p = 0.007$).

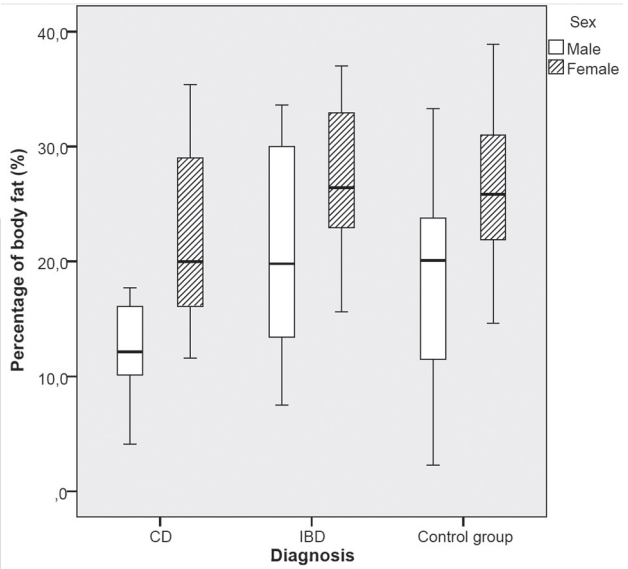

Figure 4. Percentage of body fat (BF %) in different groups (divided by sex)
Figure shows comparison in BF % in CD patients, IBD patients and control group. Patients were divided by sex. Empty bars represent boys and bars with slanted black lines represent girls. BF % did not differ significantly ($p = 0.201$ in boys and $p = 0.134$ in girls).

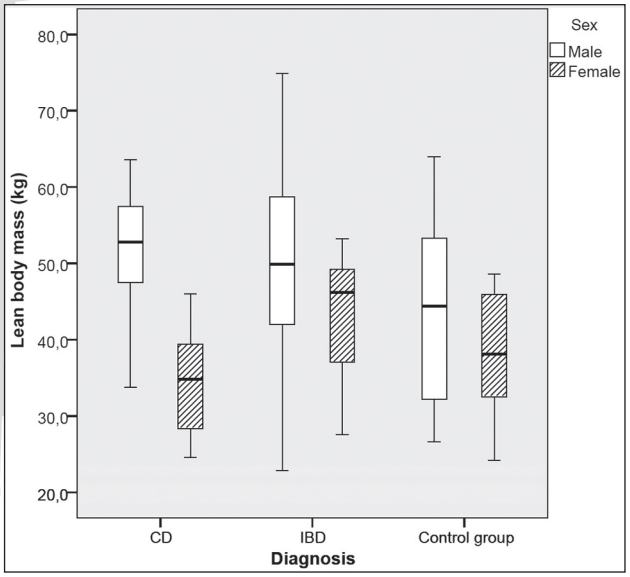

Figure 5. LBM in different groups (divided by sex)
Figure shows comparison in LBM in CD patients, IBD patients and control group. Patients were divided by sex. Empty bars represent boys and bars with slanted black lines represent girls. LBM did not differ significantly in boys ($p = 0.262$) but it did in girls ($p = 0.007$).

Furthermore, our results showed no correlation between BF % and BMD Z scores. We compared all of our patient groups, divided by sex, but also all together. None of our comparisons with lumbar spine or left hip BMD Z scores were statistically significant. That was also the case when comparing LBM with BMD Z scores in all groups of patients.

Discussion

All chronic inflammatory diseases, regardless of their etiology, can cause changes in bone metabolism via inflammation mediated pathway. Lowered BMD has been well documented in patients with chronic gastrointestinal diseases, such as CD and IBD.[1-6] However, several studies reported that treated CD patients generally achieve normal bone mineralization and bone mass.[2,13,33] This could probably be explained with the fact that children and adolescents with CD on GFD are generally eating healthier than their peers from control group. They may consume more protein from animal sources which also contain higher amounts of calcium, which is an important factor in bone health. They may also have better knowledge about the importance of balanced nutrition. Anyhow, these results are very promising. They show us that we are successful in preventing osteoporosis and decreasing the risk of bone fractures. CD patients from our study were not given any vitamin or mineral supplements, so it seems that the strict GFD sufficed. However, bone health, including BMD in children with CD is usually impaired at the time of diagnosis, as described in many studies,[1,2,12,13,28,34-36] but improves after the introduction of GFD, vitamin D and calcium supplementation.[1,2,15,33,34,37,38,40] The study of Carvalho found no difference in BMD in children with CD, but found lower BMD in adolescents with CD, compared to controls.[37] However, in clinical practice the BMD and bone health are usually worse in children with CD than in their peers.

Low BMD is frequently detected in newly diagnosed children with IBD, particularly Crohn's disease.[6] Mechanical properties of bone may worsen over time.[38] In addition, height Z-scores may not improve with conventional IBD therapy,[39] and muscle mass deficits may also persist, which can affect the accrual of bone mass, especially in patients with Crohn's disease.[40,41] Our results also show that BMD Z scores in IBD patients in stable remission are comparable to those of the control. The results suggest that we can be satisfied with the treatment of our IBD patients, because normal BMD is usually more difficult to achieve in IBD patients than in CD patients, according to others.[6,11,33] Results also support supplementation of IBD patients with vitamin D and calcium.

In our study, serum vitamin D levels in children with CD or IBD were significantly lower than in the control group. This is in accordance with previous studies.[1,17] In both CD and IBD patients the mean vitamin D levels were below reference values. This can be explained by malabsorption and gastrointestinal loss, which are the consequences of inflamed intestine, and possibly by a decreased sunlight exposure.[17,29] Low dietary intake and poor compliance with vitamin D supplementation in IBD patients could also contribute to reduced vitamin D levels in the same group of patients.

When we compare the mean value of vitamin D in the control group to some other European countries, we observe lower concentrations of vitamin D (49.9 nmol/L vs. 57.1 nmol/L in other European countries),[42] which can be attributed to different diets (less foods rich in vitamin D, like oily fish), less exposure to sunlight or seasonal variations in vitamin D concentration.

The extent of vitamin D's role in bone mineralization in pediatric patients with chronic inflammatory gastrointestinal diseases has yet to be determined. A pediatric study of Sentongo et al.[43] reported no association between BMD and vitamin D status in children with Crohn's disease. It is not clear whether pre-existing vitamin D deficiency was the initiating event leading to disease severity, or vitamin D deficiency was the consequence of severe underlying illness.[44]

Previous studies reported the effect of vitamin D and calcium supplementation in BMD of children with specific chronic diseases. They reported increases in BMD of various skeletal sites with calcium and vitamin D supplementation, which was not observed without it.[17] Adolescent with CD, who were treated from an early age have BMD values similar to those of control.[12] Moreover, an appropriate diet is very important in these patients, as naturally gluten-free products

are often low in B vitamins, calcium, vitamin D, iron, zinc, magnesium, and fiber.[45] Also, a study of dietary habits among children and adolescents in Slovenia revealed that dietary vitamin D intake was less than optimal and that calcium intake in boys was adequate, but bellow recommendations in girls.[48] Therefore we advocate supplementation of pediatric patients with vitamin D and calcium, not only those with IBD, but also those with CD. Regular follow-ups are also important in ensuring patients follow their diet, prescribed medications and supplements.

Adolescents with IBD have unique issues such as poor medication compliance, growth failure, peer influence and risky behavior, which make managing teenage IBD patients more challenging.[47] Similar challenges arise in children and adolescents with CD, especially if their symptoms are not prominent while not being on GFD. If they have no symptoms it is less likely that they will comply with strict GFD. Therefore it is critical to ensure and control appropriate treatment and diet compliance.

Girls had mean BF % values higher than boys, which is anthropometrically expected. We observed lower BF % in CD patients compared to IBD patients and controls. Lower BF % in boys and girls with CD was expected because their disease and diet.[48,49] BF % increases with implementation of GFD;[49,50] however, the exact mechanism is not yet explained.[51] Several other studies reported lower as well as higher values of BF % in children with IBD,[40,52] which can also be seen in our results.

Girls had lower LBM which is expected due to their lower body weight and higher BF %. Girls with CD had lower LBM when compared to controls which is in concordance with previous studies.[49,53] Boys with CD had LBM values similar to boys with IBD and both were higher than boys in control group. Basically normal LBM values in boys with CD could again be explained with healthy diet and lifestyle.

Furthermore, we wanted to explore the possibility of using BIA as a diagnostic tool for lowered BMD. Studies have shown positive correlation between body weight and BMD.[54-56] It has been debated which of LBM or fat mass has more influence on bone stimulatory effect.[57,58] Petit et al.[59] suggest that bone strength is primarily determined by dynamic loads from muscle force and not static loads such as fat mass.

BIA is a lucrative method because of its ease of use, low costs and resources. Regarding its advantages, it would make sense to expand indications of BIA use. Some studies implicate, that body composition (and with this BF %) correlates with BMD.[60]

Our results showed no statistically significant correlation between BF % and BMD or LBM and BMD in any of the groups, not even when subjects were divided in groups by sex. Using BIA for determining body composition was found only in smaller number of studies. In those studies, one showed negative correlation between BF % and BMD in younger women, whereas other authors found positive correlation in pre- and postmenopausal women, and some found positive correlation in adult men and women.[60-62] In one of more recent studies, positive correlation was found between BF % and BMD in prepuberty girls.[56]

BIA has potencial in clinical setting due to its practicality. However, the method will have to be studied more throughly. Body composition depends on many different factors and especially in pediatrics we have very heterogenous population, which differs by sex, age, height, weight, as well as level of development of various organ systems.

Conclusion

Early diagnosis and correct treatment are of utmost importance in preventing pediatric patient's suboptimal peak bone mass, osteoporosis and increased risk of fracture in the future. Satisfactory BMD in our CD and IBD patients warrant strict compliance with GFD in CD and necessity of good disease control in IBD patients. Even though we did not confirm applicability of BIA in determining changes in BMD, due to its practicalities, further studies are needed to determine its indications and position in the diagnostics of lowered BMD.

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Caffeic acid phenethyl ester (CAPE) protects lung epithelial cells against H_2O_2-induced inflammation and oxidative stress

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Abstract

Background and the objectives: The development of many inflammatory lung diseases such as chronic obstructive pulmonary disease, cystic fibrosis and asthma is characterized by excessive oxidative stress and inflammation. The aim of this study was to investigate the potential protective role of caffeic acid phenethyl ester (CAPE) on stress-induced damage in human lung epithelial cells (A549).

Methods: The cellular protective effect of CAPE was assessed by MTT assay, and oxidative stress was determined by nitric oxide (NO) production and total oxidant capacity measurement by spectrophotometric methodologies. The cytokine mRNA expressions were analyzed by qRT-PCR technology. The cells were pretreated with 5 μM CAPE for 2 h and exposed to 100 μM hydrogen peroxide ($H_2O_{2)}$ for 24 h, and then the samples were prepared further analyses.

Results: H_2O_2 exposure resulted in significant cellular loss (41 %), and induced oxidative stress and inflammatory cytokine expressions. Incubation with CAPE protected 65 % of cell death caused by H_2O_2, significantly suppressed NO production, and improved enzymatic and non-enzymatic antioxidant status. Inflammation was determined by pro-inflammatory cytokine mRNA analyses. CAPE treatment not only significantly enhanced H_2O_2-suppressed glutathione (GSH) levels and catalase activity but also supported total antioxidant capacity. Real time qRT-PCR analyses revealed that exposure of the cells to H_2O_2 did elevate the mRNA expressions of tumor necrosis factor alpha (TNF-α), interleukin 18 (IL-18), interferon gamma (IFN-γ), and inducible nitric oxide synthase (iNOS). While TNF-α and iNOS expressions were completely suppressed by CAPE treatment, IL-18 and IFN-γ mRNA levels were decreased by 26 and 50 %, respectively.

Conclusion: These results suggest that CAPE might thus offer protection against oxidant-induced inflammation and cellular damage in lungs.

Key words: A549, caffeic acid phenethyl ester, CAPE, inflammation, lung epithelial cells, oxidative stress

Introduction

Alveolar epithelial cell injury and repair are important in the pathogenesis of oxidant-induced lung damage and fibrosis caused by various agents. Generation of reactive oxygen (ROS) and nitrogen (RNS) species in response to oxidative stress, or as byproducts of cellular metabolism, induce lung injury in part by causing enzyme inhibition, damage to proteins, DNA, and lipids (1-3). On the other hand physiological levels of ROS also can act as second messengers in signal transduction cascades (2). Oxidative and nitrative stress and the potential injuries that result from it have been implicated in a wide number of disease processes, including inflammation, ageing, atherosclerosis, neuronal degeneration and cancer (4). Over time, oxidative stress leads to cell death through either apoptosis or necrosis or some combination of the two. As a means of self-protection, cells and tissues have developed several enzymatic and non-enzymatic systems that regulate the concentration of these species inside and outside of the cells (1). The enzymatic antioxidant defense systems comprise catalase, superoxide dismutase and glutathione peroxidase, glutathione reductase (4,5).

Caffeic acid phenethyl ester (CAPE), a natural derivative of the honeybee propolis, including is a small lipid soluble potent flavonoid with multiple

biological effects (6,7). In recent studies, a wide range of pharmacological properties were demonstrated for this substance, including anti-inflammatory, antioxidant, antiproliferative, antibacterial and immunomodulatory activities (6-8). The prominent protective property of CAPE makes it a potential therapeutic compound against damage to the kidney, pancreas, brain, heart, and other tissues or organs (3,9,10). CAPE is a specific and potent inhibitor of the activation of NF-κB (11) and potent inhibitor of leukotriene biosynthesis, which implicates it as having a potential therapeutic effect for treatment of inflammatory diseases (12). It has also been shown that caffeoyl groups of propolis as well as CAPE are important constituents that give rise to the antioxidative activities (8). Since CAPE has well known anti-inflammatory and antioxidant properties, we hypothesize that CAPE may be able to protect lung epithelial cells against oxidative and inflammatory stress-caused damage and can be used as an augmentative therapy for preventing cell death and damages in humans. The effect of H_2O_2 on alveolar epithelial cells has been intensively studied in previous researchers using various concentrations and different culture conditions (7,13,14). In the present study, we used the H_2O_2 mediated cell death model of human derived A549 adenocarcinoma cell line which are related to alveolar epithelial cells, as previous studies have shown (7,14).

Materials and methods

Cell culture

The human pulmonary adenocarcinoma cells (A549) were maintained in DMEM (low glucose, Sigma) supplemented with 10 % fetal bovine serum and penicillin/streptomycin 100 IU/ml and 100 mg/ml, respectively and incubated at 37 ºC and 5% CO_2 in a 75 cm^2 tissue culture plates. Cells were digested with 0.25 % trypsin and subcultured at 70 % to 80 % confluence. Exponentially growing A549 cells were used for all assays.

Treatments

The A549 cells were cultured overnight at a density of 10 x 10^4/well or 4 x 10^4 /well in a sterile 12-well or 24-well plate, respectively (Costar, Corning Inc., USA). To measure the concentration of exogenously applied H_2O_2 in the culture medium over time, the maintenance medium was removed, medium containing 10 - 5000 μM H_2O_2 was added, and the cells were incubated for 24 h. Following incubation the medium was removed and the cells were washed twice with PBS (Ca^{2+} and Mg^{2+} free; pH 7.4) and viability was determined by MTT assay.

CAPE is a structural relative of flavonoids and is an active component of propolis from honeybee hives. To test the cytoprotective effect of CAPE against H_2O_2-mediated cell death, the cells were pretreated for 2 h with 0 to 40 μM of CAPE (Sigma Chemical Co., St. Louis, MO, USA), then the cells were stressed with 100 μM of H_2O_2 (Sigma, St. Louis, MO) for another 24 h. Cell viability was determined by both MTT assay and trypan blue dye exclusion. In some cases, the remaining adherent cells were photographed using an Olympus phase contrast microscope (CKX41, Japan) and Olympus digital camera to assess the cells in the cultures (pictures 40x objective, scale bar: 50 μm). The concentrations (5 μM CAPE and 100 μM H_2O_2) used for the treatments were chosen in order to obtain maximal effects.

Cell viability assessment

Upon exposure to H_2O_2 for 24 hours, cell survival was quantified by the colorimetric MTT assay (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Fluka, USA) (15). A549 cells were seeded in a 12-well flat-bottom plate in triplicate and cultured in media containing CAPE, which was diluted in dimethyl sulfoxide (DMSO). The same amount of DMSO was also added to the control cells. Following culture at 37 ºC, 1 ml/well of MTT (1 mg/ml) was added, followed by incubation for an additional 90 min for each experiment. The viable cells produced a dark blue formazan product, whereas no such staining was formed in the dead cells. The resulting formazan product was dissolved in 100 μl/well of acid-isopropanol, and absorbance read at 570 nm. The cell viability was calculated by the normalization of optical densities (OD) to the negative control. Cellular viability was also determined by trypan blue dye (0.05 % in PBS) exclusion assay by a hemocytometer (data not shown).

Homogenate preparation

After H_2O_2 and CAPE exposure for 24 h, cells were homogenized in ice-cold homogenization buffer (10 mM Tris, 1 mM EDTA, 25 mM $MgCl_2$,

0.1 mM dithiothreitol, 0.25 M sucrose, pH 7.4) containing complete protease inhibitor mixture (aprotinin, phenylmethylsulfonyl fluoride, leupeptin, sodium fluoride) (Sigma, Germany). Homogenates were centrifuged at 4 ºC, 15.000 rpm for 10 min and the soluble fraction was retained. The protein concentrations of cell extracts were measured by the Bradford reagent using bovine serum albumin as a standard.

Determination of oxidant and antioxidant status

Nitric oxide concentration in cultured cell medium was determined indirectly by measuring the nitrite levels based on Griess reaction after incubations (16). Samples were deproteinized with 75 mmol zinc sulphate. Total nitrite was determined by spectrophotometery at 546 nm after conversion of nitrate to nitrite by copperized cadmium granules.

Non-enzymatic antioxidant (GSH) content of A549 cell homogenates was determined according to the method of Sedlak and Lindsay (17).

Catalase activity in the cell homogenate was measured by the method of Luck (18), and the activity was presented as k/mg protein.

The novel total antioxidant status (TAS) (19), and total oxidant status (TOS) (20) assays have been shown to be stable, reliable and sensitive to determine antioxidant and oxidant capacity of the biologic samples, respectively. Therefore we used these automated colorimetric methods for TAS and TOS evaluation besides NO, catalase and GSH analyses. The results are expressed in terms of μmol H_2O_2 Equiv./mg protein for the homogenates.

RNA isolation and real-time QRT-PCR analyses

Real-time PCR was performed in a QPCR system (Stratagene Mx 3005P, USA). Total RNA from A549 cells were extracted using TRIZOL reagent (Sigma, USA) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed in a reaction volume of 20 μl using reverse transcriptase kit (Fermentas, EU). One microliter of each cDNA was used as templates for amplification using SYBER Green PCR amplification reagent and gene-specific primers. The human primer sets used were from Thermo Electron Corporation (Germany): catalase forward: 5'-CAG AGG AAA CGT CTG TGT GAG AC-3', reverse: 5'-CAA GTG AGA TCC GGA CTG CAC-3'; iNOS forward: 5'-GGC CTC GCT CTG GAA AGA A-3', reverse: 5'-TCC ATG CAG ACA ACC TT-3'; TNF-α forward: 5'-CAG AGG GAA GAG TTC CCC AG-3', reverse: 5'-CCT TGG TCT GGT AGG AGA CG-3'; IL-18 forward: 5'-GGC AAG CTT GAA TCT AAA TTA TGT-3', reverse: 5'-GCA TCT TAT TAT CAT GCT CTG GCA C-3'; IFN-γ forward: 5'-TCC GAG GCA AAC AGC ACA TTC-3', reverse: 5'-GGG TTG GGG GTG TGG TGA TGT-3'. The amount of RNA was normalized to β-actin amplification in a separate reaction forward: 5'-CAT CGT CAC CAA CTG GGA CGA C-3', reverse: 5'-CGT GGC CAT CTC TTG CTC GAA G-3'. β-Actin was used as endogenous control, and each sample was normalized on the basis of its β-actin content.

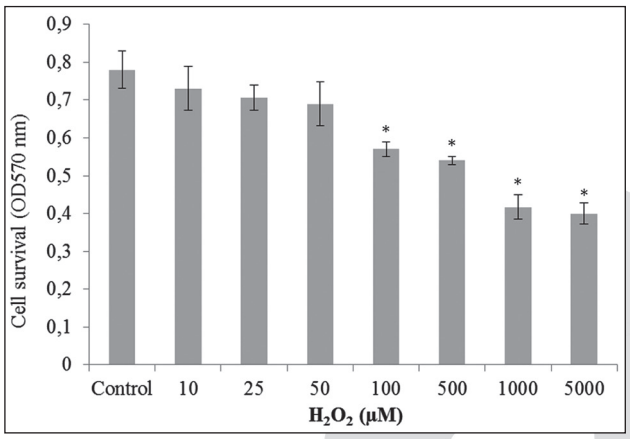
Statistical analysis

The one-way analysis of variance (ANOVA) and post hoc Duncan tests were performed on the data to examine the differences among groups using the SPSS statistical software package (SPSS1 for Windows v. 9.0). The results are presented as average ±SE. A value of $p< 0.05$ was considered significant.

Results

We used H_2O_2 treatment in order to model oxidative stress and inflammatory reactions in our cellular system. Effect of H_2O_2 on cell proliferation and viability were determined by MTT and trypan blue exclusion tests. Viability (Figures 1A and 1C) of A549 cells were decreased steadily in a concentration-dependent manner over the range of 10 to 5000 μM following 24 h H_2O_2 treatment. The data showed that 100 μM H_2O_2 killed about 41 % of cells at the end of the incubation. Concentration response course demonstrated that 5 μM CAPE protected 65 % of cell death caused by H_2O_2 (Figure 1B). For this reason 5 μM CAPE was used as a cell protective concentration for further experiments. Treatments utilizing 10 μM and higher concentrations of CAPE decreased cell viability (Figure 1B). Cells were evaluated with a microscope for density changes. It was noted that many cells detached from the flask (Figure 2)

following H_2O_2 exposure. On the other hand, the majority of cells recovered after CAPE treatment (Figure 2, CAPE+H_2O_2).



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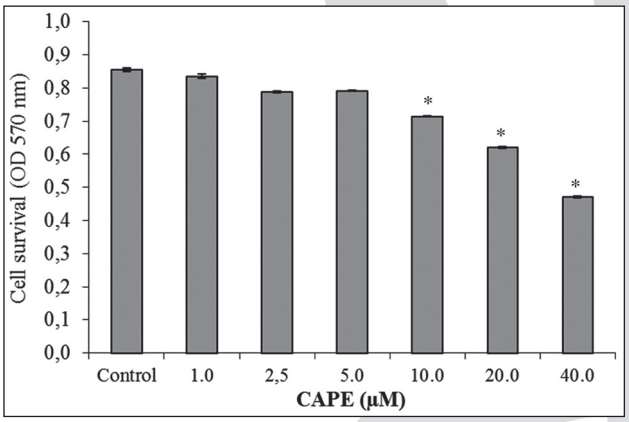

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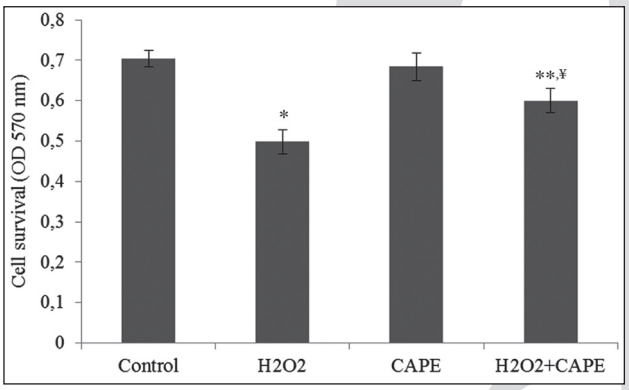

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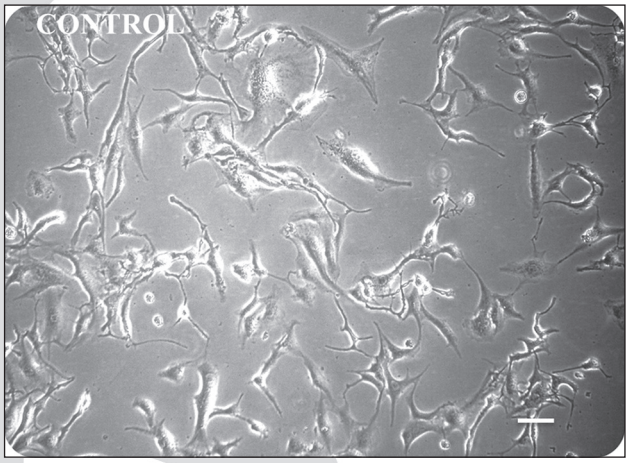
*Figure 1. Effect of H_2O_2 and CAPE on cell survival A: Concentration dependent effect of H_2O_2 on A549 alveolar epithelial cell viability. A549 cells were incubated for 24 h in the presence of various concentrations of H_2O_2 (n = 3). B: Concentration dependent protective effect of CAPE on H_2O_2-induced cytotoxicity in A549 cells. The cells were preincubated for 2 h with increasing concentrations of CAPE then incubated for a further 24 h at 37 ºC in the presence of 100 µM H_2O_2 (n = 3). C: Effect of CAPE on cell viability of A549 cells exposed with H_2O_2. Control cells were incubated with vehicle alone (DMSO), H_2O_2 groups received 100 µM H_2O_2, CAPE cells were incubated with 5 µM CAPE, and H_2O_2 + CAPE cells were incubated with 100 µM H_2O_2 + 5 µM CAPE (n = 5). *p < 0.001, ¥p < 0.05 versus control group; ** p < 0.001 versus H_2O_2-treated group.*



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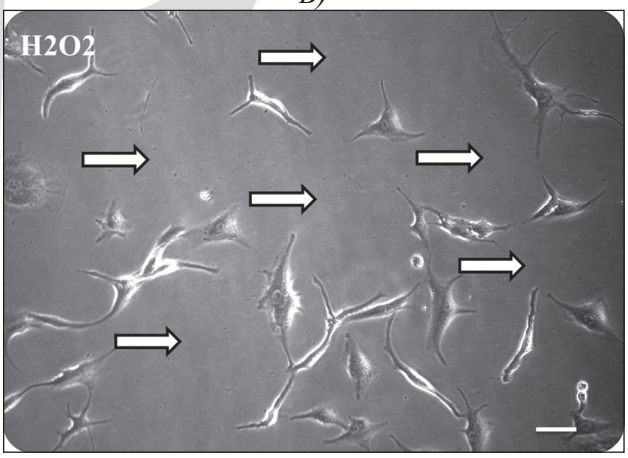

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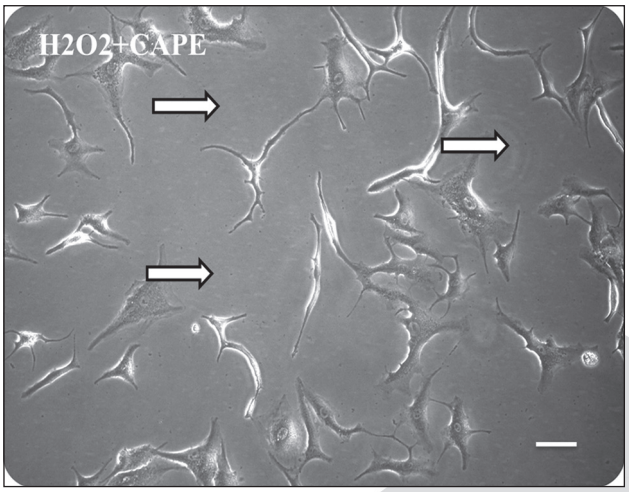
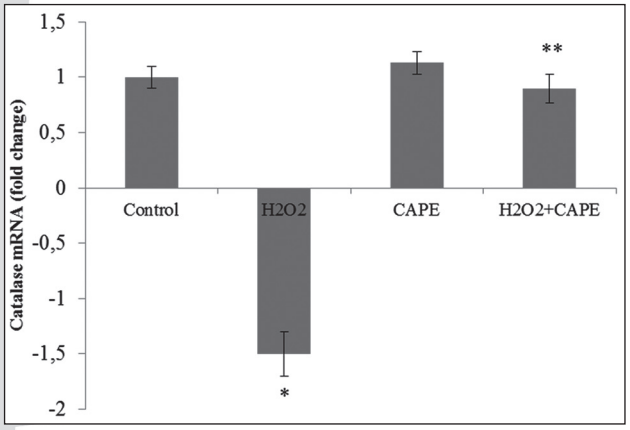


D)

Figure 2. Morphological density of A549 epithelial cells after H_2O_2 exposure and CAPE treatment. A549 cells were preincubated in the presence or absence of 5 μM CAPE for 2 h and then exposed 100 μM H_2O_2 or control (DMSO) for a further 24 h. Cells were observed with an inverted microscope for morphologic changes. It was noted that many cells detached from the flask which was indicated by arrows (Figure H_2O_2). However, CAPE treatment recovered the majority of cells from the toxicity (picture CAPE+H_2O_2). The cell view was recorded with phase contrast microscopy using a 40x objective. Scale bar: 50 μm.

To determine the effect of H_2O_2 exposure on oxidant and antioxidant homeostasis, we measured reduced glutathione (GSH) levels in A549 cells with or without H_2O_2. There was a significant (40 %) reduction in GSH levels in H_2O_2-exposed cells when compared to the control. However CAPE did preserve majority of the loss of GSH (Table 1), while CAPE alone did not alter GSH level in unexposed cells. Catalase is an important antioxidant enzyme actively involved in quenching harmful H_2O_2 radicals. The cells exposed to H_2O_2 had very low levels of catalase activity (Table 1). CAPE treatment not only supported catalase activity in unexposed control cells; but also restored in H_2O_2-inhibited activity (Table 1). RT-PCR analyses have also confirmed that CAPE elevates catalase mRNA transcription both in the control cells and H_2O_2-exposed cells (Figure 1A).

Although nitric oxide (NO) has a variety of physiological effects in mammalian cells, excessively produced NO derived from the inducible type of NOS (iNOS, NOS2) has been reported to play a pivotal role in airway and lung parenchymal inflammation. In the present study, nitrite levels in cell culture medium exposed to 24 h H_2O_2 significantly increased (9 folds) (Table 1). The effect of H_2O_2 on iNOS transcription was also evaluated by RT-PCR technology. H_2O_2 similarly increased iNOS mRNA expression by 3.5-fold (Figure 3B) though to a lesser extent than NO. A significant decrease in both NO production and iNOS transcription was observed in cells treated with 5 μM CAPE (Table 1, Figure 3B). CAPE treatment did alter neither NO production nor iNOS transcription in unexposed control cells.



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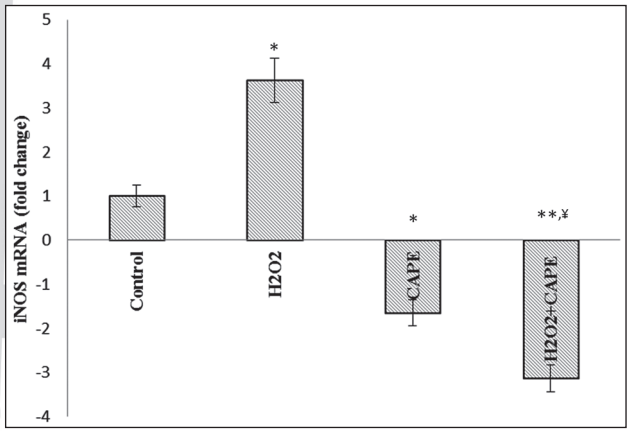

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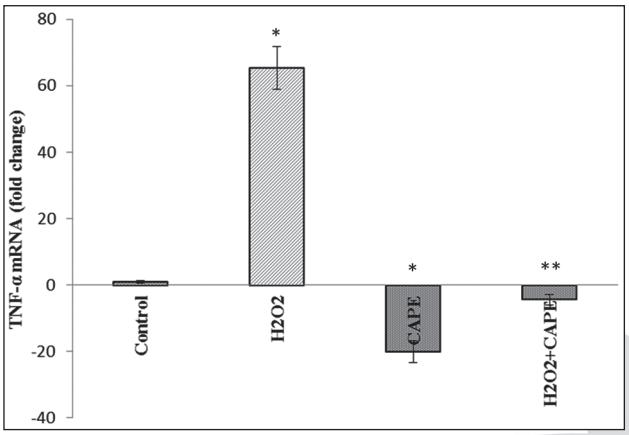

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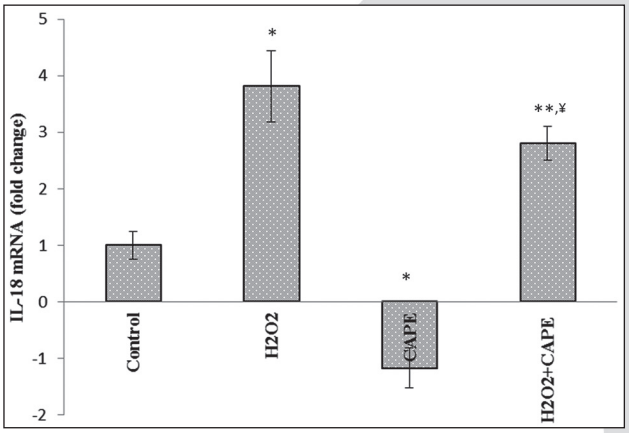

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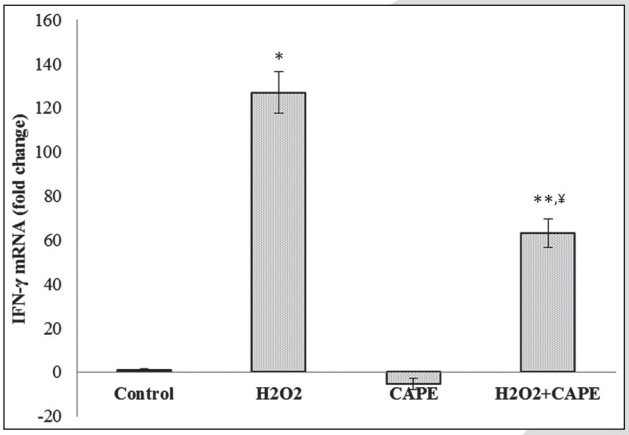

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Figure 3. Quantitative RT-PCR analyses of catalase, iNOS TNF-α, IL-18 and IFN-γ
*The mRNA levels of catalase, iNOS TNF-α, IL-18 and IFN-γ were determined by qRT-PCR in order to confirm the protective effects of 5 μM CAPE against 100 μM H_2O_2-induced oxidative stresses in A549 cells. As shown in Fig. A-C; H_2O_2-induced expressions of catalase, iNOS and TNF-α were entirely decreased by CAPE treatment. The findings have also demonstrated that CAPE significantly decreases IL-18 and IFN-γ mRNA levels by 26 and 50 %, respectively, as compared to the H_2O_2-treated cells (Fig. 3 D and E). Results were obtained using real-time RT-PCR and expressed as a relative increase of mRNA expression compared with control cells. Experiments shown are representative of three separate experiments with similar results. *,¥$p < 0.001$ versus control group; **$p < 0.001$ versus H_2O_2-treated group.*

Total oxidant (TOS) and total antioxidant status (TAS) are presented in Table 1. Total oxidant level in cell homogenates exposed to 100 μM H_2O_2 for 24 h was significantly ($p<0.001$) higher than those of unexposed cells. However, no detectable TOS data was observed in cells either exposed to H_2O_2 or in untreated cells, probably due to below the method detection limits (Erel 2005). While H_2O_2 exposure did not alter TAS ($p>0.05$) in control cells, CAPE treatment either alone or with H_2O_2 significantly ($p<0.05$) enhanced total antioxidant capacity of the cells (Table 1). These results support the antioxidant capacity of CAPE as previously implied by NO and GSH data.

The data are represented as mean ± SE. *,¥ $P < 0.05$ compared to control group; **$P < 0.05$ compared to. H_2O_2-treated group; ND: not detected. The cells were preincubated for 2 h with 5 μM of CAPE then incubated for a further 24 h at 37 ºC in the presence of 100 μM H_2O_2 (n = 3). Biochemical analyses were performed using cellular homogenates (except nitrite analyses).

To examine whether CAPE effectively inhibits oxidative stress-induced inflammation in our experimental conditions, A549 cells were exposed to 100 μM H_2O_2 for 24h. H_2O_2 exposure significantly induced TNF-α (65 fold), IL-18 (4 fold) and IFN-γ (127 fold) mRNA expressions in comparison with control cells (Figs C-E). As shown in the same figure, though 5 μM CAPE treatments entirely blocked TNF-α mRNA expression, IL-18 and IFN-γ mRNAs were decreased by 26 % and 50 %, respectively.

Discussion

Hydrogen peroxide is one of the oxidants produced through oxygen metabolism and during inflammation as part of the oxidative burst (21). Alt-

Table 1. Effects of CAPE treatment on GSH and nitrite levels, catalase activity, total oxidant (TOS) and antioxidant (TAS) status in A549 cells exposed to H_2O_2.

Groups	GSH (μM/mg protein)	Catalase (k/mg protein)	Nitrite (μM)	TOS (μmol H_2O_2 equiv./ mg protein)	TAS (mmol trolox equiv./ mg protein)
Control	3.50 ± 0.17	0.026 ± 0.01	0.124 ±0.014	0.050 ± 0.01	5.07 ± 0.25
H_2O_2	2.15 ± 0.12*	0.012± 0.001*	1.06 ±0.027*	0.100 ± 0.008*	5.05 ± 0.06
CAPE	3.12 ± 0.25	0.063 ± 0.023*	0.113 ± 0.011	ND	5.78 ± 0.10*
H_2O_2+CAPE	2.97 ± 0.11**,¥	0.043 ± 0.025**,¥	0.263 ±0.012**,¥	ND	6.02 ± 0.04**,¥

hough it had been previously shown that CAPE has many cell protective effects including antioxidant and anti-inflammatory properties, our results are the first to demonstrate CAPE could protect alveolar epithelial cells against oxidative and inflammatory stress-induced damage in A549 alveolar epithelial cells. A549 cell line is related to alveolar epithelial cells by various authors (7,14). Therefore A549 cell line has been used in this study.

The oxidative stress induced by free radicals has been implicated in the pathogenesis of chronic obstructive pulmonary disease, acute lung injury/ acute respiratory distress syndrome, hyperoxia and sepsis (22). In the present study H_2O_2 was used as a trigger for intracellular reactive species generation. Treatment with 100 μM of H_2O_2 for 24 h exerts substantial effects in reducing cell viability. However, CAPE pretreatment at 5 μM levels significantly protected the cells from H_2O_2-induced cytotoxicity. The results clearly demonstrated that CAPE could decrease the production of intracellular H_2O_2 (23), and rescues the H_2O_2-induced cytotoxicity suggesting its cell protective activity (6,23,24). On the other hand, CAPE treatment at high concentrations such as 10-20 μM for 24 h or longer time has cytotoxic effects, and it suppresses cell proliferation, colony formation, and cell cycle progression (23,26,27). This could be explained that CAPE at high doses cause gradual attenuation of the expression of the proliferating cell nuclear antigen (PCNA), which leads apoptosis-induced cell death (28) via significant loss of mitochondrial membrane potential (29). Taken together, these data suggest that CAPE at various concentrations has different effects on cell proliferation and activities.

H_2O_2-exposed cells displayed reductions of catalase activity and its mRNA transcription, and in the levels of antioxidant GSH molecule in the present study. CAPE pretreatment not only restored catalase activity and GSH levels, but also elevated mRNA transcription of catalase similar to those of control. Catalase is a scavenger enzyme that is part of the antioxidant system and catalyzes the conversion of H_2O_2 into water and molecular oxygen (30). We have demonstrated that cell protective concentrations of CAPE elicited a significant increase in activity and transcription of catalase. Though H_2O_2 is the substrate for catalase activity, its activity could be inhibited by overproduced cytokines and NO (31). Chen et al. has shown that the effects of relatively higher level of CAPE on differential cytotoxicity and apoptosis are associated with GSH depletion in A549 cells (23). CAPE may induce apoptosis through both p53-dependent and p53-independent pathways, and its antitumor activity may have occurred through the induction of apoptosis. Since GSH is a thiol antioxidant, the depleting intracellular stores of GSH by CAPE can render cells more susceptible to oxidative stress-induced apoptosis.

Reduced GSH is one of the major components of the non-enzymatic antioxidant system within the cell, and acts as a substrate for the glutathione peroxidase mediated reduction of H_2O_2 into nontoxic products (32). GSH depletion eventually induces mitochondrial GSH reduction and has been associated with increased levels of mitochondrial ROS and mitochondrial dysfunction (33). The present study demonstrated that the content of GSH decreased after exposure to H_2O_2, suggesting that oxidative injury generated, and the pretreatment with CAPE then elevated GSH levels. Such an effect of CAPE may be due to its property as an antioxidant blocking the formation of ROS, and attenuation of catalase activation and GSH consumption. It has also been demonstrated that CAPE with an-

tioxidant or free radical scavenging activity can be effective as a dual inhibitor of 5-lipoxygenase activity and arachidonic acid release from membrane phospholipids, which are important lipid mediators of inflammation (12). Compared with plasma, the antioxidant GSH is concentrated in epithelial lining fluid and appears to have an importance protective role, together with its redox enzymes in the airspaces and intracellularly in epithelial cells (32). It has been revealed that GSH and its associated enzymes present in the lower respiratory tract act as the first line of defense against external agents (34). Alterations in alveolar and lung GSH metabolism have been shown to be a central feature of many oxidative and inflammatory lung diseases, such as, cystic fibrosis and asthma (22,24).

H_2O_2-induced NO production and TOS status were also significantly suppressed by CAPE treatment. It is speculated that the higher level of GSH, TAS and catalase activity, and the lower level of NO and TOS will attenuate the ROS-induced cell damage. Recently, a number of studies have correlated the enhanced production of reactive species with H_2O_2 exposure, resulting in reduced antioxidant status (11,14,35). It has also been previously demonstrated that propolis extracts may prevent TGF-β1-induced epithelial–mesenchymal transition (EMT) of alveolar epithelial cells may contribute to airway remodeling in severe asthma and fibrotic lung diseases in A549 cells via multiple inhibitory pathways, which may be clinically applied in the prevention and/or treatment of EMT-related fibrotic diseases as well as airway remodeling in chronic asthma (35). It has recently been shown by Sırmalı and co-authors (36) that CAPE could protect the lungs from the deleterious effect of experimental contusion by directly supporting the antioxidant enzyme activity and blocking the production of free oxygen radicals. Our results contribute to existing knowledge by partially providing possible underlying molecular mechanisms of CAPE effects. Altogether, these results demonstrate that CAPE could protect alveolar epithelial cells via multiple inhibitory pathways.

As there is an active interplay between oxidative stress and inflammation, we also evaluated the anti-inflammatory activity of CAPE in H_2O_2-treated A549 cells. NF-κB, the transcription factor that regulates the expression of a number of genes involved in immune and inflammatory responses, has long been considered to be oxidant responsive (37,38). However, several studies have indicated that H_2O_2-induced NF-κB activation is cell specific and is not a universal response to oxidant signaling (38). In the present study, CAPE suppressed TNF-α, IL-18, IFN-γ as well as iNOS mRNA expressions that had been induced by H_2O_2. The pro-inflammatory cytokine TNF-α is the major inflammatory mediator that triggers a rapid rise in mitochondrial ROS and initiates necrosis and apoptosis (39). During inflammatory stimulation, translocation of NF-κB from the cytosol into the nuclei of cells induces the expression of a large number of genes such as TNF-α, acute phase proteins and enzymes such as iNOS (40). In our study, induction of iNOS mRNA expression by H_2O_2 exposure, which is similar to Zadeh et al. (41), and subsequent production of NO was significantly inhibited by CAPE pretreatment. Cao and colloquies (42) has demonstrated that LPS-induced pro-inflammatory cytokine productions and NO release were suppressed by CAPE treatment via blocking NF-κB signaling. It is suggesting that CAPE treatment would be linked to decrease in TNF-α, IL-18, IFN-γ transcriptions and NO productions as observed in our study. This, together with decreased oxidative stress and inflammatory cytokine production, may partially contribute to reduced alveolar epithelial necrosis/apoptosis. These findings support previous results regarding the beneficial effects of CAPE against oxidative stress in lungs.

The ability of CAPE to attenuate alveolar epithelial cell damage induced by oxidative stress could be attributed to the antioxidant activity and/or inhibition of free radical generation and the anti-inflammatory action of CAPE. It is suggested that CAPE is a promising agent that could help in the prophylaxis and treatment of alveolar diseases. Thus, targeting oxidative stress-inflammatory cytokine signaling by agents such as CAPE could improve therapeutic options for alveolar diseases.

Acknowledgements

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Effect of Hydrogen sulphide and Nitric oxide on left ventricular hypertrophy by altering the expression of matrix metalloproteinase

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Abstract

Background: Heart muscles acts as a functional syncytium and have highly arranged myocardial fibers in continuous fashion from endocardium to epicardium. Left ventricular hypertrophy (LVH) is an early sign of heart failure. The increase in size and mass of left ventricular myocyte is a chronic progressive inflammatory process in LVH which is activated by number of biological, chemical, mechanical and neurohormonal mediators. Continuous and consistent load on the heart initiates this debilitating process of left ventricle remodeling. Architectural complexity of extra cellular matrix (ECM) is of particular importance before and after the hypertrophy of left ventricle. The geometry of left ventricle and turnover of ECM due to collagen deposition defines the prognosis of the disease. Family of matrix metalloproteinases (MMPs) plays a significant pathological role in the turnover in the chemistry of ECM. There is another class of proteinases which controls the production or expression of MMPs is called as tissue inhibitory metalloproteinase's (TIMPs). Gaseous mediator like hydrogen sulphide (H_2S) and nitric oxide (NO) have been reported to have cardioprotective role.

Methodology: Data was obtained by using scopus, pubmed, science direct and google scholar to validate the information.

Purpose: This review article will discuss the role of gaseous mediators with a special focus on hydrogen sulphide (H_2S) and nitric oxide (NO) by altering the extra cellular matrix (ECM) in LVH by the activation or inhibition of MMPs and TIMPs.

Key words: MMPs Matrix metalloproteinase, LVH left ventricular hypertrophy, ECM extra cellular membrane, H_2S hydrogen sulphide, NO nitric oxide.

Introduction

Left ventricle hypertrophy (LVH) is increase in mass and size of left ventricles due to increase in work load on heart. This increase in size and mass is initially adaptive and supportive for heart to perform normal function in overload status. Left ventricle is most busy chamber of the heart and faces increased after load due to vasoconstriction in peripheral parts and increase heart rate. These two consistent effects on left ventricle result in left ventricle hypertrophy (LVH). LVH is the first step of cardiomyopathy which leads to symptomatic heart failure. Local or systemic presence of inflammatory cytokines like tumour necrosis factor (TNF) -α, interleukin (IL) -1 β and IL-6 is added factor in the induction of heart failure (1-3). In developing heart failure, expression of these inflammatory cytokines is increased due to activation of β-adrenergic receptors and debilitate the function of heart. So blockade of this receptor is favourable in the failing heart. In LVH, 2 pathological progressions, hypertrophy of myocyte and fibrosis of interstitium are initiated. The fibrosis causes increased deposition of Collagen I and III (4). This fibrosis and hypertrophied ventricle lead to ventricular dysfunction. (5). Left ventricular hypertrophy is of two types (1) pressure overload hypertrophy (2) volume overload hypertrophy. Geometry as well as prognosis of both types of hypertrophies is different. Different types of medication options are available for both types of hypertrophies. Gaseous mediators like hydrogen sulphide and nitric oxide have been studied a lot in cardiovascular, renovascular therapeutic areas. Their significance in the left ventricular hypertrophy still needs more attention of researchers. The aim of this review article is to highlight the significance of these gaseous mediators in LVH, summarize the

previous work done and identify some grey areas for future prospect.

Left ventricular hypertrophy and Matrix metalloproteinase

Prognosis of left ventricular hypertrophy (LVH) is always heart failure and it should be equally treated as heart failure. Prompt diagnosis and aggressive therapy may lead to the regression of LVH. Heart failure (HF) is a major public health problem in the United States. Nearly 5 million patients in this country have HF, and nearly 500, 000 patients are diagnosed with HF for the first time each year. During the last 10 years, the annual number of hospitalizations has increased from approximately 550, 000 to nearly 900, 000 for HF as a primary diagnosis and from 1. 7 to 2. 6 million for HF as a primary or secondary diagnosis (6) heart failure is one of the major cause of mortality and morbidity worldwide (7, 8). Myocardial infarction seems to be major cause of heart failure (7). Left ventricular hypertrophy (LVH) is increase in mass of left ventricles due to increase in work load on heart as shown in Figure 3. Another parameter responsible for LVH is myocardial fibrosis which is presumed to be initiated by Renin angiotensin aldosterone system (RAAS) as

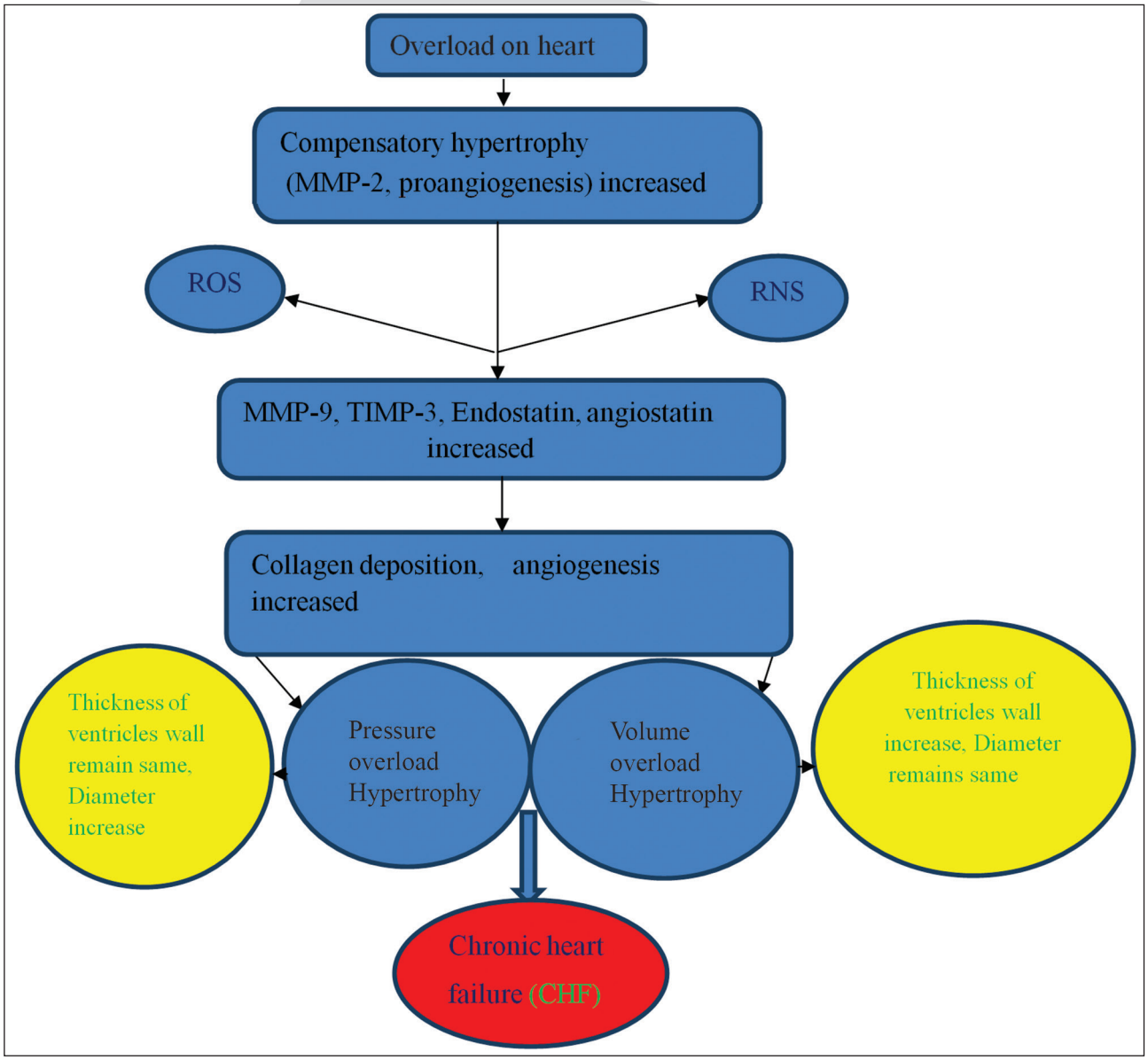

Figure 1. Flow chart showing sequence of events occurs in LVH leading to heart failure
MMP: Matrix metalloproteinase
ROS: reactive oxygen species RNS: reactive nitrogen species
TIMPs: Inhibitory matrix metalloproteinase

shown in figure 2 (a&b) adopted from (9). This relation between RAAS and fibrosis justified the role of angiotensin converting enzyme inhibitors and angiotensin receptor blockers in heart failure (10).

LVH is subdivided into 2 types (I) Pressure overload hypertrophy (POH) (ii) Volume load hypertrophy (VOH). Change in wall stress of LV is different in both POH and VOH. Difference between POH and VOH is shown in Figure 1.

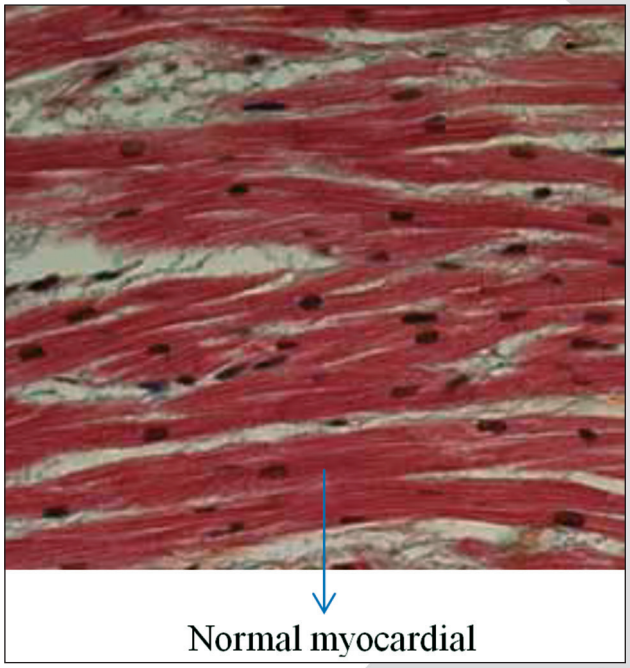

Figure 2 (a). Symmetrical geometry of fibres

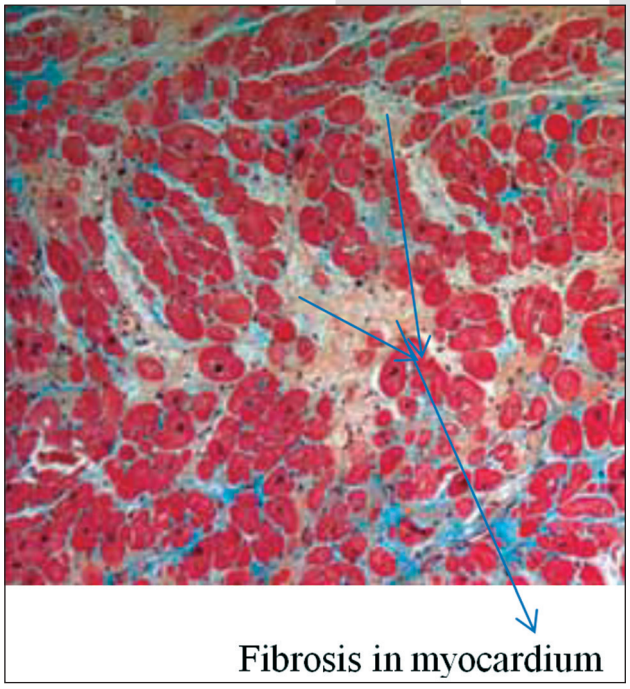

Figure 2 (b). Fibrosis Adopted from (9)

MMPs in their inoperative state control this elastin to collagen ratio by matrix synthesis and its catabolism. However, under stressful conditions when reactive oxygen species (ROS) and reactive nitrogen species (RNS) come into play their actions, MMPs get out of hibernation period and start deposition of excessive metalloproteinase (Collagen), disturb the elastin collagen ratio that causes fibrosis which ultimately leads to CHF (11, 12).

Matrix metalloproteinase (MMPs) have been found to play many physiological role like healing of wound, angiogenesis, tissue remodelling but their altered expression lead to many pathological conditions like rheumatoid arthritis (11). Accumulation of MMPs lead to disturbance in E/C ratio lead to many disease of kidneys like diabetic nephropathy and glomerulusclerosis (12). Elastin to collagen ratio determine the dysfunction of the heart in chronic cases of heart failure (13).

Following specific cardiovascular stress, cascade of reactions start in myocardium which eventually leads to left ventricular dysfunction, left ventricular hypertrophy and terminating to heart failure as shown in figure 1. Myocardium architectural complexity is maintained with suitable composition of elastin and collagen ratio (E/C). Elastin provides stretching for the myocardium during diastolic phase of cardiac cycle while collagen provides strength to myocardial cell. This E/C ratio is maintained by matrix metalloproteinases (MMPs), a family of 25 zinc dependent species. These MMPs maintain normal composition of extra cellular membrane (ECM) in their latent state by its proteolytic activities. As a result of external stimuli like oxidative stress or inflammation, some changes occur in left ventricle geometry. These changes are called myocardial remodelling (14, 15). In systolic heart failure, MMP-9 rather MMP-2 is responsible for left ventricular dilatation and may be target for prevention of left ventricle remodelling. In myocardial remodelling many reactions take place like apoptosis, necrosis and angiogenesis. Geometry of myocardium starts to turn over the E/C ratio due to activation of MMPs which are called signalling molecules and play basic role in myocardial remodelling (16-18). Table 1 below contains the name of MMPs, their number and molecular mass identified in myocardium (19).

Table 1. Showing different classes of MMPs

Name	Number	Molecular mass kDA
Collagenase Interstitial collagenase	MMP-1	52/57
Collagenase 3 Neutrophil collagenase	MMP-13 MMP-8	54 75
Gelatinase Gelatinase A Gelatinase B	 MMP-2 MMP-9	 72 92
Stromelysins Stromelysins A Matrilysin 1	 MMP-3 MMP-9	 52/58 28
Membrane type MMP MT1-MMP	 MMP-14	 66

MMP-2 and MMP-9 are the two important gelatinises that play an important role in the development and remodelling of the heart and vasculature (20). Few studies demonstrated the presence of gelatinase MMP-2, MMP-9 (21, 22) few others likeMMP-1, MMP-13 and MMP-8 in normal culture of human (21, 23, 24).

In order to control these activated MMPs, a family of inhibitory proteins called inhibitors of metalloproteinase (TIMPs) play their role to control this E/C ratio. There are four types of TIMPs, TIMP-1, TIMP-2, TIMP-3 and TIMP-4. Out of these four species, TIMP-1 & TIMP-2 are well studied. Increased expression of TIMP-1 and TIMP-2 have been proved as a result of fibrosis in chronic pressure over load (POH) human heart (25). TIMPs are actually low molecular weight proteins (190 amino acids) which can bind to MMPs covalently in 1: 1 ratio. (26, 27). TIMP-4 is present in cardiomyocyte and called cardiac specific inhibitor of metalloproteinase (CIMP), responsible to prevent activation of MMP-2 and MMP-9. TIMP-1 and TIMP-3 increase with the oxidative stress. TIMP-1 is accused for cardiac fibrosis (28) while TIMP-3 induce apoptosis in smooth muscular muscle cell (29). Previous clinical studies have demonstrated that there is robust increase in TIMP-1 level in POH (30, 31). Increase in TIMP-1 level is associated with increase in mortality (32)

Activation of Metalloproteinase by different mediators

Matrix metalloproteinase is a family of 25 members which differ from each other on the basis of promoter regions. Different mediators like angiotensin II, catecholamine, ET, cytokines, growth factors like tumor growth factor (TGF) are responsible for the up regulation or down regulation of MMPs as shown in figure 3. These mediators cause the induction of Janus kinase-signal transducers and activators of transcription (JAK-STATs) pathways. Transcription factors activated by this pathway includes AP-1 family, STAT, NF-kB, binding protein-3polyoma enhancer A-3 (PEA-3) and miscellaneous group containing SP-1 and TGF inhibitory element (TIE). These factors are present on the promoter region of genes of each MMPs as shown in figure 5.

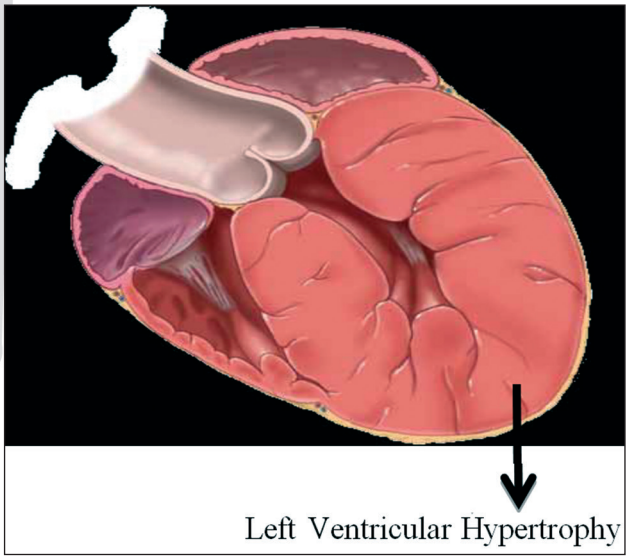

Figure 3. Showing left ventricle hypertrophy

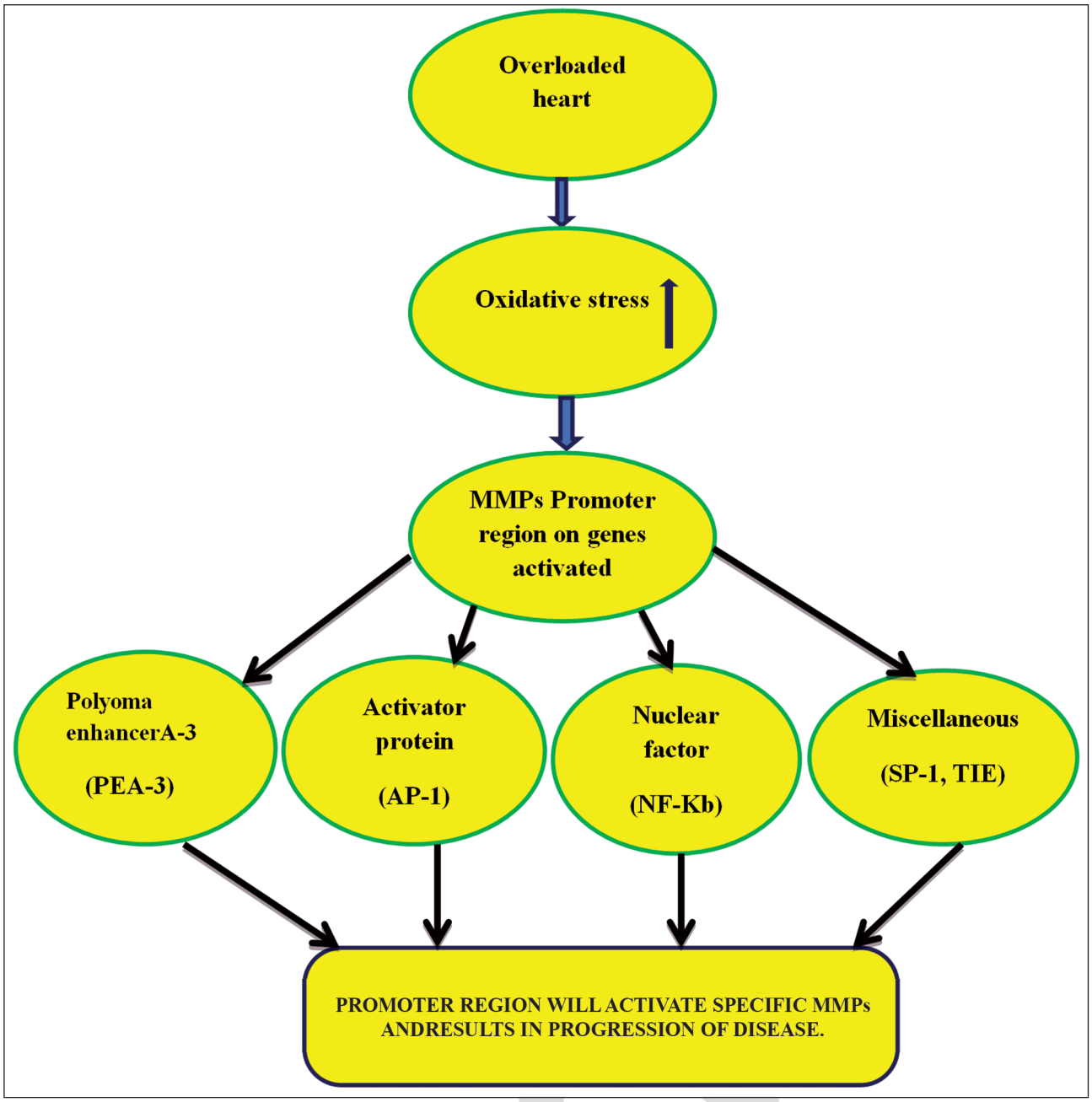

Figure 4. Showing the mechanism of activation of metalloproteinase MMPs

Different MMPs have different promoter regions on their genes so only specific mediator bind to particular promoter region on gene and up regulate that particular type of metalloproteinase. Difference in promoter regions of each protein is shown in figure 5.

Therapeutic interventions affecting MMP induction

As it is clear from the figure 4, chemical mediators and inflammatory pathways are responsible for the alteration in synthesis of matrix metalloproteinase. Inhibition of rennin-angiotensin aldosterone system have been proven effective in reduction of relative MMP levels within myocardium (33, 34) (35). ACE or MMP inhibition can reduce left ventricle dilation in rat model of heart failure (36). ACE inhibitors in POH model of rats has direct inhibitory effect on MMP and inhibit left ventricle remodelling and dysfunction (35). Inhibition of β-adrenergic receptors can altered the MMP levels in myocardium (37). It is obvious from the figure that inflammatory mediators induce the MMP levels. A large body of evidence endorsed the fact

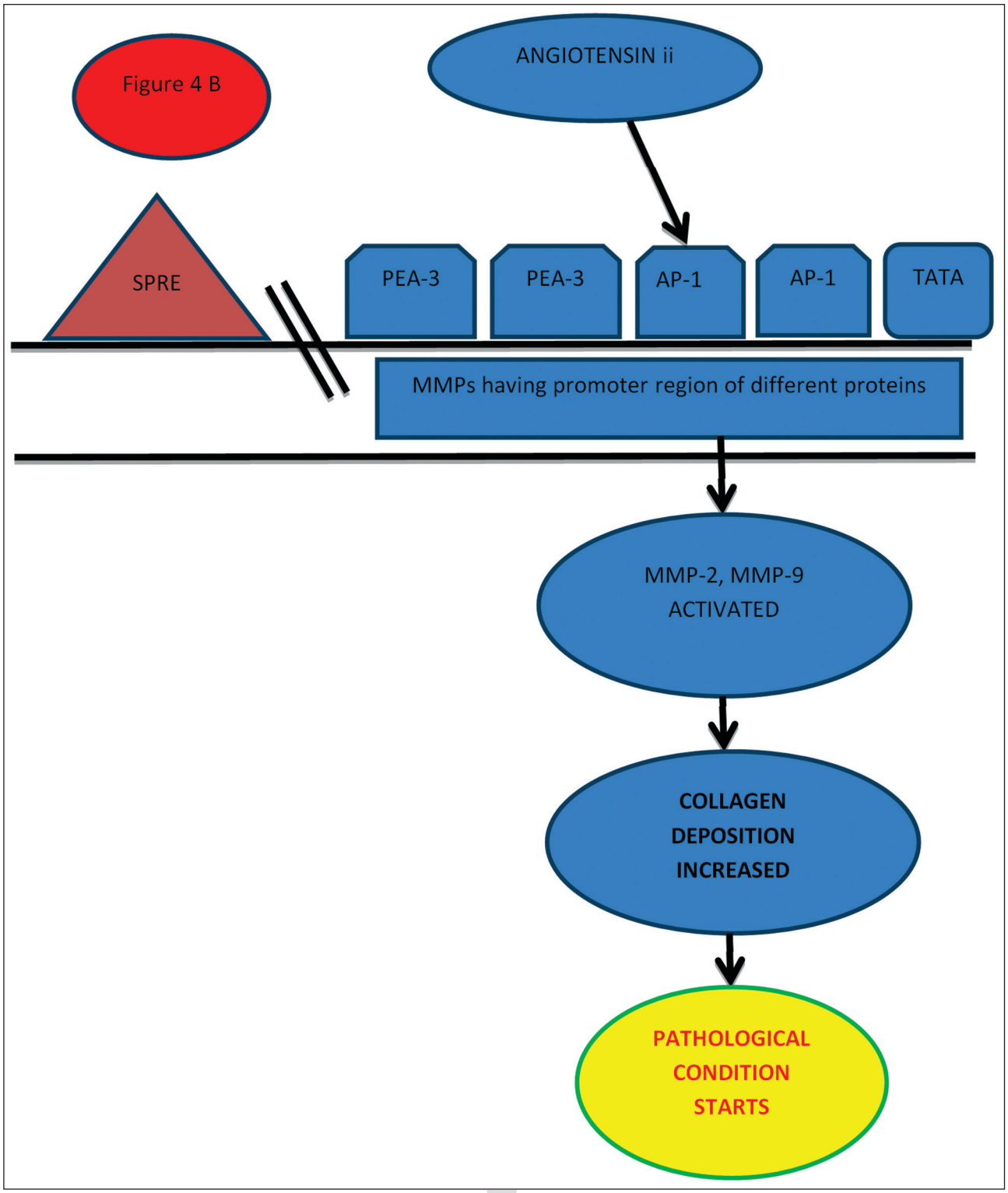

Figure 4 (B). Showing possible way for activation of (MMP-2 and MMP-9 as an example)

that modification in the mediators of inflammation can alter the local MMP in myocardium (38-40). Among other pharmacological interventions like use of statins may attenuate the local inflammatory pathways which ultimately lead to the alteration in MMP levels (41, 42)

Effects of gaseous mediators in left ventricular hypertrophy

Hydrogen sulphide (H_2S), nitric oxide (NO) and carbon monoxide (CO) are the lipid soluble gaseous molecules which are produced inside the body (43). These molecules are given the name of gaso-transmitter. Previously it was believed that

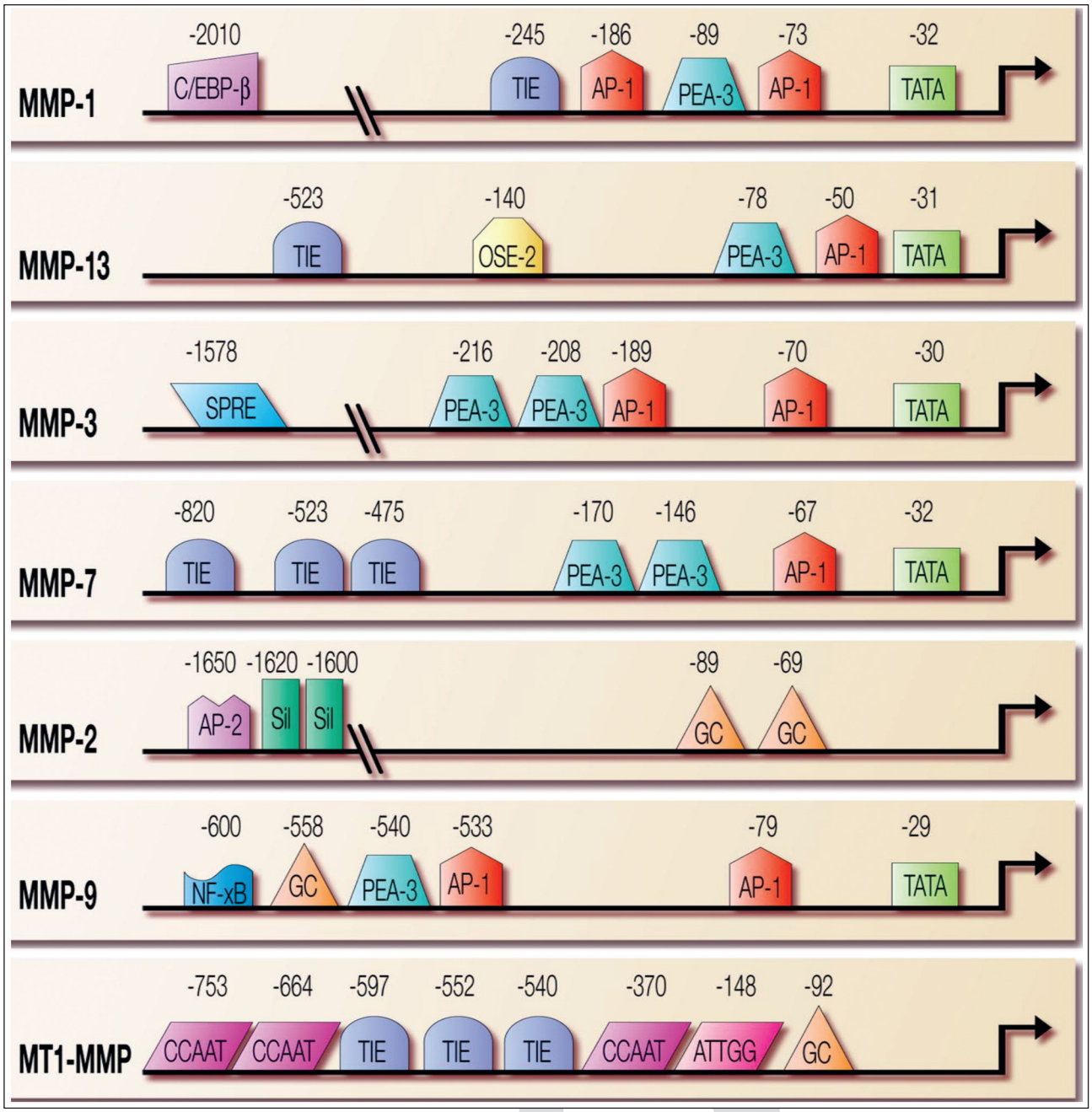

Figure 5. Adopted from (19) showing different promoter region on MMPs gene

these gaso-transmitter are toxic in their actions but research from the past few decades have proved that these mediators are endogenously produced in physiological concentration and produce pharmacological responses. These findings open a gate of research for researchers to re-evaluate these gaso-transmitters in different systems of body. Large body of evidence has endorsed the beneficial effects of these mediators in hypertension, renal failure, cancer and joint. Among all these mediators, role of hydrogen sulphide and nitric oxide can be well studied in left ventricular hypertrophy and heart failure. Literature review regarding the role of H_2S and NO in LVH and HF is described so that area of gap can be find out.

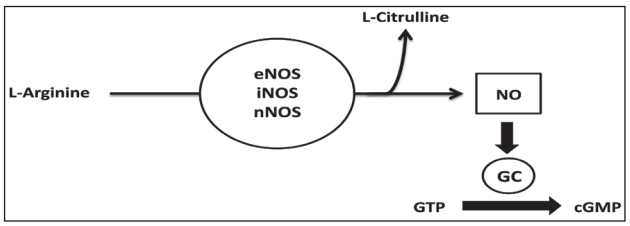

Figure 6. Showing the formation of NO is mediated by its 3 isoforms

Review of hydrogen sulphide (H_2S) in LVH leading to Heart Failure

From the literature survey, it is conceived that H_2S mitigate the transition from MI to HF by promoting angiogenesis by stimulating the production of VEGF, inhibiting antiangiogenesis process by inhibiting endostatin and angiostatin, altering the expression of MMPs and TIMPs and ameliorating LV dysfunction (44). H_2S acts on K_{ATP} channels which are widely distributed in coronary vascular and cardiomyocyte and these channel produce vasorelaxation and –ve inotropic effect that is beneficial for heart (45). H_2S which inhibit the TIMP-1 in heart failure are non-invasive predictor or markers of left ventricular diastolic dysfunction (28). Sodium thiosulphate ($Na_2S_2O_3$) is recognized to play crucial role in regulating the function of MMP-2, MMP-9 and TIMP-1 in failing heart (46). In comparison between SHF and DHF, it was observed that MMP-9 instead of MMP-2 is responsible for left ventricular dilatation in SHF (47). Normally DHF is not associated with LV dilatation but it develops collagen content. TNF-α is found to have mechanistic link between induction of MMPs and remodelling in heart failure (48). H_2S is found to inhibit apoptosis of cardiomyocyte through mitochondrial pathway and increases the survival and inhibiting the ventricular dysfunction in progressing heart failure (49). Hydrogen sulphide not only weekend pathological prognosis of ischemic heart disease but also play an important role in cardiovascular protection (50). (50) Exogenous administration of H_2S or endogenous regulation of it can protect from heart failure induced by ischemia (51). (52) demonstrate that infusion of hydrogen sulphide in IR provides better protection that bolus dose. It come to knowledge that inhalation of H_2S also provide some response to body like decrease in cardiac output and heart rate but MAP and SV is maintained (53). Hydrogen sulphide is responsible to play proangiogenic role by the phosphorylation of Akt (PKB) (54). H_2S is also found to have some impact on MMP-3 and MMP-14 in other pathological conditions. In rheumatoid arthritis and osteoarthritis, H_2S up regulates MMP-3 and down regulates MMP-14 after 6hrs of exposure to H_2S by using qRT-PCR (55).

Review of Nitric oxide (NO) in LVH leading to Heart Failure

Nitric oxide produces vasodilatation by activating cyclic guanosine mono phosphate (cGMP) on blood vessels. Normally its synthesis is controlled by three isoforms of NOS as shown in fig. 6

Because of its vasodilator an activity, NO is used in heart failure as it reduces the work load of heart by pooling the blood to the downstream. Increased production of NO is deleterious as it causes negative inotropic response which is not favourable for patients (56). NO which is produced from endothelial NOS, reduces the contractility of heart by inhibiting the influx of Ca^{++} (57, 58). Neuronal NOS, produces Nitric oxide which enhances the magnitude of bradycardia by facilitating acetylcholine release (59). Recent evidence for a bradycardiac role of NO derived from neuronal NO synthase (nNOS). Neuronal NOS facilitate chronotropic and dromotropic effects on heart (60). So, systemic or endogenous production of NO in heart failure case can provide protection by its vasodilator and –ve inotropic response. In support of this statement, it is evident that low dose of beta blockers (especially carvedilol) have been found to have protective role in case of heart failure by decreasing heart rate (61). Matrix metalloproteinase has an imperative role in vascular remodeling and apoptotic role in heart failure; we need to study the effect of NO on MMPs and TIMPs. NO has been found to have effects on extra cellular membrane turnover (62, 63). NO is reported to have modulating role on cytokines induced MMP-9 and TIMP-1 expression in rat MCs (64). Nitric oxide inhibit cell proliferation (65) and reduces blood pressure dwindle end stage heart failure. *In vivo*, decreased NO production will lead to increased MMP activity (66). *Invivo* treatment of SHR with L-arginine reduces cardiac mass and but no effect on blood pressure (67). Metalloproteinase when attached with ROS in the presence of NO acts as antioxidant activity. So, no significant data regarding to interaction between NO and MMPs in heart failure and LVH is available. Data from chemistry point of view showed that stable complex can be formed between sulphur, zinc and nitrogen containing compound.

Future prospects

In any comprehensive review, it is compulsory to provide some future directions so that the field of

matrix remodelling and degradation may progress with conclusive results. However, it must be noticed that research into the functional role of the myocardial matrix itself is undergoing a metamorphosis.

Specifically, the general thought that the matrix is a static structure that provides scaffolding for cells has evolved to one that the interstitium is a dynamic entity that is under constant change and is highly responsive to biological and mechanical stimuli.

Keeping in view the above mention literature survey of gasotransmitters (H_2S and NO) in LVH and HF by altering the MMPs, there is still a lot more to study to establish these mediators are therapeutic agents in left ventricular hypertrophy.

1. Hydrogen sulphide should be used in rat by producing POH and VOH models of left ventricular hypertrophy. MMP types and their concentration should be measured in both types of model of hypertrophy. It is also required to identify the different MMPs and TIMPs in both models of hypertrophy. Effect of hydrogen sulphide on all types of MMPs, POH and VOH should be studied to establish this drug in LVH.
2. Elastin to collage ratio is important to maintain the normal function of myocardium. Concentration of elastin in normal and hypertrophied heart needs some attention to be paid. MMPs alter this E/C ratio by increasing or decreasing the collagen deposition but no effort has been made to check the effect of MMPs on elastin.
3. Some herbal remedies like medicinal plant should be selected and screened for hydrogen sulphide and related drugs like sulphur containing compound should be isolated. These compounds should be used as herbal drugs to cure LVH.
4. Nitric oxide is vasodilator and has powerful dromotropic effects. NO has some effects on MMPs. Response of NO in LVH can be studied by considering its role in altering the production of all MMPs in hypertrophied heart. Furthermore, dromotropic effect of NO can be beneficial for $^+$ve inotropic response in debilitating heart. This work is in progress in our laboratory.
5. Some molecular mechanism need to be elucidated in myocardium regarding the production of enzymes like CSE by H_2S and iNOS by NO in normal and hypertrophied heart. This work is in progress to make it conclusive.
6. Deletion of particular MMP mRNA in particular individual is still challenge for biotechnology which can be fruitful for a patient with hypertrophied heart.
7. Some signalling pathways like RAAS (rennin angiotensin-aldosterone system) is responsible for MMP induction. Another signalling molecule like bradykinin is also stimulus of this pathway. 8. So research can be done in these receptors pathways to alleviate the MMPs production.
9. Some synergistic response of H_2S and NO in LVH by altering the MMPs induction can be studied. This work is in progress in our laboratory. Thus clear understanding of matrix remodelling process and its degrading pathways can be a therapeutic switch for LVH.

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The effect of 1% chlorhexidine gel on the shear bond strength of two self etch adhesive systems on primary tooth dentin

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Abstract

Aim: The aim of this in vitro study was to evaluate the effect of 1% chlorhexidine gel on dentin bond strength of composite resin applied with two self etch adhesives, in primary tooth dentin.

Materials and Methods: 40 sound primary molars were used. The buccal or lingual surface of each tooth was polished with waterproof polishing papers to create a flat dentin surface. The specimens were randomly divided into 4 groups of 10 each. In group 1 Clearfil SE Bond, in group 2 Clearfil S^3 , in group 3 Chlorhexidine gel + Clearfil SE Bond and in group 4 Chlorhexidine gel + Clearfil S^3 were applied. Composite cylinders were built up on the prepared surfaces and light-polymerized. After the specimens were stored in an incubator for 24 hours, the shear bond strength was measured using an instron universal testing machine at a crosshead speed of 0.5*mm/min*. The bond strength data were analyzed with ANOVA and Tukey-HSD tests. Failure mode was determined using a stereomicroscope with 20X magnification.

Results: There was no significant difference between pretreated 1% chlorhexidine gel group and control group in either self etch adhesives

($P>0.05$). On the other hand, the shear bond strength of two-step self etch adhesive (Clearfil SE Bond) was significantly higher than one-step self etch adhesive (Clearfil S^3 Bond) ($P< 0.05$). Failure mode was predominantly adhesive.

Conclusion: The findings of this study showed that applying 1% chlorhexidine gel did not adversely affect the shear bond strength of self etch adhesives in primary teeth. According to the results of this study, using disinfectants before self etch resin restorations may be suggested.

Key words: Bond strength, Chlorhexidine gel 1%, Primary teeth, Self etch adhesive.

Introduction

These days, the application of different disinfectants before restoring cavities is becoming an accepted trend, as disinfectants are assumed to reduce the potential risk of bacterial activity. Yet, there are doubts about using them with dentin bonding agents because they may have an adverse effect on the bond strength (1).

Chlorhexidine is widely accepted as an antibacterial agent. It can also –even in low concentrations- restrain the collagenolytic activity of the dentin and improve the integrity of the hybrid layer. But its interference with dentin adhesive systems is potentially a problem (2).

Among adhesive systems, self etch systems are specially valuable in pediatric dentistry because they have less steps and shorter working time (3). Two kind of self etch bonding systems have been presented. Sixth generation adhesives which contain an acidic primer and a bonding resin, and seventh generation adhesives with the etchant, primer, and adhesive resin in one bottle (4). Controversial results have been published regarding the application of chlorhexidine solution with self etch systems. Some studies have reported a reduction in shear bond strength (1,5). Some other studies found no adverse effect on 24-hour bond strength

(6). In recent years, a few studies have evaluated the effect of chlorhexidine gel on resin bond strength to dentin (1,5,7). According to the results of these studies, the application of chlorhexidine gel with self etch systems does not have any adverse effect on bond strength to permanent dentin.

The purpose of the present study is to evaluate the effect of 1% chlorhexidine gel on the shear bond strength of two self etch adhesive systems: sixth generation (Clearfil SE Bond) and seventh generation (Clearfil S^3 Bond) to primary tooth dentin.

Materials and Methods

In this in vitro study, forty caries free primary molars were collected by simple sampling. The teeth were kept in 0.9% normal saline in room temperature for a maximum of three months before the experiment. The teeth were immersed in 0.5% chloramine T, in the temperature of 4°C for 48 hours. The teeth were mounted in auto-polymerizing acrylic resin mold with dimensions of 342412 mm.

The buccal or lingual surfaces of teeth were wet-polished using 400 grit silicone carbide papers by polishing machine to expose the dentin. The dentin surfaces were wet-polished using 600, 800, and 1200 grit silicone carbide papers.

The teeth were randomly divided into 4 groups of 10 each. The groups were prepared as following:

- Group 1. Clearfil SE Bond (Kuraray, Japan) was used. According to manufacturer instructions, primer was applied on dentin surface and remained for 20 seconds. After air drying, SE Bond agent was applied on dentin surface and distributed evenly by mild air pressure. The agent was cured for 10 seconds by LED (Ivoclar Vivadent AG, FL-9494) with the intensity of 1000 mW/cm^2. Composite material (Clearfil APX, shade XL, Kuraray, Japan) was insereted into the dentin surface in two layers by packing through cylindrical plastic matrices with an internal diameter of 2.3 mm and height of 3 mms. Each layer was cured for 40 seconds, and the cylinders were peripherally cured for 60 seconds to ascertain complete polymerization.
- Group 2. Clearfil S^3 Bond (Kuraray, Japan) was used. According to the instructions, the bonding agent was applied to the dentin surface after 20 seconds. The agent was air dried for 5 seconds and cured for 10 seconds. The rest of the procedure was done as in the first group.
- Group 3. Chlorhexidine gel (Corsodyle, GlaxoSmithKline U.K.) + Clear SE Bond were used. The gel was applied on dentin surface, remained intact for 20 seconds, and air dried for 10 seconds. Clearfil SE Bond and composite were applied in the same manner as the first group.
- Group 4. Chlorhexidine gel (Corsodyle, GlaxoSmithKline U.K.) + Clear S^3 Bond were used. The gel was applied on dentin surface, remained intact for 20 seconds, and air dried for 10 seconds. Clearfil S^3 Bond and composite were applied in the same manner as the second group.
- The specimens were placed in a universal testing machine (Zwick/Roell Z050, Germany) and the shear bond strength was measured at a crosshead speed of 0.5 mm/min. The shear bond strength was recorded in Newtons (N) and calculated in MPa. After this part of the experiment, the fractured surfaces were observed with a stereomicroscope (Nikone SMZ800, Japan) at a magnification of 20X to determine the failure modes which were categorized as adhesive, cohesive, and mixed failure modes.

One-way ANOVA followed by Tukey Post Hoc tests were used to verify the effect of applying chlorhexidine before the self etch adhesive type.

Results

In this experimental study four groups of 10 primary molars were used to verify the effect of applying 1% chlorhexidine gel on the shear bond strength of Clearfil SE and Clearfil S^3 self etch adhesives, in primary tooth dentin.

The mean shear bond strength of four groups are shown in table 1.

The mean values of shear bond strength ranged from 11.77 to 22.96 MPa. The maximum mean shear bond strength was found in the Clearfil SE Bond group.

Table 1. The mean shear bond strength of dentin adhesives Clearfil SE and Clearfil S³ to primary teeth, with and without applying chlorhexidine.

	CHX applied	CHX not applied	P Value	The effect of gel application (difference between groups)
SE	16.10 ± 6.72	22.96± 7.10	0.08	-6.86± 10.05
S³	11.77 ± 5.31	13.08± 5.36	0.96	-1.31± 8.54
P Value	0. 4	0.005		0.2

Table 2. The frequency of failure mode types

Failure Mode / Adhesive Type	Applied with chlorhexidine			Applied without chlorhexidine		
	adhesive	mix	cohesive	adhesive	mix	cohesive
SE Bond	7(70%)	3(30%)	-	8(80%)	2(20%)	-
S³Bond	5(50%)	5(50%)	-	6(60%)	4(40%)	-

Although applying chlorhexidine gel before Clearfill SE bond reduced the mean shear bond strength, this reduction was not statistically significant (P=0.08). Reduction of shear bond strength was similarly seen in Clearfil S³ bond group, either not statistically significant (P=0.96).

Statistical analysis showed that, when chlorhexidine gel was not applied, sixth generation adhesive had a significantly higher shear bond strength to primary dentin comparing to seventh generation adhesive (P=0.005). But when chlorhexidine gel was applied in both groups, the shear bond strength of two groups to primary dentin did not show a statistically significant difference (P=0.4).

The application of chlorhexidine gel had a higher effect in SE bond comparing to S³ bond group, but the level of difference was not significant (P=0.2).

Failure modes are shown in table 2. The predominant mode found was the adhesive mode. No cohesive failure modes were observed in specimens.

Discussion

In the present study, the shear bond strength to dentin was significantly higher in sixth generation self etch adhesives (Clearfil SE bond) in comparison to seventh generation self etch adhesives (Clearfil S³ bond), in primary tooth dentin. The same results were found by Agostini *et al* in a comparison between one stage and two stage self etch adhesives in primary teeth (8). In a study by Perdigao *et al,* Clearfil SE adhesive showed significantly higher microtensile bond strength, comparing to clearfil S³ adhesive in permanent tooth dentin (9). In the studies by Inoues *et al* (10) and Knobloch *et al* (11) microtensile bond strength was higher in sixth generation adhesives than in seventh generation adhesives, but the difference was not statistically significant. In the study of Yaseen *et al* on primary tooth dentin, tensile bond strength was higher in seventh generation adhesives comparing to sixth generation adhesives, but the differences were not statistically significant(4).

Despite all limitations, the shear bond strength test is now considered as the most common technique to evaluate the bond strength of adhesives and resin restorations (12). In our study, the shear bond test was used to evaluate the bond strength. The most frequent failure mode was the adhesive failure. No cohesive failure mode was observed.

Clearfil SE bond and Clearfil S³ bond are two self etch adhesive resins categorized as mild adhesives. In mild self etch adhesives, surface demineralization is incomplete and residual hydroxyapatite remains connected to collagen. Although enough surface porosity exists for micromechanical retention, the residual hydroxyapatite inside the submicrone hybrid layer may act as an extra receptor for chemical bond between functional monomers and tooth structure which improves bond retention and stability(13,14) These adhesives produce a thin hybrid layer which contains smear plug and smear layer in its structure. As eliminating these layers by these systems is incomplete, there is less permeation of tubular fluid, and thus less post- restoration sensitivity (15).

In the present study, we found that the shear bond strength of Clearfil bond S^3 was lower than Clearfil SE bond adhesive. One-step self etch adhesives have problems associated to incomplete evaporation of the solvent and water before polymerization and the residual water in acidic and hydrophilic monomers which may inhibit complete polymerization of adhesive monomers (15). The ability of one-step self etch adhesives to seal the dentin surface has also been under question as they make a permeable membrane. One important characteristic of two-step self etch adhesives is that they form a hydrophobe resin layer on the primer which can reduce water absorption. The existence of this layer may explain the higher bond strength of Clearfil SE bond comparing to Clearfil S^3 bond (16).

Incomplete elimination of caries affected enamel and dentin _especially when indirect pulp therapy is intended_ is a concern in restorative dentistry, because some bacteria may be left in the cavity. One proposed solution is to use disinfectants in the prepared cavity (17).

Chlorhexidine, is a wide spectrum disinfectant which has a good suppressive effect on streptococci mutans and streptococci subrinus (2,7). A potential problem with chlorhexidine is that it may have an adverse effect on the bond strength of composite resins (5). In our study, we evaluated the effect of 1% chlorhexidine gel on the shear bond strength of sixth and seventh generation self etch adhesives in the primary tooth dentin. Primary and permanent teeth are different in both morphology and structure. Dentin layer is thinner in primary teeth, which may be the factor responsible for lower bond strength in primary teeth(2). Besides, peritubular and intertubular dentin contain less concentrations of calcium and phosphate, thus primary teeth are less mineralized and have a reduced hardness which influences the bond strength (18). Although the shear bond strength reduced by 1% when chlorhexidine was applied, this reduction was not statistically significant in either self etch adhesive groups. This result is in accordance with similar studies on permanent teeth (5,7,19). As far as our knowledge, this is the first study which evaluates the effect of 1% chlorhexidine gel on bond strength in primary teeth.

In the study of Sharma *et al* (1) and Ercan *et al* (5) chlorhexidine was used in two forms of gel and solution. The results showed that chlorhexidine did not have an adverse effect on bond strength when used in gel form. But when used with self etch adhesives in the solution form, it reduced the shear bond strength. The authors declared that the reason for non-interference with bond strength in the gel form could be the limited penetration to dentin structure and also its limited affinity to tooth structure.

In conventional adhesive systems, there is incomplete hybridization and thus exposed residual collagen fibrils because of incomplete resin penetration to prepared dentin. This phenomenon makes the bond susceptible to hydrolytic degrade. Active forms of Matrix MetalloProteinases (MMPs) are supposed to cause the self-degradation of unprotected collagen fibrils of the hybrid layer even in the absence of bacteria. MMPs, are proteolytic enzymes which exist in dentin, dentinal fluid, and saliva. These enzymes are able to degrade the dentinal collagen in reduced pH. This happens when the tooth surface is treated by primers and adhesives when a biochemical process of caries is activated (19). The hybrid layer degradation in primary teeth may take as short as only six months(20).

Chlorhexidine acts not only as a distinct disinfectant, but also as a potential matrix metalloproteinase (MMP) enzyme inhibitor (21). These enzymes can degrade the demineralized organic matrix of the dentin and consequently reduce the bond strength. It has been shown that self etch adhesives may activate hidden MMPs to a maximum level (22). In an in vivo study by Hebling *et al* on primary teeth, the teeth which had been treated by chlorhexidine showed a normal structural integrity in the collagen network of their hybrid layer after a six month period, while a progressive degrade of the fibrillar network was observed in the hybrid layer of the control group. Their study showed that chlorhexidine can interrupt the self destruction of the collagen matrix as a MMP inhibitor (21).

Recently, various in vitro (22,24-27) and in vivo (21,28) studies have shown the positive effect of chlorhexidine on bond retention in etch and rinse systems. Also a few studies have been done on the long term effect of chlorhexidine on the self etch adhesive bonds, which have similarly shown its positive effect on bond retention (6,22,29).

Conclusion

Sixth generation adhesive Clearfil SE Bond made a stronger shear bond strength to primary tooth dentin in compare with seventh generation adhesive Clearfil S^3 bond.

The application of 1% chlorhexidine gel with self etch adhesives Clearfil SE bond and Clearfil S^3 bond did not have an adverse effect on the shear bond strength to primary dentin thus it may be used as a cavity disinfectant and MMP inhibitor with self etch systems. According to the results of this study, using disinfectants before resin restorations by self etch adhesives Clearfil SE bond and Clearfil S^3 may be suggested.

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Construction of aneurysm animal model based on hemodynamics

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Abstract

Objective: To explore a method for the establishment of a stable, ideal and fast intracranial aneurysm model, and to provide a reliable aneurysm model for aneurysm related studies.

Methods: Modified microsurgery technology was applied to establish aneurysms on sidewall, bifurcation, and terminal of artery and merged saccular aneurysm, and each type of aneurysm model included seven dogs. At 2 weeks after operation, color Doppler ultrasound, magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and intra-arterial digital subtraction angiography (IADSA) were employed for examination, and computational fluid dynamics (CFD) was used for simulation analysis of aneurysm models. Cell culture method was applied to analyze the biomechanical properties of aneurysm wall tissues, and the examination and pathological analysis were performed again after the aneurysm was embolized by micro coil (MC).

Results: All models were successfully established, and the MC embolization of aneurysm cavity achieved good efficacy. The proliferation of vascular endothelial cells (VECs) in vitro was good, and the VECs successfully adhered to the surface of biodegradable polymeric materials to grow into VEC layer structure. DSA combined with noninvasive imaging could improve the diagnosis and comprehensive understanding of aneurysm, and hemodynamic analysis could imitate the hemodynamic changes of aneurysm in dog neck.

Conclusion: Our study obtains hemodynamic parameters of various types of aneurysm. Risk factor assessment combined with biomechanical properties of aneurysm wall is helpful to clarify the relationship between the mechanisms of hemodynamic changes and biological behaviors of intracranial aneurysm.

Key words: Hemodynamics; aneurysm; animal models.

Introduction

Intracranial aneurysm (AN) is cerebrovascular tumor-like protrusion induced by local pathological expansion of arterial wall, and its rupture is the leading cause of spontaneous subarachnoid hemorrhage (approximately 79%-89%) in humans. The AN accounts for 25% of intracranial hemorrhage with high mortality and morbidity, and seriously affects people's health and lives [1]. AN, once occurs, appears "aneurysm behavior" including the trends of growing, thrombosis and rupture. More and more evidences prove that the aneurysm behavior is closely related to its internal hemodynamic factors such as blood flow, shear stress, arterial pressure and pulsatile blood flow [2] and the supporting force of vascular wall itself. The hemodynamic study of intracranial AN is important for the control and treatment of AN. Because the limitation of experimental study on human body, establishing a good and stable AN animal model that meets scientific and experimental requirements has important practical significance [3].

Materials and Methods

Establishment of different types of AN models

Twenty-eight dogs (9-12 kg) were anesthetized by intraperitoneal injection of 4% sodium pentobarbital (1 mL/kg body weight) and fixed at supine position to expose subcutaneous external jugular vein (EJV) and bilateral deep common carotid artery (CCA). The EJV was spared and the proximal and distal blood flow of CCA was blocked. The EJV capsule size was controlled to anastomosed with bilateral CCA under a microscope to construct sidewall, bifurcation, and terminal and mer-

ged saccular aneurysms. There were seven dogs in each group. The specific procedures for each type of modeling referred to our previous work [4-7].

Vascular endothelial cell culture and MC construction supporting the VECs

① Dog VEC proliferation activity: Dogs jugular vein was isolated and primarily cultured with tissue explant method. The VEC was confirmed by immunohistochemical staining of coagulation factor VIII related antigen, and the VEC of 3-5 generations was used for experiment. MTT assay was used to detect cell proliferation activity.

② Amplification and cultivation: VEC was grown on a porous composite film MC, and an optimal cell density was measured. Cell counting was referred to Zund method, and MTT assay was used to reflect cell adhesion and growth.

Hemodynamic analysis of AN under different hemodynamic conditions

After color Doppler ultrasound examination, the dogs with AN were given femoral artery puncture, and the pressure in the AN was measured with micro-catheter. The dogs were divided into four groups for DSA examination (A: sidewall AN; B: bifurcation AN; C: terminal AN; D: merged saccular AN; n=7). The steps were shown as follows. After each step color Doppler ultrasound was used to detect blood flow and flow rate, and DSA was applied to detect the actual morphological changes of AN cavity.

① Controlled hypotension: Inhalable anesthetics isoflurane was used, and the drug dose was controlled along with the blood pressure control. The MAP value was controlled to decrease from the basic 17 Kpa to 6 Kpa. The blood pressure was maintained at the low level between 30-60 minutes, and then the examination was performed.

② 3H treatment: Crystal solution and normal saline was intravenously infused with 100-150 mL, and low molecular weight dextran 500 mL and plasma substitutes 50 mL were used for blood volume expansion. Vasopressors such as dopamine or metaraminol were used to elevate the preoperative systolic blood pressure by 20%-30%. The central venous pressure was maintained at 8-12 mmH_2O, hematocrit (HCT) at 33%-38% and pulmonary artery wedge pressure at 2.00-2.40 kPa (1 kPa = 7.5 mmHg), and the examination was performed.

③ Nimodipine: The examination was performed at 2 h after intravenous injection of nimodipine at a speed of 4 mL/h.

④ Vasospasm: The catheter was used to stimulate the carotid artery with AN in the DSA to induce spasm, and local infusion of papaverine was applied after examination.

Biomechanical properties of intracranial AN wall tissues

Generally, the AN wall tissues were considered as homogeneous, incompressible and isotropic (the mechanical properties at any point within the AN tissues were the same in all directions) viscoelastic substances. According to traditional biomechanical principles and practical test, the Von Mises stress of the AN wall can withstand was estimated.

During the surgical clipping of intracranial AN, the AN specimens were collected at the operating table side. Half of the specimens were not fixed, and was used for biomechanical properties measurement within 5 hours in vitro. The relationship between stress and deformation was analyzed (pressure-volume relationship). Tissue strain energy equation: $W = \alpha (IB23) + \beta (IB23)$, where W was the strain energy density, α and β were the mechanical parameters of AN wall tissues, and IB was the measured elasticity tensor.

Another half of the specimens were placed in OCT and stored in liquid nitrogen in 30 min. Reagents: ST series kit, peroxidase-labeled streptavidin staining kit (ZYMED company), benzidine (Sigma), monocyte chemoattractant protein-1 (MCP-1) monoclonal antibody and macrophage inflammatory protein 1 (MIP-1α) monoclonal antibody (CHEMCON company). The thickness of frozen sections was 6 μm, and continuously cut for 4 pieces, which were used for HE staining and immunohistochemical staining using MCP-1 monoclonal antibody, MIP-1α monoclonal antibody and phosphate buffer (negative control), respectively. Immunohistochemical sections were also stained with methyl green. Brown granules in cells were considered as positive results, and the cells with positive signal expression and the location were observed.

The relationship of various pathological types of AN wall (cell tissue ingredients comprised: intimal layer thickening, edema degeneration, collagen

degeneration, residual muscle fibers, hemorrhage/thrombus/organization, outer fibrous membrane, calcification, intra-wall bleeding lesions, inflammatory cell infiltration, infiltration of phagocytes, and endothelial changes) and AN wall microstructural changes with the AN wall tension force was studied to obtain the elastic modulus parameters. The MCP-1 and MIP-1α expression in AN wall cells was detected and the relationship with Von Mises stress was examined.

Hemodynamics of AN models after embolization

Imaging: Dogs with sidewall, bifurcation and terminal AN were given femoral artery puncture, and the MC tightly filling in the AN through the micro-catheter. DSA was used to examine the AN cavity, and the DSA and color Doppler ultrasound were performed after the experimental treatment (3H and nimodipine treatment same as the former).

Mechanical evaluation of AN after embolization: the changes of growth, metabolism and biomechanics of endothelial cells on extracellular matrix were observed. The postoperative carotid artery specimens at treatment site were collected. Mouse anti-dog VEGF monoclonal antibody was used for immunohistochemical staining (ABC staining), the light microscopy and semi-quantitative computer image analysis were applied. Five visual fields were randomly selected in each slice, and brown area and vascular cross-sectional area were determined at 200 times magnification. The positive particle density (brown granule area/vascular cross-sectional area in unit visual field) was calculated. Scanning electron microscopy was used to observe the histological changes of intimal endothelial repair, cell components and cell matrix after MC embolization. The number of cells and morphological change index were estimated. The expression of MCP-1 and adhesion molecule α-4 on cell surface was examined.

Prediction of AN re-growth, re-rupter and hemodynamic trend. According to the above mentioned method, the imaging data was analyzed for vascular wall stress distribution and the stress distribution in different shapes wall to determine the active parts of AN residual stress mechanical factors under dynamic condition. Combined with the pathophysiological changes of implanted endothelial cells, the position that was easy for AN grow and rupture was predicted.

Results

Model construction

All the dogs were alive after operation, and the sidewall, bifurcation, and terminal and merged saccular aneurysm models were successfully constructed (n=7). The sidewall AN model was shown in Figure 1.

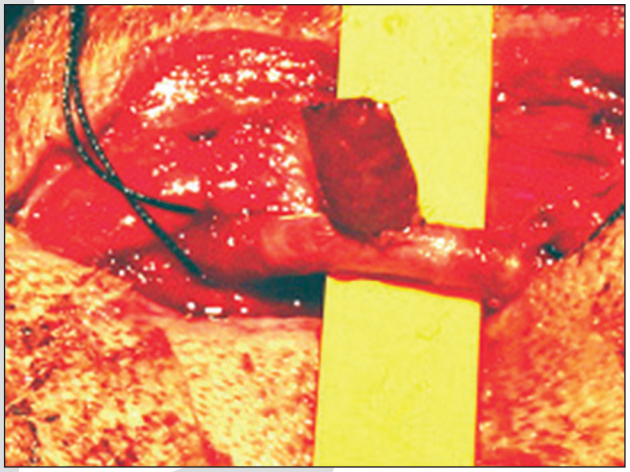

Figure 1. Sidewall AN with maximum diameter of 1.2 cm

Results of VEC culture and construction of MC supporting the VEC

The VEC proliferation in vitro was good (Figure 2). VEC vessel formation experiment proved that VEC could form vascular endothelial layer on the MC scaffold. The VEC successfully adhered to the MC surface in vitro, and further grew into VEC layer structure (Figure 3).

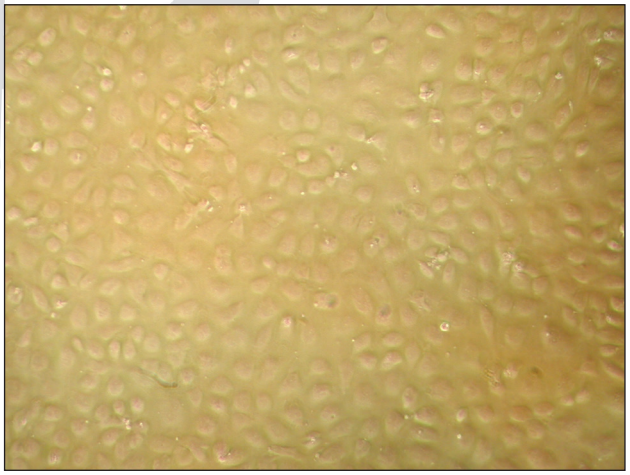

Figure 2. Dog VEC culture in vitro

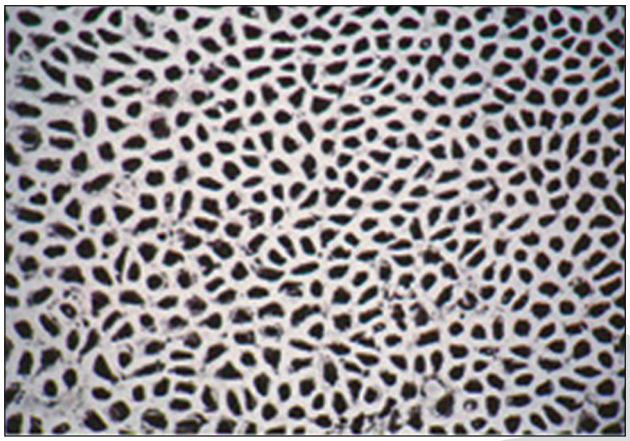

Figure 3. Formation of vascular layer stucture by VEC on artifical vessesl in vitro

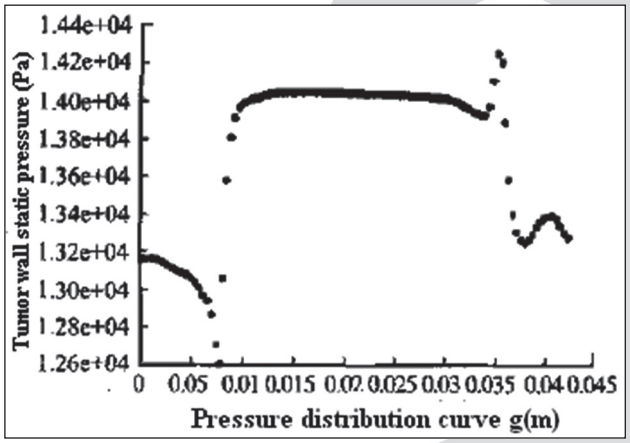

Figure 4. Distributing curve of AN wall upper pressure (absolute value) along with the length of AN wall curve (from distal AN top proximal)

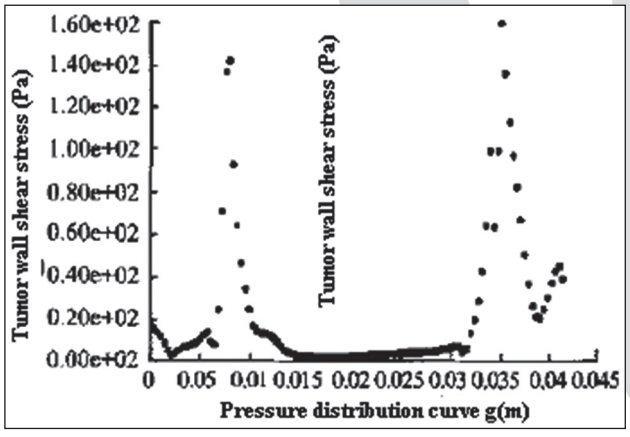

Figure 5. Distributing curve of AN wall upper shear stress (absolute value) along with the length of AN wall curve (from distal AN top proximal)

Biomechanical properties of intracranial AN wall tissues

The pressure was high on top of the AN, and the shear stress was the lowest. The pressure in the proximal inflow tract was high, and the peak pressue was also in the section as well as the shear stress. The changes of AN wall upper pressure and shear stress along with the changes of AN wall curve length were shown in Figure 4 and 5.

Hemodynamic analysis of AN model before and after embolization

The data of color Doppler ultrasound and DSA of AN models was collected, and was used for computational fluid dynamics (CFD) simulation.

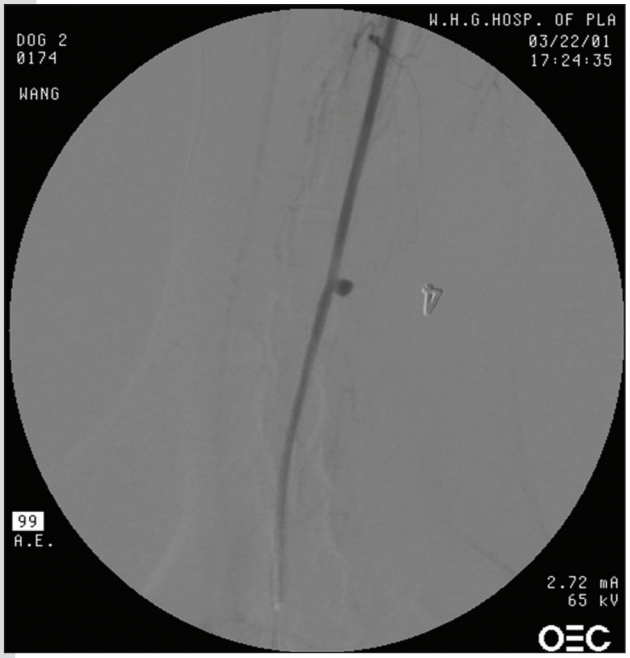

Figure 6. DSA result of sidewall AN model

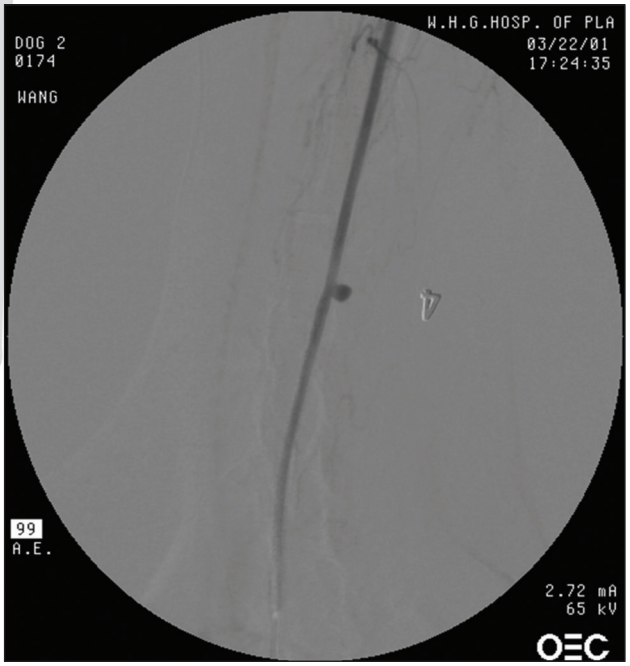

Figure 7. DSA result of bifurcation AN model

Depending on the flow line and velocity gradient graphs of blood flow characteristics of different types of AN model, the effect of possible risk parameters such as the pressure and shear stress at the proximal end of AN, AN top and distal AN wall on the flow characteristics in the AN was analyzed. The simulation results provided flow region shape in the calculated area as well as the relevant hemodynamic parameters including blood flow velocity distribution range in AN of 2-7 cm/s and blood flow velocity distribution range in the artery with AN of 8-26 cm/s. The following Figure 6-15 were the typical results.

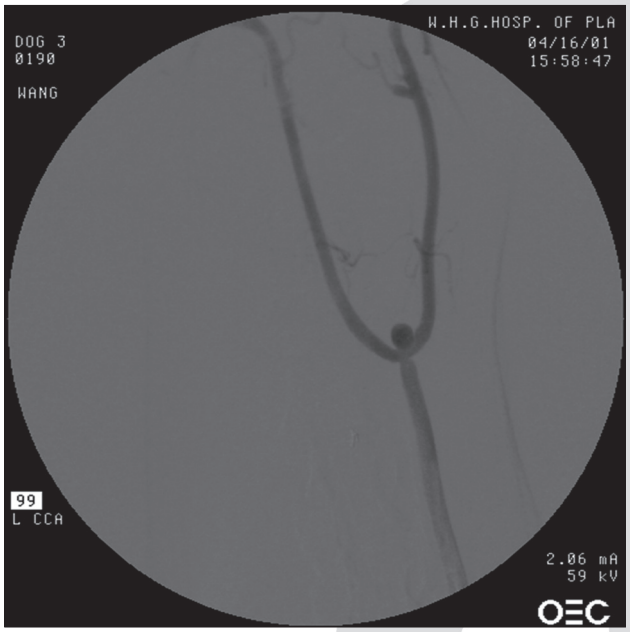

Figure 8. DSA result of treminal AN model

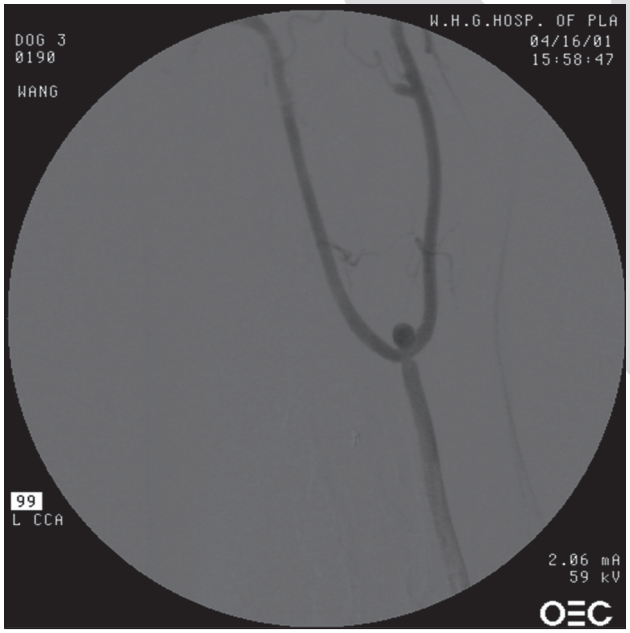

Figure 9. Velocity gredient graph of sidewall AN model

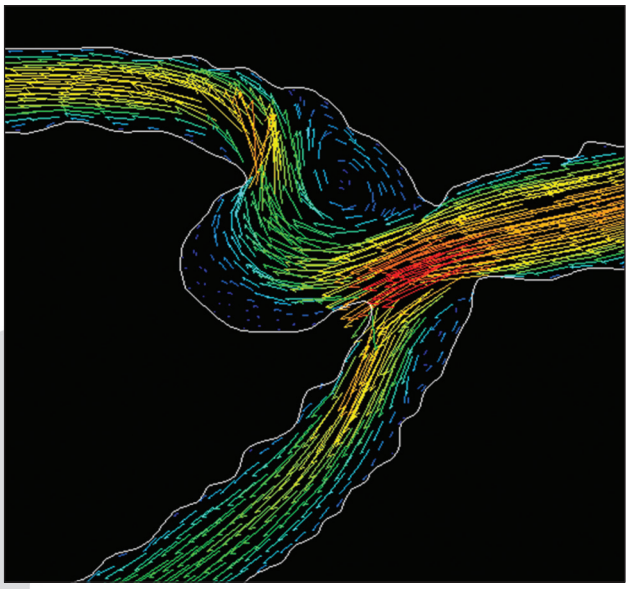

Figure 10. Velocity gredient graph of bifurcation AN model

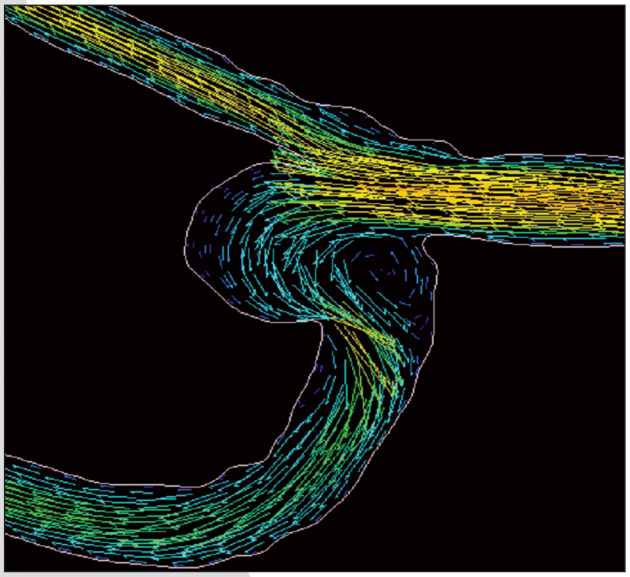

Figure 11. Velocity gredient graph of treminal AN model

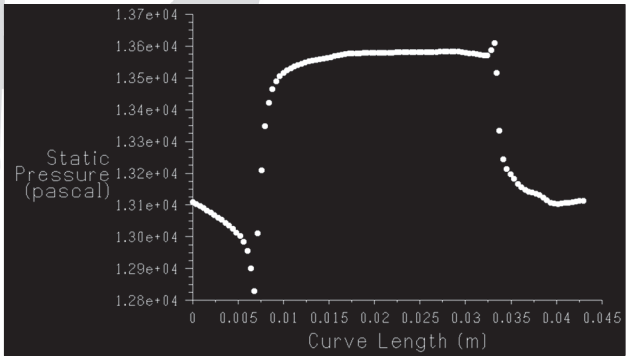

Figure 12. Distributing curve of bifurcation AN upper wall pressure (absolute value) along with the changes of AN wall curve length (distal→AN top→proximal)

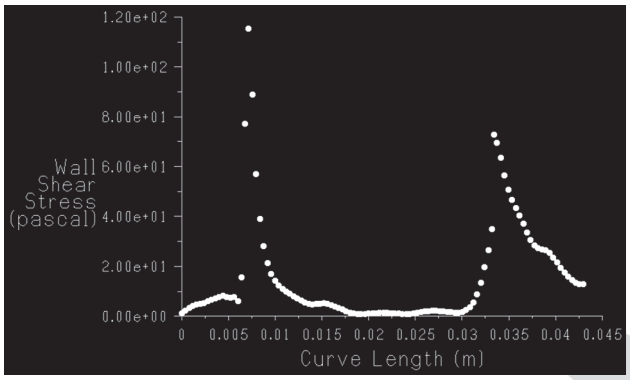

Figure 13. Distributing curve of bifurcation AN upper wall shear stress (absolute value) along with the changes of AN wall curve length (distal→AN top→proximal)

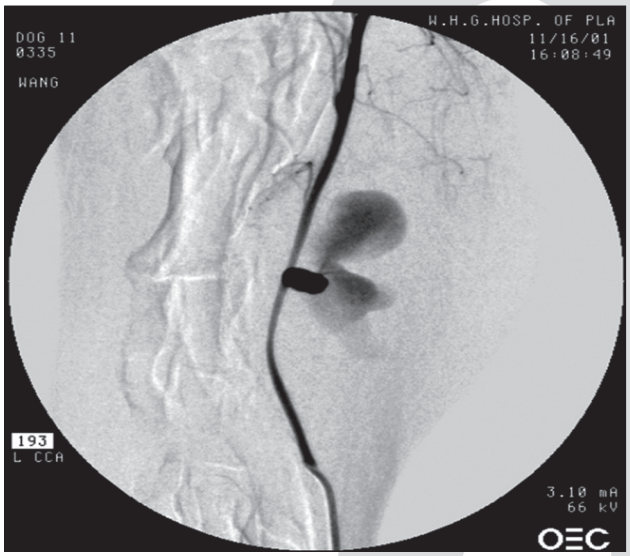

Figure 14. LCCA imaging of LCCA sidewall AN (straight arrow) complicated with pseudo-AN (curved arraw)

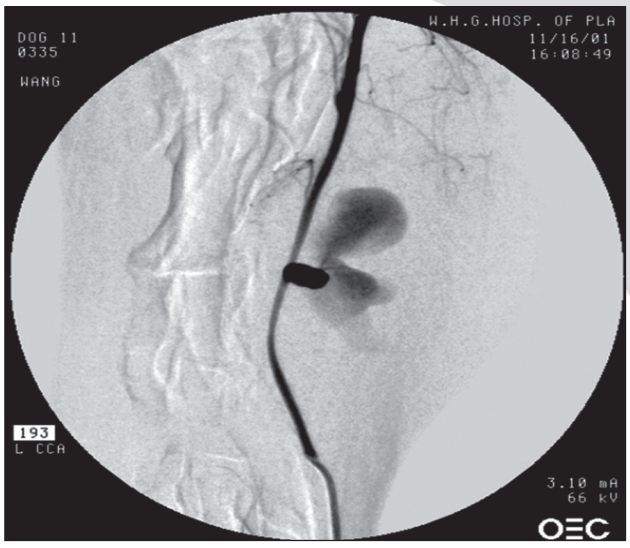

Figure 15. Velocity gredient graph of merged saccular AN

Discussion

Intracranial AN is the most important reason for acute spontaneous subarachnoid hemorrhage (SAH), and it is also one of the important reasons for human death. Brain arterial structure is characterized by less medial smooth muscle, poor elastic fibers, and thin outer membrane, and those vessels have weak contraction ability but strong expansion ability.

Arterial pressure is the direct driving force of AN formation, and it is derived from blood pressure and vascular elastic recoil tangentially passing to vascular wall and vertical to the long axis of vessel or through it [8]. Although endothelial cells are considered as a barrier to lateral conduction of stress, and the effect is weakened when endothelial cells are injured. Then the pressure will be transformed into potential tension of vascular wall acting on local vessels [9], and the changes of elastic layer and media layer occur. When the tension increases or local blood vessel tolerance decreases, the pathological condition can induce blood vessel expansion and subsequently AN formation [10]. Peripheral vascular wall tension is the main stress for AN formation. When stenosis and occlusion occur during vascular repair and cell proliferation, local pressure gradient increases, which is easy to induce AN [11]. The shear stress is derived from friction of blood flow to vascular wall, also known as the viscous stress or shear forces. It contains two parts: (1) parallel to long axis of vessel, opposite to blood flow direction, and originated from blood viscosity. (2) The reaction of vascular wall to shear stress with the same direction to blood flow. The shear stress increases along with the increase of blood flow velocity. The laminar and vortex motion adjacent to AN and changes of blood flow direction and velocity form various blood shear stress on the intima of AN wall. When the shear stress reaches a predetermined threshold, the endothelial cells are injured, leading to the AN formation [12]. In addition, the pulsatile blood flow (velocity) exerts impact force on arterial walls without supporting. The intravascular blood flow includes laminar flow, reverse flow, vortex flow and turbulence are hemodynamic factors affecting AN formation [13].

The biological behavior of AN is closely related with its hemodynamics. Pulsatile flow impact

force (velocity) is a prerequisite for AN rupture [14], which most occurs on top of AN but rarely on AN neck. Because of strong impact force of blood flow to the AN wall, AN body with blood flow direction pointing along the direction of AN artery blood flow is more prone to rupture than the AN body with other blood flow directions [15].

Foutrakis proved AN is prone to occur at the sites with great speed/pressure gradient and high shear stress [16]. The development of AN capsule aggravates the blood turbulence at this part. Shear stress within the AN at different levels (maximum on AN neck and minimum on AN top) and the direction repeated changes affect the adaptive mechanism of endothelial cells [17]. The blood flow of adjacent artery loaded with AN is also affected by the blood flow within the AN [2]. Since the reflection of AN wall to pulse wave resulting in multiple peak velocities in a cardiac cycle, the interaction with the vascular wall may induce oscillation even resonance, and promote its degeneration or lead to AN rupture.

Ferguson [8] and Gobin [18] determined the pressure in patients with intracranial AN and in vitro model of AN, and confirmed the pressure inside of the AN has no difference with the pressure in the artery loaded with AN except for the pressure distribution on the AN wall. AN intraluminal pressure forms a cyclic expansion and retraction, and affects arterial wall endothelial cell function and properties of local blood flow. Meanwhile, intra-arterial pressure fluctuations will inevitably cause pressure changes in the AN. Dum confirms the relationship between the increase of pressure and AN broken with computer cycle simulation system, which is the theoretical basis for the application of antihypertensives to prevent AN rupture. The objective of surgery or interventional treatment is designed to reduce, modify or eliminate hemodynamic status in the AN and local artery loaded with AN, terminate AN behavior and prevent AN growth and rupture.

The biological behavior of AN can be predicted based on the hemodynamic changes. Previous hemodynamic study showed that AN shape extension mostly occurs at the sites with active mechanical properties. The changes of blood flow state and significan increase of local shear stress induce AN growth, while blood flow impact force (velocity) and significant increase of AN internal pressure may cause AN rupture. The size and distribution of force on AN wall can predict the growth AND rupture site of AN. The observation of injured endothelial cells or cell repair can predict the possible site of AN re-growth and re-rupture [19].

The hemodynamics of AN is directly related to pathology and morphology, for example: pathological condition of AN, geometry properties of AN [20,21], the opening width etc. According to the results of imaging observations, intracranial AN shows relatively small shape changes, clear geometric boundary, and less affection by fluid-structure interaction induced by contractile force of AN wall compared with abdominal aortic AN, so the hemodynamic changes is closer to the real situation [22]. Meanwhile, the brain small arteries have no nourishing blood supply, and their nutrition is from arterial lumen. Continuous hemodynamic affection also cause gene expression changes in local AN wall, and even the release of substances can induce the further damage to blood vessels [23,24,25]. Quantification of the wall stress state of different shapes of AN, developing individualized study and understanding the hemodynamic characteristics are important for the hemodynamic control and treatment of AN.

Meanwhile, hemodynamic parameters change in the occurrence, growth and rupture of intracranial AN, vasospasm after subarachnoid hemorrhage, common controlling hypotensive therapy, 3H therapy (high blood volume, high blood pressure and high blood dilution degree), vasodilators (nimodipine) as well as other therapeutical measures for AN, and those factors can affect the efficacy of treatment. Thus, the study on the stress changes of AN wall under the changes of hemodynamics is necessary for clarifying the developing trend of AN biological behaviors.

This study established four different types of AN model, investigated the relationship of blood flow, shear stress, pressure and velocity with the growth and rupture of different type of AN (sidewall, bifurcation, terminal and merged AN) in combination with biomechanical properties of intracranial AN wall at different flow patterns (hypotension, hypertension, vasodilators, cerebral vasospasm), determined hemodynamic parameters in different types of AN, and predicted the response to the choice of

treatment, in order to control the growth and rupture of untreated AN with appropriate measures targeting to the AN hemodynamic characteristics. The application of micro-coil loading with endothelial cells for AN embolization provided evidences for further exploration of cell biological changes and AN re-growth and re-rupture in different types of AN [25] and for improving the efficacy of surgery and interventional treatment.

Our study started from hydrodynamics, and obtained AN hemodynamic parameters under various blood flow patterns. Risk factor assessment combined with biomechanical properties of AN wall clarified the relationship between the mechanism of hemodynamic changes and biological behavior of intracranial AN, which was helpful for the control and treatment of AN. Taken together, the changes of the biological behavior of AN is a dynamic process, involving both the characteristics of AN and the hemodynamics, and solving these problems is very important in clinical medicine.

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The significance of early cytogenetic response to Imatinib in Chronic Myeloid Leukemia

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Abstract

Introduction: Imatinib mesylate is remarkably effective in treatment of patients with Philadephia chromosome-positive chronic myeloid leukemia. Objective. We analyzed the probability of achieving early complete cytogenetic responses and baseline characteristics that might be associated with achievement of this response.

Methods: We reviewed 35 patients with chronic phase chronic myeloid leukemia treated with 400mg of imatinib mesylate daily.

Results: In the study group complete cytogenetic response rate was 46% at 6 months, 54% at 12 months and 60% at 15 to 18 months of imatinib treatment. For patients who did not achieve complete cytogenetic response, the probability of achieving that response decreased from 80% to 33% at each time point. The univariate analysis selected the following to be variables associated with lower probability of achieving early complete cytogenetic response: lower hemoglobin, higher peripheral blood and bone marrow blast percentage, and splenomegaly. The multivariate analysis identified lower hemoglobin, higher peripheral blood blast and basophils percentage to have independent adverse prognostic significance ($p<0.001$). After 5 years of treatment 70% of all complete cytogenetic responders were still in complete cytogenetic response, whereas estimated 5 years rate remaining in stabile complete cytogenetic response was 79% for early versus 67% for late responders. Therefore, the probability of complete cytogenetic response loss has tendency to decrease over time.

Conclusion: These data confirmed that the patients treated with imatinib who achieved early complete cytogenetic response maintain the high percentage of that response with long-term follow-up. From this analysis we defined factors before therapy which were independent adverse factors for achieving early complete cytogenetic response and could accurately predict patients who ultimately achieved this response.

Key words: Chronic myeloid leukemia, imatinib mesylate, cytogenetic response.

Introduction

Chronic myeloid leukaemia (CML) is a clonal disorder arising from neoplastic transformation of hematopietic stem cells, most of which are characterized by the presence of Philadelphia chromosome (Ph) and by constitutive activation of Bcr-Abl tyrosine kinase. Imatinib mesylate is selective inhibitor activeness of the Bcr-Abl tyrosine kinase. Imatinib inhibits the binding site for adenosine triphosphate to the Abl kinase, thus blocking the phosophorylation of tyrosine on substrate protein. Imatinib has demonstrated effectiveness in all three CML phases: chronic, accelerated and blastic [1, 2].

Most of the available data of efficacy of imatinib in patients with CML in chronic phase are based on results of single multicenter trial, the International Randomized Study of imatinib mesylate versus interferon-alpha and low dose cytarabine (IRIS) study. In the IRIS study, after 12 months of imatinb therapy, 69% of patients with CML in early chronic phase were projected to achieve a complete cytogenetic response [3]. This response rate improves to 82% after 5 years of continued therapy [4]. However, recent analyses showed that the risks of disease progression among patients who achieve a complete cytogenetic response within and after 12 months are similar [5].

Imatinib has proven highly effective in the treatment of CML and is now became the first line therapy of newly diagnosed CML. By use of this drug, complete hematologic remission can be achieved in almost all Ph chromosome positive patients with CML in chronic phase. What is more important, major cytogenetic response can be achieved in more than 50% who begin the treatment in late

chronic phase and in more than 80% of patients who are treated in early chronic phase [3, 6].

Objective of the study

The objective of this study was to evaluate rate of achieving a complete cytogenetic response early during the treatment with imatinib. We investigated the possibility of improving the cytogenetic response for patients who did not achieve complete cytogenetic response at different time points. Finally we analyzed baseline characteristics of patients and probabilities of achieved early complete cytogenetic responses.

Patients and Methods

In this paper we present single–institution experience in the treatment with imatinib mesylate of newly diagnosed patients with CML. We have analyzed 35 consecutive adults with Ph-positive early chronic phase CML who received imatinib between June 2006 and July 2011. All patients had received prior hydroxiurea therapy. These patients were treated on frontline therapies with 400mg daily dose of imatinib mesylate orally. Dose escalation to 600mg/800mg has been required in cases of previous therapy failure, i.e. in patients with cytogenetic refractoriness or cytogenetic relapse.

The main clinical and hematologic characteristics at diagnosis are reported in Table 1. The median age of patients was 52.5 years (range 19 to 73 years), and 19 patients (54.3%) were male. The median time from diagnosis to imatinib therapy start was 2.5 months (range 1 to 7 months). Patients had a median follow-up of 30 months (range 12 to 60 months) and no patient was lost to follow-up. Sokal risk group stratification for patients with evaluable data was as follows: low risk 54%, intermediate risk 37% and high risk 9%.

Before the start of treatment patients were evaluated with history by a physical examination and complete blood cell count with differential and blood chemistry. All patients had pre-treatment bone marrow evaluation for morphology and cytogenetic analysis. After treatment was started patients were evaluated with complete blood count monthly, comprehensive biochemistry panel monthly during first 6 months and then once every 6 months. Bone marrow aspirations for morphology and cytogenetic were repeated every 6 months.

Chronic-phase CML was defined as the presence of blasts in the peripheral blood of less than 15%, basophils less than 20%, blasts together with

Table 1. Clinical and laboratory features at diagnosis

Parameter	Value, median (range)
Median age, y (range)	52.5 (19-73)
Median time from diagnosis to imatinib, mo (range)	2.5 (1-7)
Median WBC $\times 10^9$/L (range)	38.5 (3.5-254)
Median Platelets $\times 10^9$/L (range)	375.5 (140-1165)
Median Hemoglobin, g/dL (range)	122.5 (74-145)
Median percentage of PB blasts (range)	2.7 (0-7)
Median percentage of PB basophils (range)	3.0 (0-9)
Median percentage of BM blasts (range)	3.2 (0-6.5)
Median percentage of BM basophils (range)	3.5 (0-10)
Splenomegaly, n (%)	27 (77)
Dose, mg, n (%)	
400	23 (66)
600	5 (14)
800	7 (20)
Sokal risk score, n (%)	
Low	19 (54)
Intermediate	13 (37)
High	3 (9)

WBC indicates white blood cell; PB, peripheral blood; BM, bone marrow;

promyelocytes less than 30%, and platelets more than 100×10^9/L. Definition of chronic phase and response criteria were as published according to recommendation of LeukemiaNet panel 2006 [7]: complete hematologic response (CHR) was defined as a white blood cell count of less than 10×10^9/L, a platelet count of less than 450×10^9/L, no immature cells (blasts, promyelocytes, myelocytes) in the peripheral blood, and disappearance of all signs and symptoms related to leukaemia (including palpable splenomegaly) lasting for at least 4 weeks. Cytogenetic response was evaluated by standard cytogenetic analysis. Cytogenetic responses, based on metaphase analysis of at least 20 cells, were defined as: complete Ph-positive 0%, partial Ph-positive 1% to 34%, minor Ph-positive 35% to 95%, and absent Ph-positive >95%. Major cytogenetic response includes complete plus partial cytogenetic response - Ph-positive less than 35%.

Descriptive statistic (number, proportion, median, range) were used to summarize the pre-treatment characteristics of the patients and response to imatinib mesylate therapy. Differences among variables were compared by the χ^2 test and Mann-Whitney U test. Univariate and multivariate analyses were performed to identify potential prognostic factors associated with achievement of complete cytogenetic response. The Kaplan-Meier method was used to calculate the duration of complete cytogenetic response from the date of achieving to date of complete cytogenetic response loss or of last cytogenetic control, whichever came first.

Results

Of the 35 patients analyzed in this study, 34 (97%) achieved a complete hematologic response within 6 months. During 12 months from the start of therapy 74% patients achieved major cytogenetic response and 54% achieved complete cytogenetic response. At the time of data analysis 6 patients (17.1%) had permanently discontinued imatinib after median time of 18 months (range 12 to 24 months). Reason for discontinuation included cases of cytogenetic response absence, after the dose of the drug was increased to 600mg/800mg daily. One patient was excluded from treatment with imatinib because of progression to the phase of acceleration.

Table 2. Association between response to imatinib at specific time points and later complete cytogenetic response

Cytogenetic response	n	Complete CgR, n (%)
At 6 months		
Ph > 95%	4	1 (25)
Ph 35-95%	5	3 (60)
Ph 1-34%	10	8 (80)
Ph 0%	16	16 (100)
At 12 months		
Ph > 95%	6	1 (17)
Ph 35-95%	3	1 (33)
Ph 1-34%	7	5 (71)
Ph 0%	19	18 (95)
At 15 to 18 months		
Ph 35% or more	3	1 (33)
Ph 1-34%	5	3 (60)
Ph 0%	21	20 (95)

The associations between response at 6, 12 and 15 to 18 months and later complete cytogenetic response are shown in Table 2. In the study group complete cytogenetic response rate was 46% at 6 months, 54% at 12 months and 60% at 15 to 18 months of imatinib treatment. The probability of eventually achieving a complete cytogenetic response decreases if complete cytogenetic response has not yet been achieved after 6, 12 and 15 to 18 months on imatinib. Thus, for instance, a patient who has achieved a partial cytogenetic response in same analyzed period had decreased chance to obtained complete cytogenetic response from 80% to 60% with continuation of therapy. Also, among patients who had achieved only a minor cytogenetic response after 6 and 12 months of therapy, the subsequent complete cytogenetic response rates were 60% to 33%.

We analyzed the factors associated with achievement of response at 6 months of the start of treatment. Significant adverse factors for achieving early complete cytogenetic response to imatinib are shown in Table 3. A univariate analysis identified the following variables associated with lower probability of achieving early complete cytogenetic response: lower hemoglobin, higher peripheral blood and bone marrow blast percentage, and splenomegaly. In a multivariate analysis, summarized in Table 4, lower hemoglobin, higher peripheral blood blast and basophils percentage were identified as predic-

Table 3. Univariate analysis for the probability of achieving a complete cytogenetic response on imatinib at 6 months of therapy

Parameter	Effect	CCgR at 6 months median (range) or no. (%)		P
		Yes (n=16)	No (n=19)	
Age, y	NS	50.3 (30-68)	50.4 (19-67)	0.96
Lower hemoglobin (g/L)	Adverse	109.2 (74-133)	101.7 (87-144)	<0.001
Higher WBC, x10^9/L	NS	76.4 (4.4-254)	70.5 (3.5-90.2)	0.007
Platelets, x10^9/L	NS	328.7 (142-681)	367.7 (140-863)	0.51
PB basophils (%)	NS	2.5 (1-5.5)	1.9 (0-6.5)	0.88
Higher PB blasts (%)	Adverse	1.9 (0.5-6.5)	3.2 (0-8.5)	<0.001
BM basophils (%)	NS	2 (0-6)	3 (0-8)	0.11
Higher BM blasts (%)	Adverse	2.5 (0.5-6.5)	3.7 (0-8.5)	<0.001
Presence of splenomegaly	Adverse	9 (56.3)	16 (84.2)	<0.001
Longer CML duration, mo	NS	2.2 (1-6)	3.3 (1-12)	0.6
Dose (400 vs 800mg/day)	NS	14 (87.5)	11 (62.5)	0.09
Sokal risk score				
Low	NS	10 (62.5)	9 (47.4)	0.22
Intermediate	NS	6 (37.5)	7 (36.8)	0.78
High	NS	0 (0)	3 (15.8)	0.83

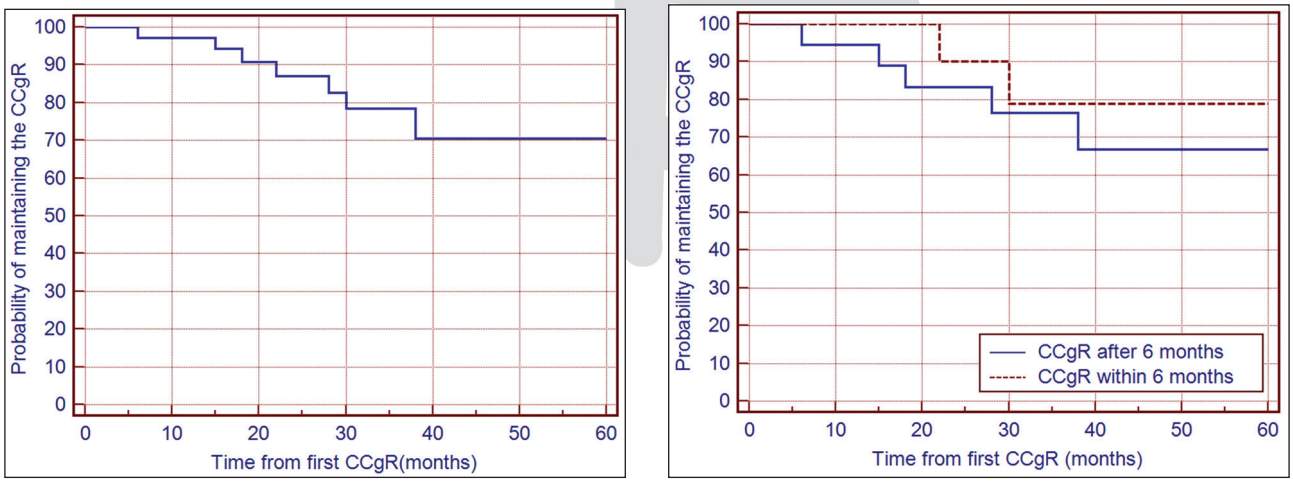
NS indicates not significant

Table 4. Multivariate analysis for the probability of achieving a complete cytogenetic response on imatinib at 6 months of therapy

Factor	Estimate of coefficient	Estimate of odds ratio	P
Higher PB blasts	-0.87	0.42	<0.001
Higher PB basophils	-1.12	0.66	<0.001
Lower hemoglobin	0.32	0.99	<0.001

tors of a decreased probability of achieving complete cytogenetic response at 6 months.

In Figure 1. probability of maintaining the complete cytogenetic response for all patients (A) and (B) in patients whose achieved that response within 6 months or later during treatment (n=16 and 19, respectively) is shown. After 5 years of treatment, 70% (95 Cl, 67% to 75%) of complete cytogenetic re-



A *B*

Figure 1. Kaplan-Meier estimates of the duration of the complete cytogenetic response in all analyzed patients (A), and (B) in patients whose achieved that response within 6 months or later during treatment

sponders were still in complete cytogenetic response. The proportion of patients that remain in stable complete cytogenetic response after 5 years was similar in both analyzed groups (79% versus 67%).

Discussion

The introduction of imatinib mesylate, a potent and selective tyrosin kinase inhibitor, into chronic myeloid leukemia therapy has marked a major advance in treatment of this disease. Imatinib mesylate induces complete cytogenetic responses in a high proportion of chronic myeloid leukemia patients [2].

The phase III International Randomized Study of INF versus STI571 trial compared imatinib and IFN plus cytarabine, in 1106 CML patients in early chronic phase. This study demonstrated, follow up at 8 years, cumulative best complete cytogenetic response rate is 89%, the estimated survival rate is 85% and freedom from progression rate is 93%, with event-free survival of 81%. IRIS study and other significant studies reported that cytogenetic landmark are the cytogenetic response at 12 and 18 months and also the early response at 3 and 6 months are important [4, 8, 9].

In this paper we have estimated the rate of achieving early complete cytogenetic responses to imatinib on patients with chronic myeloid leukemia in chronic phase and analyzed influencing factors to early complete cytogenetic response.

In the paper of Kantarjian et al. [10] it was shown that patients in partial cytogenetic response after 12 months of therapy still had a 73% incidence of later achieving a complete cytogenetic response, while that rate decreased to only 10% in patients who still had a minor cytogenetic response after 12 months of therapy. Our analysis showed that patients with partial cytogenetic response after 12 months of therapy had a 60% incidence of later achieving a complete cytogenetic response, compared to those with minor cytogenetic response who had an incidence of 33%. Kantarjian et al. [10] also demonstrated in their work that patients without a cytogenetic response at 6 months and those with only a minor cytogenetic response at 12 to 18 months, had worse estimated 4-year survival rates of 70% and 79% respectively, compared with those with better cytogenetic response, who had estimated 4-year survival rates 88% and 100%. In later works Kantarjian et al. [11] reaffirmed that cytogenetic response to imatinib mesylate at 12 months was predictive of prognosis. Patients who had achieved less than a major cytogenetic response at 12 months had a worse prognosis.

In the study of Cardema et al. [12] it was evaluated whether the achievement of early response confers a prognostic advantage. This study showed that the possibility of achieving a complete cytogenetic response during imatinib therapy declines if complete cytogenetic response had not been yet reached after 6 and 12 months of treatment. For example, patients who have not achieved a complete cytogenetic response at 6 months were 57% able to eventually achieve complete cytogenetic response over time with continued therapy. After 12 months of continuous imatinib therapy without a complete cytogenetic response the probability of achieving complete cytogenetic response decreases to 42%. Thus, for instance Cardema et al. [12] identified the following characteristics to be independent adverse prognostic factors for achieving early complete cytogenetic response: lower hemoglobin, higher peripheral blood and bone marrow blast percentage, splenomegaly, and imatinib therapy at standard dose of 400mg. Similar, the analysis of our results selected the following adverse prognostic factors: lower hemoglobin, higher peripheral blood and bone marrow blast percentage, and splenomegaly.

Kantarijan et al. [10] reported that in univariate analysis they identified the following to be independent adverse prognostic factors for achieving a complete cytogenetic response: the presence of blasts and basophils in peripheral blood, the presence of more than 5% blasts in bone marrow, white blood cell count more than $10x10^9/L$ and platelet count more than $450x10^9/L$. A multivariate analysis identified high platelet count and Ph positivity percentage of more than 90% prior to starting imatnib mesylate therapy as important adverse prognostic factors for achieving a complete cytogenetic response.

Several studies have established that cytogenetic response as the most important prognostic factor for long-term out come in CML patients. Druker BJ et al. [6] showed that the 5-year update of the IRIS study indicates that PFS is better for patients who achieve a complete cytogenetic response irrespective of whether this is achieved at 12, 18, or 24 months, thus indicating that time to achieve

cytogenetic response is of minor importance. Similarly, Iacobucci I. et al. [5] in their analysis have shown that patients treated with imatinib who obtained a complete cytogenetic response after 12 months of imatinib therapy had PFS and estimated 4-year overall survival rates similar to those who achieved a complete cytogenetic response within 12 months. Although patients who have not achieved a complete cytogenetic response can improve the response during the course of therapy with imatinib, they have basically two possibilities: either to achieve complete cytogenetic response or to progress. Likewise, Cardema et al. [12] in their study analyzed the outcome of patients who did not achieve a complete cytogenetic response at different intervals of time during therapy with imatinib. His study showed that failure to obtain a complete cytogenetic response within the first 12 months of imatinib therapy is associated with a high rate of disease progression and that the risk is noticeable early in therapy with imatinib.

Conclusions

The patients treated with imatinib who early achieved a complete cytogenetic response maintain the high percentage of that response with long-term follow-up. Our data confirms that depth of response obtained during imatinib therapy is the most important prognostic factors for outcome in CML.

From this analysis, we conclude that the adverse prognostic factors for early achievement of complete cytogenetic response are low hemoglobin, high peripheral blood and bone marrow blast percentage and splenomegaly.

On the basis of these results it is possible to produce a prognostic model which could exactly predict patients who early achieve complete cytogenetic response.

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Prevalence of lymphedema in patients following breast surgery in Hungary

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Abstract

A postoperative cross sectional study of patients who underwent breast surgery between 1st January 2008 and 30th December 2010 was conducted in Hungary. Our aim was to survey the occurrence of postoperative arm swelling and limb complaints together with their most frequent locations on the arm. 364 randomly selected patients were questioned using our questionnaire. Data were collected about the type of breast surgery and axillary lymph node removal, postoperative radiotherapy and chemotherapy, occurrence of wound healing problems, postoperative physiotherapy and the onset of limb complaints and their treatment

The circumference of the arm was measured at 5 standard locations on the arm with a measuring tape. Lymphedema was diagnosed if the difference on at least 1 out of the 5 measured locations exceeded 2 cm, when compared to the same location on the control arm. 23.4% of the 364 patients studied showed signs of lymphedema. 21% of the sampled patients had been operated on within 1 year prior measurements. The rate of those undergoing surgery more than 10 years before was 18.7%. Increase in circumference was most frequently observed at the 4th and 5th measuring points i.e. 5 cm above the elbow and at the axillary line. In cases with breast preservation surgery the occurrence of lymphedema was significantly lower than that of the mastectomy cases. Similarly, correlation was found between cases with axillary block dissection and removal of sentinel lymph nodes and the development of lymphedema as well. When the increase in circumference exceeded 2 cm, the correlation between lymphedema and complaints was found significant. Considering the complaints in the light of the post- surgical interval, the rate of patient's complaints was 2-3 times higher in cases with lymphedema than without. According to the results obtained by a multiple logistic regression model, the incidence of lymphedema was affected by the time of neither the surgery nor the radiotherapy. On the other hand, however, the risk was significantly increased by axillary block dissection and mastectomy.

Key words: Secondary arm lymphedema, breast cancer, quality of life, arm circumference.

Introduction

During the last decades significant changes have been taking place in the diagnosis, surgical treatment and oncotherapy of malignant breast tumours. Indeed, the increased chances of survival have focused the attention on quality of life issues. The results of early diagnosis are increasingly making breast conservation surgical techniques possible and render mastectomy with its well-known functional, aesthetic and psychological problems redundant. Radical lymph node block dissections that have been routinely performed in the past are replaced by the initial removal of sentinel lymph nodes. One of its obvious advantages is the reduction of the development of arm lymphedema caused by the damage to the axillary lymphatic vessels.(1) Following radical surgery, increase of the arm circumference, volume and/or some kinds of arm complaints have been reported on the operated side in 5-70% of patients (2,3,4,5). Circumference increase of at least 2 cm or volume increase of at least 200 ml are usually regarded diagnostic by the literature (6,7). According to Földi, however, the asymptomatic, latent, so called 0 phase lymphedema also can lead to manifest disturbances of lymph circulation(8).

Postoperative limb complaints and lymphedema after breast surgery and oncotherapy have been extensively studied in the literature, but not in the Hungarian population. Therefore, our aim was to perform a cross-sectional, quantitative and qualitative survey of occurrence in our country.

Patients and methods

The survey was performed between 1st January 2008 and 30th December 2010 assessing a major group of patients living the southwestern part of Hungary (population of 1 million peoples) who underwent operation and treatment for breast cancer (n = 364). According to oncological statistics, in this region approximately 6200 patients are registered with this diagnosis. After obtaining the necessary authorizations, data collections were carried out in oncological centers by questioning patients who were scheduled for follow-up within a given period of time, as well as liaising with civil organizations of these patients. The participants were selected into the sample randomly. Those who underwent bilateral surgery were excluded. Surgical interventions and oncological treatments were not carried out in the same institution but were scheduled according to the current protocols.

We obtained information about the time and type of surgery and the treatments, as well as the complaints related to lymphedema via our questionnaire. Then, with the help of medical professionals, we measured the circumference of arm on both sides, at the same five pre-defined measuring points, using a measuring tape under standard tensile force. The readings were given in cm.

Apart from anatomical knowledge of lymph circulation in the upper limb, our previous experience we obtained during the treatment of lymphedema of the upper extremity, were also of importance when the five measuring points had to be determined. The database of our previous circumference measurements helped to select the points where the greatest differences were expected. The use of a quick and simple measuring method was also of primary importance.

The measuring points were as follows:

1. The line of the metacarpo-phalangeal joints, without the pollex
2. The midpoint of the distance between the radial bone's styloid process and the olecranon
3. The midpoint of the distance between point 2 and the olecranon
4. 5 cm above the olecranon
5. At the axillary line.

An increase in circumference exceeding 2 cm at least at 1 out of these 5 measuring points or a difference in circumference of more than 10% when compared to the corresponding point of the control arm, was considered lymphedema.

Statistical analysis

Data are presented as count (percent), or mean and standard deviation.

Association between variables were tested using the chi-square test, nonparametric Friedman-test, Wilcoxon's paired test and Mann-Whitney U-test. All tests were two-tailed. $p < 0.05$ was taken as evidence of statistical significance.

Finally, logistic regression was used to examine the association between presence of lymphedema and treatment. Statistical procedures were performed using the statistical package SPSS 15.0 for Windows.

Results

The surgery-related characteristics of sample participants are shown in Table 1.

Table 1. Medical characteristics

Period after breast surgery	1 - 35 years
< 1 year	84 (23.1 %)
2 - 3 years	81 (22.3 %)
4 - 5 years	71 (19.5 %)
6-10 years	60 (16.5 %)
> 10 years	68 (18.7 %)
Type of surgery	
mastectomy	176 (48.4 %)
breast conservation	188 (51.6 %)
Type of axillary lymph node removal	
axillary block dissection	225 (61.8 %)
sentinel lymph node removal	139 (38.2 %)
Radiotherapy	288 (79.1 %)
Cytostatic therapy	213 (58.5 %)
Postoperative physiotherapy	196 (53.8 %)
Wound healing disorder	56 (15.4 %)
Postoperative limb complaints	160 (44.0 %)

Number of sample components, n=364

Incidence rate of increase in arm circumference

The occurrence of increase in circumference by more than 2 cm at least at one of the five measuring points depending on the time is shown in Table 2.

Table 2. Incidence of arm edema assessed by time interval after surgery

Time interval after surgery (year)	Increase in circumference by more than 2 cm measured at least at 1 measuring point		
	Number of cases	%	$CI_{95\%}$
0-1	16	19.0	12.1 – 28.7
2-3	19	23.5	15.6 – 33.8
4-5	14	19.7	12.1 – 30.4
6-10	15	25.0	15.7 – 37.2
10 <	21	30.9	21.1 – 42.6
Total	**85**	**23.4**	**19.3 – 27.9**

The frequency of edema in the group of patients who had operation within 1 year is reasonably less, while in the group of those who underwent surgery more than 10 years ago, this rate is comparatively higher.

In the entire sample, 38 patients (10.4%) showed a decrease in circumference by more than 2 cm at least at one measuring point, however, besides the decrease, in 12 cases an increase at certain measuring points could also be registered. The most common change following "0" change is a change of +1cm and of -1cm, respectively, which is within the limits of measuring error.

When an increase in circumference of the arm by more than 4 cm was measured at least at one of the five measuring points it was considered to be a serious case of lymphedema. The prevalence of serious cases in the whole sample is 40 (10.9%) .

Comparison of circumference measuring points

During the analysis of occurrence of increase in circumference at several measuring points, the we found that an increase by more than 2 cm mostly occurs at the measuring points No 4 and 5 (in 43 and 44 cases ~ 12%), while at the measuring point No 1 only in 9 cases (~ 3%). The extreme values and mean values (as well as the standard deviation) of the relative change in circumference at the measuring points are shown in Table 3, including the whole sample (n = 364). The relative change in circumference at the measuring point No 1 is significantly less than at the other points (significance level of the Wilcoxon's test in all comparisons: $p = 0.001$, while the values at the measuring points 2-5 did not show a significant difference (according to the Friedman's test).

In light of the time interval after surgery, the rate of patients with complaints is 2-3 times higher among those with edema than those without edema (Table 4).

Table 3. Relative change in circumference at the measuring points

Measuring point	Minimum (%)	Maximum (%)	Mean (%)	Std. Deviation (%)
1.	-13.6	25.0	1.3	4.8
2.	-16.0	43.8	2.6	7.3
3.	-17.2	42.9	2.6	5.9
4.	-12.5	39.9	2.8	6.1
5.	-12.5	40.9	2.4	5.6

Table 4. Distribution of patients with arm complaints according to incidence

Time interval after surgery (year)	Incidence of limb complaints developing on the affected side		Significance level (χ^2-test)
	No increase in circumference by more than 2 cm	Increase in circumference by more than 2 cm at least at 1 measuring point	
0-1	26 (38.2 %)	14 (87.5 %)	0.000
2-3	21 (33.9 %)	12 (63.2 %)	0.023
4-5	14 (24.6 %)	10 (71.4%)	0.001
6-10	22 (48.9 %)	9 (60.0 %)	0.456
10 <	17 (36.2 %)	15 (71.4 %)	0.007

When assessing the incidence of complaints in correlation with the severity of edema it can be established that the rate of patients with symptoms were rising in parallel with the severity of edema, especially in patients with a circumferential increase by more than 5 cm at least at one of the measuring points. Of these 14 patients only one answered that she had not perceived a swelling, numbness or weakness in the affected arm.

The most common limb complaints following breast tumor surgery in the form of pain, numbness and arm swelling, occurred in 67 cases (35.6 %) after total mastectomy and in 93 cases (52.8 %) after breast preserving intervention (χ^2=10.92; p = 0.001).

Assessing the incidence of symptoms (pain, numbness, arm swelling) by the types of axillary lymph node dissections, we found 50 patients with complaints in the entire sample (36.0%), and 110 cases (48.9 %) after partial mastectomy. This constitutes a significant difference (χ^2=5.82; p = 0.016).

The correlation between the total or partial breast removal and occurrence of the edema is significant in the entire sample (χ^2 =17.56; p < 0.001), edema developed in 58 patients (33.0 %), after total mastectomy and in 27 patients (14. %) after partial breast removal.

In case of complete axillary dissection, edema developed in 65 patients (28.9%) and after partial axillary dissection in 20 patients (14.4 %) (χ^2 =10.09; p = 0.001).

Statistically significant correlation can be demonstrated between the arm swelling and the type of breast or lymph node surgery when groups with different elapsed postoperative time intervals were compared.

When the sampled population was divided according to the date of surgery into three groups i.e. pre 2004 cases, cases operated between 2004-1006 and post 2006 cases it becomes obvious that the number of total mastectomy cases is decreasing with the progress in time (54.1%, 50.5%, 38.3%,). The same goes for the number of cases with total lymph node dissections (69.2%, 61.3%, 52.3%, respectively).

The relation between oncotherapy (radiotherapy, chemotherapy, physiotherapy) and lymphedema within the groups defined by the type of breast surgery and the date of the procedure, was evaluated in only three samples (pre 2003, 2004-2006, and post 2006 cases) due to the relatively small number of patients. The frequency of radiotherapy between the groups defined by the type of surgery was found significantly different in the post 2006 group only. On the other hand the frequency of chemotherapy was higher in all the groups where breast preserving surgical techniques had been used (chi-square test p < 0.001).

Approximately half of the patients questioned participated in postoperative physiotherapy.

No statistically significant correlation was detected between the occurrence of lymphedema and the use of radio- chemo- and physiotherapy within the homogenous groups defined by the time and the type of surgery.

Postoperative wound healing disturbances were also studied and did not reveal any detectable association with the occurrence of lymphedema.

Discussion

Arm swelling due to damage of lymph circulation caused by surgery for malignant breast tumours is a complication of primary importance. This complication may develop months, years or even decades after surgery and can influence the quality of life . Therefore, patients should be warned of the symptoms and possible dangers of this complication in the perioperative period. (9, 10) In our study we found significant correlation between the lymphedema and arm complaints. According to the literature local scar formation caused by radiotherapy and wound healing disturbances can be regarded as risk factors. (11) Based on our results we can't confirm that view. In its early stage, an almost unnoticed few cm increase in arm circumference can lead to functional problems and pain. In an advanced stage painful swelling, weakness, fatigue, sensation of pins and needles, arm movement limitation, are regarded as characteristic signs. According to Armer the frequency of clinical symptoms varies in parallel with the occurrence of lymphedema. (12) During our study we found similar results. Early diagnosis, special treatments aiming to improve lymph circulation by all means may reduce complaints and complications. (13) Patient education should include information on known risk factors and instructions how to avoid them (avoid taking BP measurement, iv injecti-

ons, blood sampling, iv infusions on the affected side, prevent skin lesions, fatigue, preserve normal skin pH etc.). Patient information should also include diagnostic and treatment possibilities that are available and necessary in case circumference increase is suspected. (14) The simplest method of diagnosing lymphedema due to lymph circulation disturbance is the comparable measurement of arm circumferences at standardized identifiable points, although, other methods are also available. (15) Our 5-point comparable arm circumference measurement is simple and quick. It can be used in asymptomatic patients as well as at the time of the onset of complaints. We would recommend its use during the patient's oncology follow up and control appointments. Measuring the arm circumference at the two most suitable location, the 4th (5 cm above the olecranon) and 5th points (axillary line), where the difference is most likely the highest when compared to the control.

Early treatments are offering good effect. In advanced stages therapeutic problems, the risk of inflammatory complications may lead to secondary deterioration of quality of life. (16)

Therefore, timely diagnosis is of paramount importance. In our work a simple method to promote this aim is offered.

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The effectiveness of different methods of emergency contraception education for University students

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Abstract

Aim: This study was made between February 6th-May 4th 2012 as a single blind and controlled, non-randomized intervention study which aimed to make an evaluation of the emergency contraception educational activities given with different types of methods.

Methods: The intervention group of the study was formed by 87 students, and its control group was formed by 87 students. Personal information survey and another survey which was prepared to determine the level of knowledge about emergency contraception were used as data collection surveys. Both groups were given education about the emergecy contraception, and a brochure was given to the intervention group in addition to the emergency contraception survey and related questions were answered. The evaulation of the data was made with percentile calculations, Kruskal Wallis test, t-test, Man Whitney U test and t-test in dependant groups.

Results: A statistically significant difference was found between the means of knowledge scores of the first and last application between the intervention group and the control group ($p<0.001$). Moreover, the correlation between these mean scores was determined to be in the positive direction and in a highly-related way ($r=0.806$; $r=0.719$). In the statistical evaluation of the students in the intervention and control groups in the aspect of hearing about the concept of emergency contraception and having knowledge about the emergency contraception, a statistically significant difference was found in the mean scores and it was determined that the last application had increased the knowledge scores in a statistically-significant way ($p<0.01$).

Conclusion: In the light of these results, due to knowledge levels about emergency contraception being low, it is advised that education programs towards young people should be planned with the cooperation of the health department of the university and wide-research in the subject should be made with the usage of different education techniques.

Key words: Emergency contraception, educational intervention, knowledge.

Introduction

According to United Nations Organization, adolescent is defined as someone who is between the ages of 15-25, getting education, not working to earn his/her life and who do not have a seperate accomadation. Adolescence is an important period of life in an individual's life and physical growth and sexual development with cognitive and psychosocial change occur in this term, and the individuals find out about their gender roles. In adolescence, sexual health problems occur with the development of sexual functions and the adolescent being active sexually (Koc 2003; Koksal 2009). In a study made by WHO in 1994 to determine the health-related problems of the adolescents, the main problems of the adolescents were found as smoking, consumption of alcohol, drug addiction and the sexual health problems (Koksal 2009). In our country, in a study made by Ozcebe and Akin between the university students, it was determined that %75 of the male and %25 of the female students have active sexual lives and the mean age of losing virginity was determined as 17.2 in males and 19.9 in females. Yanikkerem determined that %51.7 of the students have sexual experience and determined the mean age of losing virginity as 17.6 (Ozcebe 1993; Yanikkerem 2003).

The behavioral tendency to be sexually active is increasing among young people and the age of losing virginity is shifting to younger ages. However, adolescents lack of knowledge about sexuality and contraception and this causes unwanted pregnancies in young ages. These pregnancies cause high mortality and morbidity risk both for the mother and the baby. Moreover, they cause spiritual and social problems (Giray 2004; Askin 2005, Koksal 2009).

Although it is not a contraceptive method, emergency contraception (E.C.) is one of the methods used in adolescents to prevent unwanted pregnancies. According to American Obstetrics and Gynaecology Organization (ACOG), E.C. is a medical intervention to prevent pregnancies after sexual activities (including rape). The day after pill or postcoital contraception are the terms used for emergency contraception; however emergency contraception (EC) is more widely used due to both terms not defining the situation completely (Aksu and Karaoz 2008; Koksal 2009; Acog 2002).

E.C. is an important opportunity to prevent unwanted pregnancies among adolescents. The risk of getting pregnant after sex in an healthy couple is %8 and E.C. decreases this risk by %75 when it is used properly. So in literature it is said that this method may decrease unwanted pregnancies and the intentional miscarriages by %60. However, the usage of EC by the adolescents is determined to fairly low according to several studies (Karaduman 2004; Tokuc 2002; Celik 2006; Grimes 2002; Koksal 2009). In the study made by Civil and Yildiz (2010), it was determined that %64.1 of the male students had sex and their mean age of losing virginity was 17.28+-1.81. According to Turkish Population and Health Survey (TNSA) 2003 data, %15.5 of the adolescents between 15-19 and %60.7 of the adolescents between 20-24 had sexual experience (Civil and Yildiz 2010). In the study made by Koksal (2009), it was determined that %46.4 of the university students in the 20-22 age group had sexual experience (Koksal 2009).

The lack of knowledge about contraception, prejudices, wrong beliefs and attitudes are the factors which effect its usage in a negative way. Health care personnel and especially nurses have an important responsibility in giving information to negate these factors. One of the main aims of nursing is to help the indiviuals, familes or groups in determining their health care needs and present them the suitable solutions to solve their problems. Nurses, in this aspect, should act as counselors towards adolescents who are under pregnancy risk and not using any kind of contraceptive method (Koksal 2009; Civil and Yildiz 2010).

The aim of this study is to make an evaluation of the emergency contraception educational activities given with different types of methods.

Materials and Methods

This study was made between February 6th-May 4th 2012 as a single blind and controlled, non-randomized intervention study which aimed to make an evaluation of the emergency contraception educational activities given with different types of methods. The sample of the study was formed by 174 students taking a course named "Health and Life" in a university on the Anatolian side of Istanbul. The intervention group of the study was formed by 87 students, and its control group was formed by 87 students. Consent was granted from the related institution before the study and information was given to the students orally and they were asked to participate in the study in this way.

As data collection surveys; a personal information survey which consisted of 12 questions and an emergency contraception knowledge survey prepared by the researcher and consisted of 10 questions were used. The questions in the knowledge survey were prepared as multiple-choice questions and each correct answer was calculated as 5 points. The minimum score in the knowledge survey is 0 (zero) and the maximum score is 50 (fifty). The interpretation was that the more the score is, the more the knowledge level is. The students were asked to put a nickname or a mark which they can remember later on the survey. The first application of the research was made between February-March 2012 and its last application was made between April-May 2012. Both groups were given education about the emergecy contraception, and a brochure was given to the intervention group in addition to the emergency contraception survey and related questions were answered.

The evaulation of the data was made with percentile calculations, Kruskal Wallis test, t-test, Man Whitney U test and t-test in dependant groups.

Findings

In Table 1, findings related to several traits of the individuals in the intervention and control groups are given. In the research, it was determined that %47.1 of the students in the intervention group were in the 21-23 age group and %50.6 of the students in the control group were in the 18-20 age group. It was found out that %51.7 of the students of the intervention group were male, and %59.8 of the students in the control group were female. Mo-

Table 1. The distribution of several individual traits of the participants

Individual Traits	Groups Intervention		Control		Analysis*
	Number	%	Number	%	
Age groups					
18-20	33	37.9	44	50.6	x^2=3.599
21-23	41	47.1	39	44.8	p= 0.463
24 and over	13	14.9	4	4.6	
Gender					
Female	42	48.3	52	59.8	x^2=0.847
Male	45	51.7	35	40.2	p= 0.242
Current Accomadation					
Dormitory	12	13.8	15	17.2	x^2=6.360
Family Home	55	63.2	54	62.1	p= 0.703
Living with Friends	11	12.6	13	14.9	
Other (with relatives etc.)	9	10.3	5	5.7	
Longest accomadation place					
Village-town-county	18	20.7	16	18.4	x^2=2.386
City	18	20.7	19	21.8	p= 0.665
Metropol	51	58.6	52	59.8	
Educational status of the mother					
Literate / elementary school graduate	32	36.8	32	36.8	x^2=4.149
High school graduate	34	39.1	33	37.9	p= 0.386
University graduate	21	24.1	22	25.3	
Educational status of the father					
Literate / elementary school graduate	20	23.0	15	17.2	x^2=1.571
High school graduate	39	44.8	37	42.5	p= 0.814
University graduate	28	32.2	35	40.2	
TOTAL	**87**	**100.0**	**87**	**100.0**	

**Chi-square analysis was made (p<0.05).*

reover, majority of the students in both groups live with their families (I:%63.2, C:%62.1). It was determined that majority of the students lived in metropol cities (I:%58.6; C:%59.8), their mothers are high school graduates (I:%39.1;C:%37.9) and their fathere are also high school graduates (I:%44.8; C:%42.5). The age groups, genders, current accomadations, longest accomadation places, and the educational status of their mothers and fathers of the intervention and control groups were found to be statistically similiar (p>0.05).

The distribution of the opinions of the participants about emergency contraception of the intervention and control groups are given in Table 2. In the research, it was determined that % 48.3 of the students have not heard of the emergency contraception, and this percentage was determined as % 54 in the control group. Moreover, in both groups it was found out that majority of the students do not have knowledge about contraception (I:%51.7; C: % 64.4), want to be informed (I:55.2; C: % 79.3) and think that each individual should have knowledge about contraception (I:% 70.1; C: % 70.1). While % 63.2 of the students in the intervention group stated that they do not think contraception is a sin, % 55.2 of the students in the control group stated they were undediced on this subject. The hearing of contraception status, being informed about contraception status, the desire to be informed status, the idea of each individual should have knowledge of contraception status and the views towards contraception being a sin or not are all similiar statistically between the two groups (p>0.05).

The distribution of the mean knowledge scores of students in the intervention and control groups is shown in Table 3. While the first application mean knowledge score of the intervention group was x=27.73+-16.14, their last application mean knowledge score was 43.44+-6.16. These were determined as x=23.27+-16.65 and x=36.14+-9.81

Table 2. The distribution of the opinions of the participants about emergency contraception

Opinions	Groups				Analysis*
	Intervention		Control		
	Number	%	Number	%	
Have you ever heard of "Emergency contraception" concept?					x^2=2.031
I have	45	51.7	40	46.0	p= 0.198
I have not	42	48.3	47	54.0	
Do you have any knowledge about "Emergency contraception"?					x^2=0.000
He/she does have	42	48.3	31	35.6	p= 0.988
He/she does not have	45	51.7	56	64.4	
Do you want to be informed about "Emergency contraception" ?					x^2=0.324
He/she wants	48	55.2	69	79.3	p= 0.606
He/she does not want	39	44.8	18	20.7	
Do you think every individual should be informed about emergency contraception?					x^2=4.845
Yes	61	70.1	61	70.1	p= 0.304
No	8	9.2	7	8.0	
Undecided	18	20.7	19	21.8	
Do you think emergency contraception is a sin?					
Yes	5	5.7	5	5.7	x^2=0.546
No	55	63.2	34	39.1	p= 0.969
Undecided	27	31.1	48	55.2	
TOTAL	**87**	**100.0**	**87**	**100.0**	

**Chi-square analysis was made (p<0.05).*

Table 3. The distribution of the mean knowledge scores of the students

Groups	Evaluation Time	n	X ± sd	Analysis*	r**
Intervention group	First application	87	27.73±16.14	t:-11.484 p:0.000	0.806
	Last application	87	43.44±6.16		
Control group	First application	87	23.27±16.65	t:-17.412 p:0.000	0.719
	Last application	87	36.14±9.81		

**For dependant samples, t-test and correlation analysis have been made (p<0.01).*

in the control group, respectively. A statistically significant difference was found between the first application mean knowledge scores and last application mean knowledge scores of the two groups (p<0.001). Furthermore, the correlation between these scores was determined to be in a positive and high-related way (r=0.806; r=0.719). Correlation values were found to be statistically significant at the p<0.001 significance level.

The distribution of the first and last application mean knowledge scores of the intervention and control group according to several variables is shown in Table 4. A statistically significant difference between the first and last application mean knowledge scores in the aspect of the hearing of contraception status and being informed about contraception status was found and it was determined that last application increased the mean knowledge scores in a statistically-significant way (p<0.01). Increase in the intervention group is more striking compared to the control group. Moreover, correlation values between the first and last application mean knowledge scores of the intervention group were found to be statistically significant. They are statistically significant at the 0.01 significance level.

Discussion

The aim of this study is to make an evaluation of the emergency contraception educational acti-

Table 4. The distribution of the first and last application mean knowledge scores of the intervention and control group according to several variables

Variables	Groups	First application			Last application		r*
		n	X±sd	Analysis	X±sd	Analysis*	
Have you ever heard of "Emergency contraception" concept?	Intervention	45 42	33.00±13.80 10.57±15.21	t: 6.870 p:0.000	45.00±7.43 40.95±9.45	t: 5.532 p:0.000	$r_{1=}$ 0.597 $r_{2=}$ 0.515
I have I have not	Control	40 47	33.25±11.18 14.78±15.87	t:6.165 p:0.000	41.87 ±4.78 31.38±6.48	t: 3.619 p:0.001	$r_{1=}$ 0.556 $r_{2=}$ 0.365
Do you have any knowledge about "Emergency contraception"?	Intervention	42 45	31.66±11.18 16.33±16.63	t:5.009 p:0.000	40.47±7.55 32.11±10.02	t:4.370 p:0.000	$r_{1=}$ 0.477 $r_{2=}$ 0.428
He/she does have He/she does not have	Control	31 56	35.64±10.22 16.42±15.57	t:6.166 p:0.000	46.93±4.01 31.51±6.31	t:4.310 p:0.000	$r_{1=}$ 0.556 $r_{2=}$ 0.423

**For dependant samples, t-test and correlation analysis have been made (p<0.01).*

vities given with different types of methods and the age groups, genders, current accomadations, longest accomadation places, and the educational status of their mothers and fathers of the intervention and control groups were found to be statistically similiar (p>0.05) (Table 1). This shows that the study groups had similiar traits. In the aspect of wanting a sample with different characteristics, this is not a desired situation.

It was determined that half of the students in the intervention and control groups have not heard of the emergency contraception concept and majority of the students in both group do not have knowledge about this concept (Table 2). In the study made by Koksal (2009), it was found out that %71.8 of the university students have not heard the emergency contraception concept and %79.3 of them have no knowledge of this concept. In the study made by Gungor et al. (2006), it was determined that %53.9 of the women participants knew nothing about emergency contraception (Gungor ve ark., 2006). According to the latest research, it is obvious that the public lack of knowledge about this subject. In the study made by Motlagh, Moradi and Nougjah (2006), it was stated that %73.2 of the women participants have not heard of EC and %78.9 of them did not get any information about EC. The studies show that this situation is more or less the same for adolescents and majority of the adolescents have no knowledge of EC.

It was determined that majority of the students wanted to be informed about emergency contraception and they think that each individual should have knowledge about this subject (Table 2). There are several studies which show similiar results in the literature. In the study made by Koksal (2009), it was determined that %69 of the university students wanted to be informed about contraception and %62.4 of them think that each individual should have information about this subject. In the study made by Uzuner et al., it was found out that the health care personnel lacked of knowledge about EC and this situtation was fixed with interactive education (Uzuner 2005). The lack of knowledge about EC may cause concerns about the methods and decrease the preference rate of the methods.

5.7% of the students included in the study stated that they think contraception is a sin (Table 2). In the study made by Koksal (2009), %5.8 of the university students stated that the usage of emergency contraception is a sin. This result is a sign that adolescents need to be informed about the effect, efficiency and the usage rules of the E.C. methods. The percentage of people with a negative opinion about emergency contraception being low is considered a sign that people in risky groups being generally undecided about this subject shows that they need more information about emergency contraception.

When the first application and last application mean knowledge scores were compared between the intervention group and the control group, it was determined that (Table 3) the last application mean knowledge scores were higher compared to the first application mean knowledge scores in both groups (p<0.05). Furthermore, the correlation between the mean score averages which were measured before and after the education in both groups show a positive kind of relationship. This situation shows that if the first application scores of the students in the intervention and control gro-

ups is higher, then their last application scores are also higher. This result is considered an important result in the aspect that it proves the consistency of the answers given to the first application and last application knowledge evaluation surveys. For a program which is applied on a study group to be efficient and effective, the distribution of correct answers is expected to be higher in the last test compared to the first test (Kutlu 2002). In this aspect, the last application scores being higher in the intervention and control groups compared to their first application scores show that the education given was effective in both groups in increasing the level of knowledge. Moreover, the difference between the first and last application scores was found to be higher in the intervention group in both of the groups. According to this finding; it was determined that the intervention education provided more positivie attitude change compared to the standard education.

The distribution of the first and last application knowledge scores according to several variables is given in Table 4. It was determined that the last application scores of the students in the intervention group who have never heard of emergency contraception or have no knowledge of the subject were higher. This finding showed that compared to the traditional teaching method, giving brochures to the students is a more effective method in increasing their level of knowledge. In the study made by Celikli (1999), it was determined that the interative education increases the level of knowledge and skills of the students. In the study made by Ugurlu (2000) which was about the determination of the level of knowledge of the high school students about AIDS, it was determined that the increase in the knowledge scores of the students who got interactive education was higher compared to the students who got traditional education. In the study made by Kellogoz Dogan (2004), the nutritional education given with brochures was found to be providing more increase in the knowledge scores of the students compared to the traditional teaching method. In the study made by Bagdatli (2011), it was stated that the additional education and applications given with the traditional patient education also helped to get more effective results. These findings show similiarity with our study findings.

Results and Suggestions

The aim of this study was to make an evaluation of the emergency contraception educational activities given with different types of methods and here are the results we have found:

- Half of the students in the intervention and control groups have not heard of the emergency contraception concept and majority of the students in both groups have no knowledge of this concept.
- Majority of the students want to be informed about emergency contraception and they think that each individual should have knowledge about this concept.
- Last application mean knowledge scores of the intervention and control groups were both remarkably higher compared to the first application mean knowledge scores in both groups.
- The increase in the last application mean knowledge scores of the students in the intervention and control groups who have not heard of the emergency contraception concept and have no knowledge about this concept was found to be higher.

In the light of these results, these are our suggestions:

- It is advised that education programs towards young people should be planned with the cooperation of the health department of the university.
- It is advised that posters, brochures etc. should be used in the university to raise awareness about this subject, the educational activities should be repeated and different educational techniques should be used.
- It is advised that sexual health education courses should be given as elective courses in other departments in the university and emergency contraception should be included in these courses.
- It is advised that wide-research should be made about this subject.

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Cardiovascular protective effects of dark chocolate

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Abstract

Numerous medicinal properties were attributed to chocolate troughout history. Some of them were confirmed by clinical studies, which associated cocoa and chocolate consumption with lower incidence of cardiovascular disease. Chocolate is a calorie rich food with high sugar and saturated fat content. Nevertheless, it may have beneficial effects on serum lipids. Cocoa flavanols, especially epicatechin, are powerful antioxidants *in vivo*, which may improve the vascular endothelial function through nitric oxide mediated vasodilatation and contribute to decreased blood pressure, increase insulin sensitivity, reduce platelet activation and lower susceptibility of LDL to oxidation. Chocolate, especially dark, is a good source of minerals, like potassium, magnesium, phosphorus, iron, copper, manganese and zinc. Therefore, dark chocolate with a cocoa content of about 70 % is a tasty, widely available source of dietary flavonoids and minerals, which should be included even into the diet of people at risk of cardiovascular diseases, provided they keep its consumption within the limits of recommended sugar and saturated fat intakes.

Key words: Chocolate, cocoa, antioxidant, cardiovascular disease

Introduction

Chocolate has long been used for medicinal purposes; by Aztecs in the pre-Columbian era to soothe the stomach and intestines, control childhood diarrhea, reduce fevers, etc. [1]. It became widely used by European physicians during the 17th and 18th centuries for rejuvenation, as a libido enhancer, to combat emaciation, increase production of breast milk, prolong longevity, delay hair growth, promote kidney stone expulsion, clean teeth, regulate sleep and prevent syphilis, among other. The claims of the positive impact of chocolate on our health remained to these days; some of them were tested and produced encouraging results. Regular consumption of dark chocolate seems to be an effective cardiovascular preventive strategy even in a population with metabolic syndrome at high risk of cardiovascular disease [2].

There are many studies linking the consumption of dark chocolate with lower incidence of cardiovascular disease. Djoussé with colleagues [3] performed a cross-sectional study on 4.970 participants; 2.258 men and 2.712 women with a mean age of 52 years (SD=13.7). Chocolate intake was assessed by a semi-quantitative food frequency questionnaire. Results were adjusted for diabetes, weight loss, race and other lifestyle factors. Consumption of more than 5 units (1 unit = 1 oz = 28 g) of chocolate per week was associated with 57 % lower prevalence of coronary heart disease (defined as myocardial infarction, coronary artery bypass surgery and percutaneous transluminal coronary angioplasty) compared to zero consumption of chocolate. Consumption of 5 units of non-chocolate candy per week was associated with 49 % increase of coronary heart disease prevalence compared to no consumption of non-chocolate candy. The results are even more astonishing as the high chocolate intake was also associated with higher body mass and calorie intake, lower HDL, lower fruit and vegetable consumption, higher consumption of non-chocolate candy, saturated fat and cholesterol. In contrast to clinical studies and meta-analysis of Ried with colleagues [4], Djousse with colleagues [3] found no association between the chocolate consumption and lower blood pressure, possibly because of lower amounts of flavanols in milk chocolate as the questionnaires did not distinguish between the dark and milk chocolates. Beneficial effects of chocolate consumption on lower prevalence of coronary heart disease were attributed to flavanols, like epicatechin, and minerals such as potassium and magnesium [3].

Larsson and colleagues confirmed the association between the increased risk of stroke and chocolate consumption on 37103 Swedish men

[5]. During 10.2 years of follow-up they recorded 1.995 stroke cases. The relative risk of stroke for the quartile with the highest consumption of chocolate – median 62.9 g/week, was 0.83 (95 % CI: 0.70–0.99) (17 % lower) compared to the quartile with the lowest chocolate consumption (median 0 g/week). The study did not differentiate between the milk and dark chocolates, but the authors presumed most of the chocolate consumed was the milk one, as this accounts for 90 % of the chocolate consumed in Sweden. The results of meta-analysis of 5 studies, among them the one performed by the authors of this meta-analysis [5], are that the relative risk for stroke decreased 19% between the groups of people with the highest and lowest chocolate consumption. It was 0.81 (95 % CI: 0.73–0.90) in the group of highest chocolate consumption. The authors concluded that moderate consumption of chocolate was associated with a lower risk of stroke in both men and women [5].

A systematic review of 42 acute, short term and chronic randomized controlled trials of chocolate, cocoa, and their components flavan-3-ols reported consistent acute and chronic effects on flow-mediated dilatation (FMD) and insulin resistance. FMD was improved by average of 1.62 % (95 % CI: 1.33%; 1.92) two hours after the ingestion of cocoa or chocolate containing more than 50-100 mg of epicatechin and by 1.60 % (95 % CI: 0.95; 2.24) when the same amount was given chronically. Blood pressure reduction, after chronic consumption of cocoa/chocolate containing more than 50-100 mg of epicatechin per dose, was observed; the systolic blood pressure was lower for 4,48 mm Hg (95 % CI: -6.32; -2.63) and diastolic for 4.25 mm Hg (95 % CI: -5.66; -2.85). There were small changes in the contents of blood lipids upon the long term consumption of cocoa/chocolate containing more than 50-100 mg of epicatechin per dose: the reduction of LDL by 0.02 mmol/L (95 % CI: -0.08; 0.04), the increase of HDL by 0.02 mmol/L (95 % CI: 0.00; 0.04) and the reduction of triglycerides by 1.13 mmol/L (95 % CI: -0.23; -0.02) were observed. The improvement of FMD was evident with lower doses of epicatechin, while the effects on blood pressure were proportional with increased amounts of epicatechin [6]. The amount of epicatechin of less than 50-100 mg/day corresponded to epicatechin content in about 100 g of 75-85 % cocoa dark chocolate (Table 1) [7]. Epicatechin was described as the key contributor to the observed effects, while other potentially beneficial compounds were potassium, stearic acid and metylxantines [6].

To evaluate the effects of cocoa/chocolate consumption on the blood pressure, Ried with colleagues [4] evaluated 15 clinical studies of which 13 met the inclusion criteria. Subjects were given cocoa products containing 30 – 1000 mg cocoa flavanols per day, control groups were given white chocolate or cocoa-free products; alternatively low-flavanol products were used as controls in some trials. All trials revealed significant reductions of blood pressure; the systolic blood pressure was reduced by 3.16 mm Hg (95 % CI: -5.08; -1.23) and diastolic by 2,0 mm Hg (95 % CI: 3.35; -0.69). The authors also performed subgroup meta-analyses. Blood pressure reduction in pre-hypertension and hypertension groups increased. The blood pressure reduction was more pronounced in pre-hypertension and hypertension groups. Systolic blood pressure was reduced by 5.0 mm Hg (95 % CI: -7.99; -2.05) and diastolic by 2.7 mm Hg (95 % CI: -4.89; -0.58). There was no statistically significant reduction of systolic or diastolic blood pressure in normotensive group (1.56 (95 % CI: -3.81; 0.68) and -1.3 mm Hg (95 % CI: -2.88; 0.33), respectively). The authors concluded that flavanol-rich (dark) chocolate was effective in reducing blood pressure in prehypertensive and hypertensive subjects but it did not significantly reduce the blood pressure in normotensive subjects. The positive effects were attributed to flavanols and oligomers - procyanidins [4].

Tokede and colleagues performed a systematic review of 10 clinical trials from 2-12 weeks that included 320 participants [8]. The tested groups were given flavanol rich chocolate/cocoa drinks (88 to 963 mg flavanols/day), while mostly white chocolate was used in control groups. LDL was significantly lower in the flavanol group; -5.90 mg/dl (95 % CI: -10.47; -1.32 mg/dl); no statistically significant difference in the amounts of HDL -0.76 mg/dl (95 % CI: -3.02; 1.51 mg/dl) and triglycerides -5.06 mg/dl (95 % CI: -13.45; 3.32 mg/dl) were observed. The changes in lipid profiles were more evident in short-term studies, however were small. Only the reductions of LDL were

statistically significant, but it is evident that dark chocolate had no major effects on serum HDL and triglycerides [8]. Cocoa flavanols may have prevented the oxidation of LDL [9] and consequently decreased the LDL uptake by foam cells thus the formation of fatty streak [10]. Fourty grams of cocoa powder in milk was given to trial participants with a high risk of cardiovascular disease (total polyphenols 495.2 mg and 46,08 mg epicatechin). The group that received the cocoa powder had lower levels of oxidized LDL (-12.3 U/L (99 % CI: 19.3; 5.2); LDL was decreased by 14 % compared to the group that received only milk [9].

Clearly, cocoa and chocolate have measurable, beneficial effects on the human body. The compounds that may contribute to these effects are cocoa flavanols (flavan-3-ols, dimers and trimers), minerals (potassium and magnesium) and stearic acid. In the rest of the article, we shall describe the known cardioprotective compounds of cocoa/chocolate and provide the scientific background on their function in the body.

Composition of dark chocolate

Chocolate is a processed form of cocoa. Its chemical composition differs from that of raw cocoa as a result of fermentation, roasting, conching, tempering and alkalization [11]. As chocolate is the most popular and widespread cocoa product, we will hereafter focus primarily on the composition of dark chocolate, which we define as a chocolate that contains at least 70 % cocoa solids. Its main ingredients are: cocoa mass, sugar, vanilla and lecitin (emulgator) [12].

Fats

Cocoa butter is the fatty part of cocoa bean. Its special composition and structure contributes to chocolate's many desired properties: melting in the mouth, friability and shine [15]. The average dark chocolate (75-85 % cocoa solids) contains 33 % of oleic, 25 % palmitic, and 33 % of stearic fatty acids [13].

Consumption of foods rich in saturated fat used to be associated with elevated serum LDL and thought of as an important risk factor for the development of cardiovascular disease [16,17]. Many data imply that there is not enough evidence to link the ingested saturated fat with the increased risk of cardiovascular disease [18]; however, it still stands that the replacement of saturated fats with polyunsaturated fats, especially with linolenic acid decreases the cardiovascular disease mortality [19]. Stearic acid, a dominant fatty acid of cocoa seems to lower LDL and increase HDL when it replaces other saturated or *trans* fatty acids, yet unsaturated fatty acids are still more beneficial to health [16,20]. Nevertheless, dark chocolate as a whole seems to have a beneficial effect on blood lipids, because its consumption is associated with a lowered LDL [21,8,22,23,6] reduced triglycerides [22,23] and, in some studies, slightly increased HDL [9,22,23,6]. The positive effect on triglicerides and HDL may be of minor importance [8].

Phenolic compounds

Astringent and bitter taste of cocoa is attributed to its polyphenol content [24,11], mainly flavanols; flavan-3-ols and their oligomers and polymers procyanidins. Flavan-3-ols (also catechins) found in cocoa are epicatechin and catechin [25,26,27,28]. Epicatechin and catechin are isomers with a similar molecular formula ($C_{15}H_{14}O_6$); with *cis* then *trans* configuration. Each of them has two stereoisomers: (+)-epicatechin, (−)-epicatechin, (+)-catechin and (−)-catechin. They are also present as derivatives (gallocatechins) [28]. Procyanidins (also called proanthocyanidins or condensed tannins) are oligomeric and polymeric molecules of flavan-3-ols (($C_{15}H_{14}O_6$) n) [25,26,27,28].

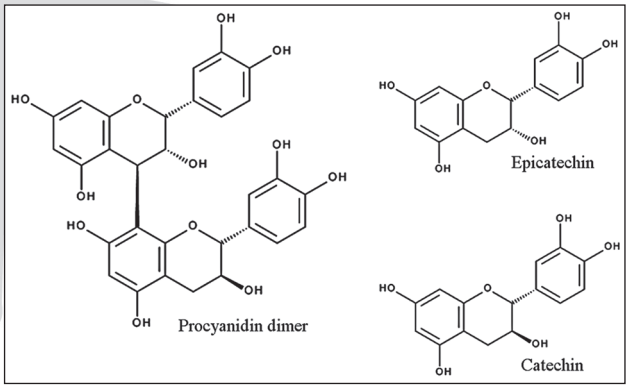

Figure 1. Procyanidins are polymers of two or more units of catechin and/or epicatechin

Flavan-3-ols, dimers and trimmers of procyanidins can be effectively absorbed into the bloodstream [29,30]. In contrast, larger oligomers and polymers cannot be absorbed, therefore their effects

Table 1. Nutritional values of the average dark chocolate (70-85 % cocoa solids). [13,7,14]

Nutrient [13]	**per 100 g**		**DRI values for comparison (♀ age 19-50).** [14].
Energy (kJ/kcal)	2504/598		
Water (g)	1.37		
Protein (g)	7.79		
Carbohydrate (g)	45.90		
Sugars (g)	23.99		
Dietary fiber (g)	10.9		
Fats (g)	42.63		
Saturated fatty acids (g)	24.49		
Stearic acid. 18: 0 (g)	13.63		
Palmitic acid. 16: 0 (g)	10.08		
Monounsaturated fatty acids (g)	12.78		
Oleic fatty acid. 18: 1 (g)	12.65		
Polyunsaturated fatty acids (g)	1.257		
Linoleic acid 18: 2 (g)	1.217		
Cholesterol (mg)	3		
Ash (g)	2.32		
Calcium (mg)	73		1000
Iron (mg)	11.90		18
Magnesium (mg)	228		310
Phosphorus (mg)	308		700
Potassium (mg)	715		4700
Sodium (mg)	20		1500
Zinc (mg)	3.31		8
Copper (µg)	1770		900
Manganese (mg)	1.95		1.8
Selenium (µg)	6.8		55
Vitamin A (µg)	11.7		700
Vitamin E (mg α-tocopherol eq.)	1.523		15
Vitamin K (fitomenadione) (µg)	7.3		90
Vitamin B1 (thiamine) (mg)	0.034		1.1
Vitamin B2 (riboflavin) (mg)	0.078		1.1
Vitamin B6 (pyridoxine) (mg)	0.038		1.3
Vitamin B12 (cyanocobalamin) (µg)	0.28		2.4
Niacin (mg)	1.054		14
Pantothenic acid (mg)	0.418		5
Caffeine (mg)	80		
Theobromine (mg)	802		
Polyphenols (mg) [7]*	1296	±510.14	mean ± SD
Monomers (mg)	108,6	±31.61	mean ± SD
Catechin (mg)	24.2	±12.76	mean ± SD
Epicatechin (mg)	84.4	±30.29	mean ± SD
Procyanidin (mg)	1293.8	±510.14	mean ± SD
2 – 3-mers (mg)	235.4	±88.18	mean ± SD
4 – 6-mers(mg)	287.6	±123.43	mean ± SD
7 – 10-mers (mg)	181	±85.02	mean ± SD
Polymers (mg)	476.2	±198.02	mean ± SD
ORAC (oxygen radical absorbance capacity)	227	±74	mean ± SD

**Calculated average based on 5 samples of commercially available dark chocolate, analyzed by Gu and colleagues [7].*
SD=standard deviation

are limited to the intestinal lumen [30]. Food matrix (sugar, milk) can also have an impact on bioavailability of flavanols. Absorption of procyanidin dimers and trimmers is lower, when carbohydrates are simultaneously present in the gut, but there is no significant effect on epicatechin and catechin [29]. Some early studies pointed to a strong inhibition of bioavailability and antioxidant activity of flavanols in plasma by milk [31], but newer research shows that milk does not have a notable effect on antioxidant activity in plasma [32,33].

The mere content of cocoa solids does not correlate with the levels of flavan-3-ols and procyanidins, as the percentage of cocoa solids of the cocoa products derives from cocoa butter, which does not contain polyphenols [26]. Polyphenol content of dark chocolate on the other hand correlates well with non-fat cocoa solids (NFCS) in plain dark chocolate. A high correlation coefficient between the cocoa polyphenols content and NFCS was reported by most studies: $r^2 = 0.73$ [26], $r^2 = 0.98$ [34], with the exception of the one by Langer and colleagues [25], $r^2 = 0.49$. NFCS can be calculated from the theobromine content [26].

In some cases the polyphenol content does not coincide with NFCS, due to the impact of manufacturing processes. Fermentation, roasting [34] and, most drastically, cocoa alkalization (dutching) influence the amount of preserved polyphenols (Figure 2) [35].

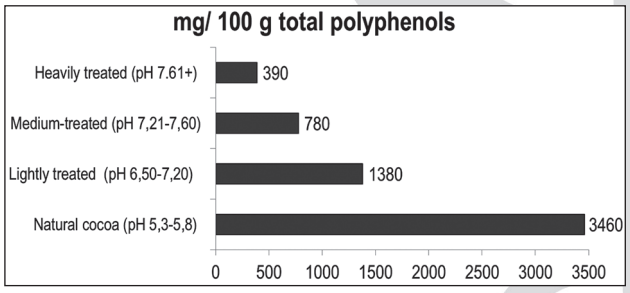

Figure 2. Average impact of cocoa alkalization on polyphenol content in cocoa [35]

Dark chocolate contains 77-158 mg of flavan-3-ols (catechins and epicatechins) and 500-1980 mg of procyanidins per 100 g. Procyanidin content is strongly correlated with ORAC, suggesting that procyanidins contribute the largest share to the antioxidant activity *in vitro* [7]. The total antioxidant capacity of cocoa (measured as ORAC) is strongly correlated with non-fat cocoa solids (NFCS), because polyphenols are only present in non-fat portion of cacao [7,34].

Flavan-3-ols and procyanidins

Phytonutrients, such as cocoa flavanols are not essential nutrients such as vitamins and minerals, but have an important role in maintaining body functions and health, especially in the adult and later phases of life. They could be described as "lifespan essentials" [36].

Flavanols in cocoa reduce the incidence and prevalence of cardiovascular disease [3, 37, 38, 39, 40]. This cardio-protective effect of chocolate may come from the ability of flavanols to inhibit free-radical mediated events – to act as *in vivo* antioxidants. Flavan nucleus and its multiple hydroxyl groups of flavanols act as chelators and free radical scavengers [41]. The result of this may be beneficial effects of flavan-3-ols and procyanidins, like improvement of endothelial function through the nitric oxide mediated vasodilatation [44,42,40,43], decreased susceptibility of LDL to oxidation [22,23,45], inhibition of platelet activation [39,46,47] and increased insulin sensitivity [48,49,6].

Cardiovascular risk factors such as smoking, aging, hypertension, hypercholesterolemia and hyperglycemia are associated with diminished endothelium-dependent vasodilatation [50]; the severity of endothelial dysfunction is linked to the risk for cardiovascular events [51]. Cocoa flavanols may improve endothelial function, which results in lower blood pressure [44,42] and increased insulin sensitivity [48,49].

Flavan-3-ols and procyanidins increase the bioavailability of nitric oxide (NO), a signaling molecule, which causes relaxation of smooth muscles of vascular endothelium. Sufficient availability of NO is associated with smooth muscle relaxation in normal endothelium; this vasodilatation maintains the blood pressure at normal level. Abnormal blood pressure and hypertension is also a result of decreased NO homeostasis. Epicatechin and oligomeric procyanidins can increase bioavailability of NO through several mechanisms e.g. its increased synthesis by activation of NO-synthase [42], by inhibition of NADPH-oxidase, which decreases NO oxidation [52,49]. Epicatechin seems to be the main contributor to most of the effects on vascular function [50].

Improvement of insulin sensitivity can contribute to increased bioavailability of NO [53,48], which increases insulin-stimulated glucose uptake and vascular tone [48]. Insulin has an important role in hemodynamic regulation in addition to its well-known metabolic roles. Insulin stimulates the production of NO in vascular endothelium. NO diffuses into vascular smooth muscle where it induces vasodilatation, which leads to increased blood flow and vascular recruitment in skeletal muscle. The glucose uptake rate is linearly increased with increased blood flow [54], the increase of blood flow in a blood vessel can be measured as flow mediated dilatation (FMD) [55]. Cocoa flavanols cause vasodilation through increased NO bioavailability, increasing blood flow to skeletal muscle, insulin sensitivity and glucose uptake, similar to exercise [49,48].

The second potential mechanism of cocoa action is the reduction of LDL sensitivity to oxidation [44]. Macrophage uptake of oxidized LDL is faster than uptake of un-oxidized LDL. Oxidized LDL binds with 40-times greater affinity to scavenger receptors. LDL deposition inside macrophages leads to development of foam cells and formation of fatty streak – the beginning of atherosclerosis [10]. Sufficient intake of cocoa flavanols may attenuate against LDL oxidation [56,22,23], as flavanols present in blood stream may act as antioxidants on the surface of LDL particles, on the border between the lipid and aqueous phase, and thus increase resistance to oxidation by free radicals [45].

The third mechanism of cocoa action may be partial inhibition of platelet function caused by flavanols [39,46]. Level of platelet activation function is important in the first stages of atherosclerosis development and in the occurrence of acute thrombosis [57,58].

When vascular wall damage occurs, the initial response of platelets is adhesion to exposed sub-endothelium. Collagen binds to platelet surface receptors α2β1 and GPVI. Because of high shear rates due to blood flow, the von Willebrand factor binds to receptor GPIb-IX-V. After the subsequent platelet adhesion, the blood vessel surface significantly increases, due to its changed shape, to an irregular sphere with multiple filopodia. After that, a series of processes occur which result in platelet aggregation. Activated platelets release aggregation factors such as ADP and TxA2 (thromboxane) and factor IIa (thrombin). Thrombin promotes aggregation and triggers exocytosis of ADP and thromboxane, which are also promoters of aggregation. Adrenaline also stimulates aggregation, but only in the presence of other agonists. The aggregation inhibitors are prostacyclin and nitric oxide [59].

Flavanols may affect many factors of homeostasis, particularly the endothelium factors - nitric oxide (NO), prostacyclin and fibrinolytic factors such as tissue plasminogen activator (tPA) [47]. The acute intake of cocoa flavanols (900 mg), inhibits clotting time by inhibiting collagen-epinephrine induced clotting for 3-11% and collagen-ADP for about 11%. In chronic (100 mg flavanols/day) intake, there was 3-11 % inhibition of collagen-ADP induced clotting; there was no inhibition of collagen-epinephrine induced clotting time [39]. Pearson with colleagues [46] observes that the anti-clotting effect of 897 mg flavanols (18.75 g of cocoa powder) was reported as only slightly less effective than 81 mg of aspirin. Aspirin and flavanols may also have additive effects on blood clotting.

Minerals

Dark chocolate is a good source of minerals (Table 1), but not a particularly good source of vitamins [13]. At least a part of protective effects of chocolate on cardiovascular disease can be contributed to its high mineral content. Chocolate is rich in magnesium, potassium [3,60,61], selenium, copper [61], iron [62,63] and other minerals.

Sufficient magnesium and potassium intake is associated with lower blood pressure [64, 65, 66, 67, 68] magnesium deficiency is associated with development of primary hypertension [64]. Magnesium supplementation in deficient persons improves the plasma levels of magnesium and may decrease the blood pressure [64, 65, 66]. Magnesium intake of 500-1000 mg/day can reduce the blood pressure; by 5.6 mm Hg (systolic) and 2.8 mm Hg (diastolic) [65]. Magnesium affects the calcium ion concentration and its availability, acting as calcium channel blocker. This results in production of nitric oxide and vasodilators prostacyclins. The result is improved endothelial function and consequently lower blood pressure [65,69]. Magnesium supplementation also increases insulin sensitivity [66], but only in magnesium deficient persons [70].

Potassium deficit has a role in development of hypertension, especially when sodium intake is high [67], as high sodium intake is associated with higher potassium excretion [71]. Blood pressure reduction as a result of potassium supplementation in hypertensive subjects is related to salt intake. When dietary sodium intake is low, the effect of potassium supplementation is low as well; the effect of potassium supplementation is higher when sodium intake is high [68].

Chocolate is relatively rich in iron, but it is also rich in flavanols (Table 1), which can inhibit iron absorption in a dose-dependent manner [72,73]. This can occur by forming the complexes that cannot be transported thought the basolateral membrane and by chelating iron and thus limiting the access to the apical surface of the enterocytes [72]. Ascorbic acid attenuates many inhibitory effects [74]. Iron from chocolate is still moderately available, despite its poor absorption because of flavanols. Its bioavailability is lower than that of ferrous sulfate and it is comparable with that of iron citrate and the iron from wheat flour. Relatively good iron availability may be the consequence of fermentation, grinding and roasting of cocoa [62]. Cocoa products, therefore, could be a source of some bioavailable iron (Table 1).

Chocolate and cocoa are rich sources of copper (Table 1). Copper is needed, for example, in copper/zinc superoxide dismutase, which is important in oxidative damage defense. Copper deficiencies are rare [75]; they occur in people that do not meet the DRI values or their needs exceed DRI values [63].

Methylxantines

Chocolate contains methylxantines; caffeine and theobromine [13]. The primary mechanism of caffeine action is competitive binding to adenosine receptors; adenosine and caffeine molecules are similar enough for caffeine to bind, but caffeine does not stimulate but only occupies adenosine receptor. The main effect of adenosine is initiation of sleep (reducing the level of consciousness); therefore, the effect of caffeine is the increase of brain activity [76].

The effects of theobromine on humans, compared to caffeine, are weak. Theobromine binds to adenosine receptors, but with approximately a 10-fold lower affinity. The effect of caffeine is undoubtedly prevalent in chocolate, despite the 10-fold higher concentrations of theobromine [77]. Methylxanthines do not seem to cause chocolate cravings. Cravings are often associated with addiction. Michener and Rozin have demonstrated that the desire for chocolate is associated with taste and other rheological properties of chocolate and not with the pharmacological activity of methylxanthines [78].

Biogenic amines

Biogenic amines are vasoactive substances that can cause symptoms ranging from redness, headache, changes in blood pressure to cardiovascular shock in susceptible persons. These effects are typically caused by inhibition of enzymes that degrade biogenic amines. The inhibitors of those enzymes are present in some medications (monoamine and D-amino acid oxidases inhibitors; MAO and DAO inhibitors) [24].

Biogenic amines occur as products of microbial decarboxylation of free amino acids during the cocoa fermentation [24]. Significant biogenic amines in cocoa are 2-phenylethylamine, tryptamine, tyramine, histamine, serotonin, and dopamine [24,79]. Their effect is significant in people using MAO and DAO inhibitors.

Conclusion

Numerous studies are reporting beneficial health effects of dark chocolate. The main contributors to these effects are monomeric and oligomeric flavanols; of those, the most important seems to be epicatechin. Other compounds that could be responsible for some beneficial effects are minerals such as potassium, magnesium and copper. Dark chocolate is also a good source of iron, zinc, phosphorus and manganese.

Chocolate and cocoa can improve insulin sensitivity, reduce platelet activation, reduce blood pressure, reduce plasma LDL and reduce LDL oxidation. Consequently, through these mechanisms chocolate/cocoa intake can substantially lower the incidence of cardiovascular diseases. It seems beneficial to consume the dark chocolate with cocoa content of at least 70% and within the recommended limits for sugar and saturated fat intake. The chocolate should be produced from less processed, non-alkalized cocoa, although even milk chocolate with low cocoa content seems to retain some effectiveness in prevention of cardiovascular disease.

Table 2. Overview of significant active components and their effects

Ingredient	Site of action	Effect	Reference
Flavan-3-ols and procyanidins	Vascular endothelium – improved endothelial function	Improved insulin sensibility	[48,49,6]
		Decreased blood clotting	[39,46,47]
		Decreased blood pressure	[44,42,4,6]
	Plasma lipoproteins	Lowered LDL	[44,42,48,49]
		Decreased suceptibility of LDL to oxidation	[9,22,23,45]
Magnesium	Vascular endothelium – improved endothelial function	Decreased blood pressure	[65]
		Improved insulin sensibility (ony in Mg deficient)	[66,70]
Potassium	Potassium supplies	Decreased blood pressure, but only if sodium intake is high.	[67,68]

Abbreviations

ADP - Adenosine diphosphate
DRI - daily recommended intake
ET-1 - endothelin
factor IIa - thrombin
FMD flow-mediated dilatation
GPVI - glycoprotein VI
HDL - High-density lipoprotein
LDL - Low-density lipoprotein
NADPH - nicotinamide adenine dinucleotide phosphate
NFCS - non-fat cocoa solids
NO - nitric oxide
ORAC - oxygen radical absorbance capacity
r2 - correlation coefficient
SD - standard deviation
tPA - tissue plasminogen activator
TxA2 - thromboxane

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Assessment of self-efficacy levels of health school students

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Abstract

Introduction: Self-efficacy is a cognitive perception factor that affects the behaviour of an individual. It is important to understand how the student's perceived self-efficacy affects the student's behaviour. Knowledge of the self-efficacy levels of the students will give educators a better understanding of the students and give the students a better understanding of themselves.

Aim: The aim of this cross-sectional study was to determine levels of self-efficacy of health schools students and factors that affect their self-efficacy.

Methods: The study population consisted of 513 students who studied at three health schools. Data were collected with the self-efficacy scale. The data analysis included the Kruskal–Wallis test, the student's *t*-test and Spearman's correlation analysis.

Results: There were statistically significant differences between the average self-efficacy scores and particular students' characteristics (health status, involvement in sporting activities, family relationship, career planning, high regard for the profession, etc.) ($p<0.05$). The age of the student's father, the mother's education and the number of siblings independently affected the students' perceived self-efficacy.

Conclusions: If students are encouraged to participate in cultural, social and sport activities during their training and if those who have health problems are encouraged to avail of health services, we believe that the self-efficacy levels of students will improve.

Key words: Health school students, Midwifery, Nurse, Self-efficacy.

Introduction

Self-efficacy is a cognitive perception factor that affects the behaviour of an individual. Self-efficacy is defined as the individual's perception/judgment of successfully realising a certain act or controlling events or the person's judgment relating to achieving a certain performance level[1,2]. Self-efficacy determines how an individual feels, thinks and behaves, and it can prevent or increase the motivation to act. It allows the individual to assess his/her capabilities and capacity in a more objective manner. Self-efficacy can influence a variety of areas, such as success at school, mental and physical health, career choices and socio-political changes[1]. As self-efficacy determines thinking and behaviour, if an individual believes that he/she can achieve an outcome, he/she acts more actively and can control his/her life[1-3]. Studies have shown that a strong sense of self-efficacy is correlated with better health, higher success levels and improved social integration[1,3]. Research has also demonstrated that individuals with a lower sense of self-efficacy have lower self-confidence levels and pessimistic thoughts about their individual achievement and development[2]. They may also experience depression, anxiety and helplessness. The degree of self-efficacy can prevent or increase the motivation to act. An individual with a high level of self-efficacy prefers more complicated and risky tasks, has higher goals and puts great effort into reaching those goals[2,4].

Previous studies reported that factors such as family structure, education, health status, culture, place of residence and social support affect the development of the perceived self-efficacy of an individual[1,2,4]. Some studies revealed that there is correlation between students' self-efficacy levels and their age and socio-economic levels[5,6], gender[6,7] and parents' educational levels[8]. Two Turkish studies reported a positive correlation between successful learning outcomes, students' regard for the profession[1,7] and their perceived self-efficacy[9]. Another

study reported that the individual's self-efficacy level affected that person's career development[10].

Today, developments in the healthcare system mean that nurses and midwives have to continuously develop themselves both personally and professionally; they also have to develop creative solutions[11,12]. In this context, undergraduate nursing and midwifery education aims to furnish students with knowledge and skills in cognitive, affective and psychomotor areas. The students should be able to utilise the knowledge and skills that they learn at school in their individual and professional lives and develop positive behaviours. It is important to understand how the student's perceived self-efficacy (a cognitive factor) affects the student's behaviour. Knowledge of the self-efficacy levels of the students will give educators a better understanding of the students and give the students a better understanding of themselves. According to the literature, some training strategies (such as simulation training) increase their level of self-efficacy[13-15.] Self-efficacy perception levels can also be increased by developing strategies that facilitate student learning. The aim of this study was to determine the levels of self-efficacy of health schools students and to identify factors that affect their self-efficacy.

Focusing on health school students, the following questions were investigated: Is there a statistically significant difference between students' self-efficacy levels and their personal characteristics (age, gender, etc.)? Is there a statistically significant difference between their self-efficacy levels and family-related characteristics (parents' education, parents' employment status)? Is there a statistically significant relationship between the student's self-efficacy level and the student's age and the ages of the parents?

Methods

Setting and Sampling

This cross-sectional study was conducted between April 1, 2009, and May 30, 2009, (second academic semester) at three health schools in Turkey. The study population (n=996) consisted of students at three health schools in three different universities: Edirne (n=467), Kirklareli (n=319) and Tekirdağ City (n =210). Five hundred and thirteen students agreed to participate in the study: Edirne (n=319), Tekirdağ (n=80) and Kırklareli (n=114).

Data Collection

Data on the students' personal and professional background were collected using a personal information form[2,3,4,6,7,14,16]. The Turkish version of the self-efficay scale was administered

Ethical Considerations

The Ethics Committee of Trakya Universty Medical Faculty Hospital approved this study. Additionally, permission was received from the each school. One of the researchers explained that participation was voluntary. All the participants were informed of the purpose of the study and were assured of confidentiality and anonymity. The students were asked not to indicate their name on the forms. Consent was assumed if the student completed and returned the questionnaire.

Self-efficacy Scale

To determine the perceived self-efficacy of the students, the self-efficacy scale developed by Sherer et al. (1982)[17] and Sherer and Adams (1983)[18] and validated by Gözüm and Aksayan (1999)[4] was administered. It is a 5-point Likert-type scale consisting of three items and four subscales (initiating, maintaining, completing the behaviour and struggling with obstructions). The score range is 23–115. A higher score reflects a higher degree of perceived self-efficacy. The Cronbach alpha coefficient of the scale was 0.81 in the validity and reliability Turkish study, and it was also 0.81 in this study.

Personal Information Form

The personal information form consisted of questions relating to the student's personal background (age, gender, grade, department, family structure, income level, employment status, place of residence, health status) and their professional characteristics (high regard for the profession, subscription to a professional journal, career planning, participation in socio-cultural activities, etc.).

Statistical Analysis

The self-efficacy scale scores were compared with the Mann–Whitney U test to identify significance differences between the students (gender, department, plans to go on to study for a graduate degree, subscription to a professional journal). The Kruskal–Wallis test was used to compare the self-

efficacy scores among the students (family income level, family relations, relations with friends, health status and participation in sports activities). Multiple linear regression analysis was used to identify independent variables associated with the students' self-efficacy scores. The linear regression model used the score on the self-efficacy scale as the dependent variable and the student's age, the education and ages of the student's parents and the number of siblings as the independent variables. The statistical significance limit was accepted as $p<0.05$. The Statistica 7.0 statistical program (Statistica, Tulsa, OK, USA) was used for statistical analysis.

Results

The average age of the participating students was 21.3±1.7 years. Among the participants, 89.9% were female, 73.3% were nursing students, and 26.7% were midwifery students. Of the students, 39.8% had one sibling, and 55% resided in a dormitory. One hundred and forty (27.3%) of the students were in their first year, 133 (25.9%) were in their second year, 123 (24.0%) were in their third year, and 117 (22.8%) were in their fourth year. The average rank of preference was 9.2±6.9 for the students' departments (Table 1).

Table 1. Personal characteristics of the students (n=513)

Variables	*n* (%) or mean ± SD[§]
Age (years)	21.3±1.7
Gender Female	461 (89.9)
Grade First year	140 (27.3)
Department	
Nurse	376 (73.3)
Midwife	137 (26.7)
Number of siblings	
One sibling	204 (39.8)
Two siblings	140 (27.3)
Employment status	
Not employed	476 (92.8)
Rank of preference Current residence	9.2±6.9
Dormitory	282 (55.0)
Shared house with friends	161 (31.4)

[§]*Mean ± SD: Mean ± Standard deviation*

The average age of the students' mothers was 46.1±5.2 years, and the average age of their fathers was 49.9±5.7 years. The majority of the students' mothers (81.5%) and fathers (61.8%) were primary and secondary school graduates. The majority of the mothers (84.6%) were housewives, and 37.6% of the fathers were retired (Table 2).

Table 2. Family background of the students (n=513)

Variables	*n* (%) or mean±SD[§]
Mother's age (years)	46.1±5.2
Father's age (years)	49.9±5.7
Mother's education	
Primary/secondary	418 (81.5)
High school and higher	95 (18.5)
Father's Education	
Primary/secondary	317 (61.8)
High school and higher	196 (38.2)
Mother's employment status	
Not Employed	434 (84.6)
Employed	70 (15.4)
Father's employment status	
Retired	193 (37.6)
Working	107 (20.9)

[§]*Mean ± SD: Mean ± Standard deviation*

The majority of the students (92.2%) had no chronic disease, 67.1% had good health, and the majority did not smoke (81.7%) or use alcohol (71.3%). Of the students, 41.9% said that they went to the cinema once a month. Nearly half (43.7%) did not go to the theatre. A total of 40.4% said that they participated in sporting activities whenever possible (Table 3 and 4).

For the majority of the students (80.7%), the family income level was average. Most of the students (79.7%) liked their department, 76% of them wanted to go on to study a graduate degree, and 38.2% subscribed to a professional journal. The majority of the students stated that they got on well with their families (87.9%) and friends (83.8%) (Table 4).

The average score for self-efficacy was 85.5±12.1. There was no statistically significant difference between the self-efficacy scores of the female and the male students ($p>0.05$) (Table 4).

The average self-efficacy scores of the students who liked their department ($p=0.001$), who wanted to go on to study a graduate degree ($p<0.001$) and

Table 3. Health status and socio-cultural characteristics of the students (n=513).

Variables	*n* (%) or Mean ± SD[§]
Presence of chronic disease	
Yes	40 (7.8)
No	473 (92.2)
Smoking	
Yes	94 (18.3)
No	399 (81.7)
Alcohol consumption	
No	366 (71.3)
Sometimes	133 (25.9)
Frequently	14 (2.7)
Going to the cinema	
Once a month	215 (41.9)
More than once a month	146 (28.5)
Going to the theatre	
No	224 (43.7)
Few times a year	158 (30.8)

[§]*Mean ± SD: Mean ± Standard deviation*

Table 4. Comparison of the students' characteristics and their average self-efficacy scores (n=513).

Variables	*n* (%)	Mean ± SD[§]		*p*
Gender			Z_{MWU}‡	0.071
Female	461 (89.9)	85.9±11.8		
Male	52 (10.1)	82.1±13.9	-1.804	
Liking of department			Z_{MWU}	0.001
Yes	409 (79.7)	86.5±11.7		
No	104 (20.3)	81.9±13.1	-3.254	
Plans to go on to study a graduate degree			Z_{MWU}	<0.001
Yes	390 (76.0)	86.7±11.6		
No	123 (24.0)	81.8±13.0	-3.631	
Subscription to a professional journal			Z_{MWU}	0.019
Yes	196 (38.2)	87.2±11.8		
No	317 (61.8)	84.5±12.2	-2.355	
Family's income level[#]			KW†	0.103
Good	46 (9.0)	86.5±12.8		
Average	414 (80.7)	85.9±11.7	4.552	
Low	53 (10.3	81.7±14.2		
Family relations[#]			KW	0.006
Good	451 (87.9)	86.2±11.7		
Moderate	56 (10.9)	82.1±11.7	10.131	
Poor	6 (1.2)	68.8±24.3		
Relations with friends[#]			KW	0.005
Good	430 (83.8)	86.3±11.7		
Moderate	78 (15.2)	82.3±11.9	10.494	
Poor	5 (1.0)	67.60±24.8		
Health status[#]			KW	0.003
Good	344 (67.1)	86.2±11.7		
Medium	160 (31.2)	85.1±12.0	11.925	
Poor	9 (1.8)	68.4±18.1		
Participation in sports activities[#]			KW	<0.001
No participation	170 (33.1)	82.4±12.4		
Only watching	104 (20.3)	85.1±12.4		
Regular participation	32 (6.2)	89.3±1.3	20.869	
Participation whenever possible	207 (40.4)	87.7±11.3		

[§]*Mean ± SD: Mean ± Standard deviation, †KW: Kruskal–Wallis variance analysis, ‡$Z_{MWU:}$ Mann–Whitney U test.*

Table 5. Multiple linear regression model on self-efficacy scale in students

	Enter method		
	Type	**Coefficients**	***p***
Age	Continuous	-0.063	0.273
Grade	Ordered	0.092	0.106
Mother's age (years)	Continuous	0.058	0.428
Father's age (years)	Continuous	-0.191	0.009
Mother's education	Ordered	0.134	0.005
Father's education	Ordered	-0.025	0.579
Number of siblings	Ordered	0.125	0.008

who subscribed to a professional journal (p=0.019) were statistically significantly higher than those who did not express these sentiments (Table 4).

The student's family income did not affect the self-efficacy score (p>0.05). The average self-efficacy scores of the students who said they got on well with their family (p<0.006) and friends (p<0.005) were significantly statistically higher than those who said they had moderate or poor relations with their family and friends (Table 4). The average self-efficacy scores of the students with good and medium health were statistically significantly higher than the students with poor health (p<0.001), and the average scores of the students who participated in sports activities were statistically significantly higher than those who did not take part in such activities (p<0.001) (Table 4).

The results of the multiple linear regression with the enter method on the self-efficacy scale are shown in Table 5. Of the seven independent variables that were entered into the linear regression model, the father's age, the mother's education and the number of siblings were significantly correlated with the student's self-efficacy score (i.e. these parameters were independent contributors to the self-efficacy scale). This study found that as the father's age increased, the self-efficacy levels of the student decreased. In contrast, an increase in the mother's education status and the number of siblings had a positive effect on the student's self-efficacy level (Table 5).

Discussion

The literature has reported that factors such as the family structure, education, social support, health status, culture and place of residence affect the self-efficacy perception[1,2]. Other factors reported to play a role are the individual's experiences, professional counselling and conditional factors (anxiety, distress, etc.) In this study, the following factors had a positive effect on the student's perceived self-efficacy: the father's age, the student's relationship with family and friends, the student's health status, the student's participation in sports activities, the student's regard for the profession and the presence of career plans. The average self-efficacy score of the students was 85.5±12.1. Similarly, some previous studies reported that self-efficacy scores of nursing and healthcare students were between 88 and 91[5,7,8,19,20]. In a study by Zalewska-Puchala et al. (2007) in Poland with first-year nursing students, the self-efficacy score of the students (53.7%) was high and of the students (38.4%) was medium[16]. In another study, the individual's age, gender, social status and family structure all influenced the person' perceived self-efficacy[2]. Self-efficacy refers to an individual's self-judgment relating to his/her capacity to achieve a certain performance level[4]. In the present study, the total average self-efficacy scores of the nursing and the midwifery students were high. Individual development, positive thoughts about their future and receiving a university-level education appeared to have a positive effect on the perceived self-efficacy of the students.

In the present study, the perceived self-efficacy of the health school students who liked their department was good. In studies conducted by Aştı et al. (2009) with first-year nursing students[19] and by Çam and Engin (2006) with nursing students found that the students who willingly chose nursing as a profession had a higher perception of self-efficacy[21]. Sergek and Sertbaş (2006) found no

significant correlation between willingly choosing the profession and perceived self-efficacy[9]. However, the same study found that nursing students who liked/regards the profession had higher self-efficacy scores. According to the findings of the current study, those who had a high regards for the profession also had a higher degree of perceived self-efficacy. This finding suggests that consciously choosing nursing as a profession has a positive influence on the student's perceived self-efficacy.

A strong sense of self-efficacy results in success and well-being. Most importantly, it allows for personal development and diversification of skills. Individuals with a strong sense of perceived self-efficacy tend to try to undertake more challenging tasks, set higher goals and attempt to reach those goals. Self-efficacy is an important factor affecting an individual's performance and success[1,2,22]. A study conducted with nurses revealed that their level of self-efficacy affected their career development[10]. In the current study, the self-efficacy levels of the students who wanted to go on to study a graduate degree and those who subscribed to a professional journal were higher than those who did not.

In the current study, the perceived self-efficacy of the students with good relationships with their families and friends was higher than that of students who had average and poor relationships with their families and friends. According to the literature besides direct experiences, indirect experiences (friends, family, etc.), advice, encouragement and support are all effective in the development of self-efficacy. Social support is an important factor in developing and maintaining an individual's self-efficacy[1]. Aştı et al. (2009) reported that students who shared their experiences/decisions with their family had a higher level of perceived self-efficacy[19]. We think that participation of students in social-cultural activities will contribute to the development of their perceived self-efficacy.

Self-efficacy is closely related to physical health. Studies revealed that the perceived self-efficacy of an individual is an important positive factor in the development and maintenance of positive health behaviours[1,14,16]. Healthcare professionals have to be role models in their lives due to their professional responsibilities[23]. In this study, the students who good health and participated in sports activities positively affected on students' self-efficacy levels. The perceived self-efficacy of students can be improved by supporting healthy lifestyle behaviours during their education.

In the multivariate analysis, the father's age had an independent effect on the perceived self-efficacy of the student, and the latter decreased with an increase in the father's age. The mother's education had an independent effect on the students' perceived self-efficacy, with students who had a mother with a higher level of education displaying greater self-efficacy. Doni et al. (2009) also found that the education levels of the students' parents affected their perceived self-efficacy and that this increased as the education levels of their parents increased[8]. In this study, we think that the higher education levels of the younger parents, including the mothers, had a positive influence on the students' perceived self-efficacy.

One previous study reported that there was no significant correlation between the number of siblings and self-efficacy scores[8]. However, in the present study, in the multivariate analysis, the number of siblings had an independent effect on the students' perceived self-efficacy. An increase in the number of siblings had a positive effect on the student's level of self-efficacy. In Turkish culture, the social support of family and friends is important. Thus, good relationships with family and friends have a positive impact on the perceived self-efficacy of students.

Conclusions

The student's father's age, the mother's education level, the number of siblings, the level of regard for the department and the desire to study for a graduate degree, in addition to subscription to a professional journal, relationships with family and friends, health status and participation in sports activities, all affected self-efficacy levels.

Implication for Practice

Promoting the nursing and midwifery profession, informing students while they make professional choices and providing them with the necessary support may positively affect health students' self-efficacy levels. We believe that the self-ef-

ficacy levels of students will improve if they are encouraged to participate in professional, cultural, social and sports activities during their education and if students with health problems are provided with more opportunities to access healthcare services. The nursing and midwifery curriculum should include strategies aimed at enhancing the self-efficacy levels of the students.

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Prognostic value of e-cadherin, β-catenin and cd44 expression in bladder cancer

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Abstract

Introduction: Loss of expression of adhesion molecules may contribute to cancer progression. Epithelial cadherin (E-cadherin) interacts with cytoskeletal proteins through the catenin complex. CD44 is also a surface adhesion molecule.

Aim: The present study was undertaken to investigate the alterations in the expression of E-cadherin, β-catenin, and CD44 in urothelial carcinoma of the bladder, and their prognostic value.

Materials and Methods: 54 patients with primary urothelial carcinoma of the bladder were included in this study. Tissue sections representative of the tumour in each case were immunostained with CD44, E-cadherin and β-catenin.

Results: Loss of membranous E-cadherin immunoreactivity was higher in invasive and high grade tumours, but this finding was not statistically significant. Loss of expression of β-catenin was significantly associated with higher tumour grade (p=0.001), but CD44 failed to show the same correlation. Both β-catenin and CD44 were not associated with invasiveness.

Conclusion: Several studies have examined the role of E-cadherin in urothelial carcinoma revealing clear associations of decreased expression, with high grade and advanced stage tumours. Our study confirms this data although not statistically significant. These findings suggest that the expression of E-cadherin and β-catenin might be useful prognostic markers for the clinical assessment of bladder cancer. Although several studies have shown an altered expression of CD44, there are other studies suggesting that it has no association with clinico-pathological variables which was also the case in our study.

Key words: Bladder cancer; CD44; E-cadherin; β-catenin.

Introduction

Urothelial carcinoma (UC) of the bladder is the second most common malignancy of the genitourinary tract, and the second most common cause of death of all genitourinary tumours. Approximately in 80% of patients with primary bladder cancer, the tumour is confined to the superficial mucosa. The risk of recurrence is as high as 70% and tumour progression to a higher grade and/or stage is about 30%. Furthermore 50% of the patients who are treated locally will relapse with metastatic disease within 2 years of treatment (1). Even in those that do not progress, regular surveillance by cystoscopy is required, making bladder cancer one of the most expensive and labour-intensive cancers to treat (2). It is very important to predict which superficial bladder tumours will recur or progress, and which invasive tumours will metastasize.

Alterations in the adhesion properties of neoplastic cells may play an important role in the development and progression of the malignant phenotype in a wide range of tumour types, including bladder cancer (3). Loss of intercellular adhesion allows malignant cells to escape from their site of origin, degrade the extracellular matrix, acquire a more motile and invasive phenotype and finally invade and metastasize (3). Cell adhesion is essential for the normal development and function of tissues. The interactions between the cytoskeleton and adhesion molecules regulate a variety of functions, including signal transduction, cell growth, differentiation, site-specific gene expression, morphogenesis, immunologic function, cell motility, wound healing, and inflammation (3,4).

The main families of adhesion molecules are the cadherins, integrins, members of the immunoglobulin superfamily, and selectins (4). The epithelium specific adhesion molecule E-cadherin is a glycoprotein of the cell surface that is responsible for calcium dependent cell adhesion. E-cadherin inter-

acts with cytoskeletal proteins through the catenin complex (5,6). β-catenin binds directly to the cytoplasmic domain of E-cadherin and regulates cadherin-mediated cell recognition and adhesion (7). Loss of the normal, membranous E-cadherin-catenin complex immunoreactivity occurs frequently in UC and correlates with high grade, advanced stage, and poor prognosis (3). CD44 is also a surface adhesion molecule and has been implicated in lymphocyte activation, recirculation, homing and tumour invasion (8) and also may have a prognostic value in predicting prolonged survival in invasive bladder carcinomas which has not been confirmed yet (9). There has been interest in the expression of CD44 as a marker of tumour aggressiveness and metastatic potential in human breast cancer, gastric carcinoma, and colonic carcinoma, as well as in UC (8,10). Studies investigating CD44 expression in UC have shown that an inverse correlation between the expression of CD44 and histological grade and tumour stage exists and loss of CD44 variants may provide an additional parameter in identifying patients with UC at risk for tumour recurrence (11).

The present study was undertaken to investigate the alterations in the expression of E-cadherin, β-catenin, and CD44 in UC of the bladder, and their prognostic value.

Materials and methods

Patient Selection

Fifty-four patients with primary UC of the bladder were included in the study. Tissue samples were obtained by radical cystectomy in patients with invasive tumours and transurethral resections in patients with superficial tumours. The histological grading was performed according to the WHO-ISUP criteria (12). Patients were grouped as low grade and high grade. The pathological staging was done according to the TNM classification (13).

Immunohistochemistry

Formalin-fixed, paraffin embedded tissue sections representative of the tumour in each case were immunostained with CD44 (Novocastra, 1:50), E-cadherin (Neomarkers, ready to use) and β-catenin (Genetex Inc, ready to use). Tissue sections were deparaffinized, and rehydrated. Endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 in methanol for 30 min. After blocking endogenous peroxidase, microwave antigen retrieval in citrate buffer was performed for 20 minutes and pH 6.0 was maintained. The sections were then incubated with primary antibodies for 60 minutes. ThermoShandon (LabVision IHC System Solutions, Fremont CA, USA) was used as the secondary system. Amino-ethyl carbazole was used as chromogen and finally Mayer's hematoxylin was used for counterstaining. Sections between all incubations, sections were washed with PBS. Evaluation of the staining was carried out by two investigators. CD44, E-cadherin and β-catenin membranous expression was evaluated in the major infltrating zone of the tumour. Membranous staining for E-cadherin and β-catenin was considered positive whereas membranous and/or cytoplasmic staining was considered positive for CD44. The proportion of stained cells and the cellular localisation of immunostaining were evaluated semiquantitatively. The immunostaining for E-cadherin and β-catenin was considered as: positive when the pattern of immunoreactivity was membranous, similar to that of normal urothelial cells; in at least 20-80% positive tumour cells), and negative when less than 20% of tumour cells were positive or cytoplasmic, nuclear distribution was deserved (14).

Statistical Analysis

Statistical analysis was carried out first on the entire group of cases (Ta, T1, T2, and T3 tumours). Differences in the expression of E-cadherin, β-catenin and CD44 according to patient and cancer characteristics were assessed by the κ^2 and Fisher exact tests. P values of less than 0.05 were considered statistically significant. The statistical analysis was performed by using SPSS 11.5 and MedCAlc ®v11.0.1 statistical software.

Results

The median age of the study group consisting of 49 male and 5 female patients was 64 (range: 29-84 years). The tumours were grouped as superficial (Ta and T1, n=42) and invasive tumours (T2, T3, and T4, n=12). Among the 54 tumours, 24 were classified as low grade and 30 as high grade.

The E-cadherin and β-catenin staining showed a membranous pattern, except for CD44 where the

staining was also found in the cytoplasm both in the basal cells of the malignant epithelium and in its more superficial cells in most specimens.

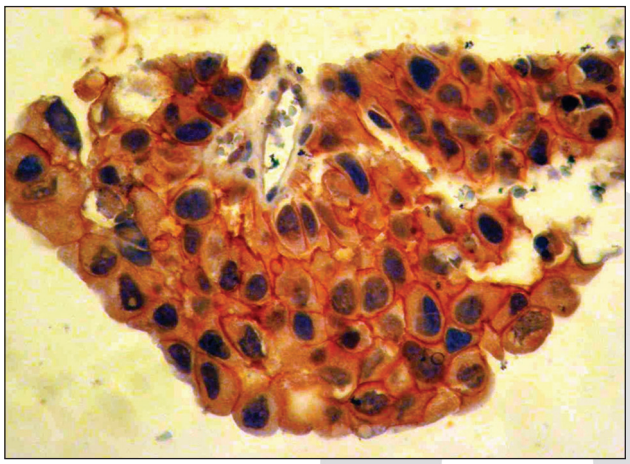

Figure 1. UC showing positive homogeneous membranous staining for β-catenin (x 400)

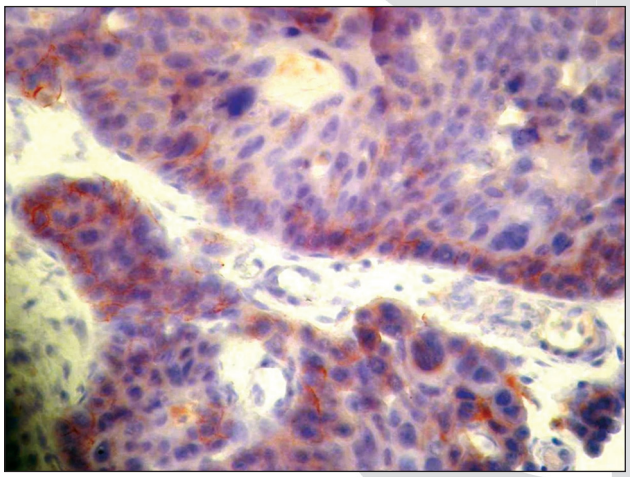

Figure 2. Tumour cells showing diffuse cytoplasmic staining for CD44 (x 400)

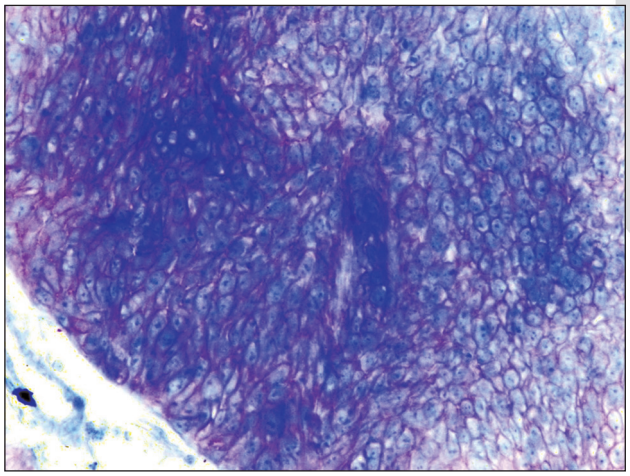

Figure 3. Tumour cells showing positive homogeneous staining for E-cadherin (x400)

In some areas there was a degree of regional heterogeneity in staining, but the trend towards staining of epithelial cells above the basal layer was still maintained. In the current study, 55,17% and 61,11% of the tumours showed negative staining for E-cadherin and β-catenin, respectively. Loss of membranous E-cadherin immunoreactivity was higher in invasive and high grade tumours, but this finding was not statistically significant. Loss of expression of β-catenin was significantly associated with higher tumour grade (p=0.001), but CD44 failed to show the same correlation. Neither β-catenin nor CD44 were associated with invasiveness.

Discussion

UC of the bladder is a heterogenous disease most of which are superficial tumours with possibly less aggressive biological behaviour than invasive ones and some as aggressive as their invasive counterparts. The mechanisms of recurrence, tumour invasion and metastasis are associated with complex biological processes that remain unclear. Staging and grading allow stratification of biological potential of the tumour to a certain degree and are also useful for clinical purposes. However, tumoural heterogenity still exists within various prognostic subgroups.

Biomarkers have not been extensively studied in UCs. P53 mutations (6) and angiogenesis (9), which are well-known markers of worse prognosis, have also been related to worse prognosis in patients with UC (15).

Preservation of intercellular adhesion is critical to the maintenance of normal tissue architecture. Cadherins are a family of transmembrane glycoproteins that are involved in homotypic calcium-dependent intercellular adhesions as well as cell signaling. E-cadherin is a 120 kDa glycoprotein located on chromosome 16. This protein is expressed in all epithelial tissues and is associated with intracellular proteins called catenins that couple cadherins to actin microfilaments (16). The E-cadherin/catenin complex is important for cell polarity, maintenance of normal tissue morphology and cellular differentiation (17). Loss of E-cadherin mediated cell adhesion is involved in tumour progression and metastasis. There is also evidence that E-cadherin may have a role in the supression of invasion (11,18). The role of adhesion molecules in UC is still controversial.

Several studies have explored the expression of cadherins and catenins and their role as prognostic factors in urothelial bladder carcinomas. Previous studies demonstrated a decreased or aberrant E-cadherin staining in high grade and advanced stage tumours (19-25). However, Reis et al found an association between overexpression of E-cadherin and tumour recurrence (15). These data contradict previous studies. Interestingly, Lim et al. found normal expression of E-cadherin in both usual and micropapillary urothelial bladder carcinomas, which was independent of tumour stage, tumour grade or presence of microvascular invasion (18). They proposed that loss of E-cadherin expression may be associated with histologically special plasmocytoid or signed ring cell UC (15). However other investigators showed that abnormal E-cadherin expression had no association with the same prognostic indicators (6,26). Although it is not statistically significant our study confirms the findings of several studies reporting that loss of E-cadherin and β-catenin expression is significantly associated with invasiveness and higher grade tumour (14).

These differences might be explained, to some extent, by different underlying mechanisms of the loss of E-cadherin expression. Among these are the association with factors at different cellular levels (mRNA or protein), with the cellular disturbances of E-cadherin-associated catenins, or with the up-regulation of N-cadherin which is also associated with adhesion (6).

β-catenin is an E-cadherin-associated protein that links the cytoplasmic tail of E-cadherin to the actin cytoskeleton of the cell, and is necessary for E-cadherin function (27). Limited data are available on altered α-, β-, and γ-catenin expression and tumourigenesis in the bladder. Abnormal expression of these cadherin cytoplasmic partners have been reported to be significantly correlated with tumour grade, advanced stage, and poor survival (25,28,29).

β-catenin is a multifunctional protein; in addition to its function in adhesion complexes, β-catenin has a role in signal transduction pathways (30) which are in some tumours, considered to be related to proliferation and invasion (31). In invasive tumours, the decreased β-catenin expression was prevalent as compared to α- and γ-catenins. The aforementioned decreased membranous β-catenin expression could be ascribed to the increase turnover of the protein via the proteasome or its nuclear localization (28). The persistent β-catenin expression, even in the absence of E-cadherin expression in invasive tumours, could allow the formation of an intercellular adhesion complex involving another member of the cadherin family or could be due to the presence of a cytoplasmic pool of the protein (28). Decreased β-catenin immuno-reactivity was found to be associated with poor outcome in bladder cancer (25). Muro et al found significant association between loss of β-catenin expression and grade, stage, tumour progression and decreased survival. However, the prognostic effect of these variables appeared to be of limited value in multivariate analysis (14). In the current study, we established an association between loss of of β-catenin expression and higher tumour grade which was statistically significantly ($p=0.001$). But we could not find an association between tumor stage and immune expression pattern either.

It is known that CD44 plays a definite role in cell–cell and cell–matrix interactions and is generated as various isoforms. Hence, differing quantities of the individual variant isoforms can also be detected in several normal tissues and organs (32). Whether the altered CD44 expression and tumour progression in bladder cancer are causally related is still unclear. Previously published data showed that the dominant pattern of CD44 expression in papilloma, papillary urothelial neoplasm of low malignant potential, or low-grade papillary carcinoma was normal or accentuated pattern (8,11). A statistically significant progressive loss of CD44 with increasing grade and stage in pTa and pT1 papillary urothelial neoplasms was also observed by the same investigators. These results suggest that the expression of CD44 is associated with differentiation and prognosis in bladder cancer (8). Patients with very early stage tumours (pTaGl) and severe dysplasia also showed abnormal CD44 protein pattern similar to the more advanced bladder malignancies. This finding suggests that the disturbances in the activity of this gene begin very early in the neoplastic process and may prove to be clinically useful (32).

Down-regulation of CD44 would facilitate loss of cell– cell cohesion, detachment from the basement membrane, and subsequent infiltration of the underlying tissues. Kuncova et al suggested that, progression to higher grades of UC was associated

with a decrease in CD44 expression, higher proliferative activity of tumour cells, and more frequent p53 over expression (33). It was speculated that increased expression of CD44 (or increased numbers of cells positive for CD44) either represented an initial phase of cancer development, or—more precisely—cancers composed of cells showing a lower degree of neoplastic transformation. Focal loss of CD44 immunoreactivity, reflecting local derangement of CD44 expression, suggests the existence of subpopulations of tumour cells, which might represent clones possessing more advanced neoplastic transformation, associated with higher risk of local recurrence and potentially more aggressive behaviour of the tumour (34). However, in several studies the relationship between overall distribution of CD44 immunostaining and tumour grade was confirmed only for a few isoforms. The presence of areas with squamous differentiation showing strong immunoreactivity for CD44 in some poorly differentiated UC could be one of the explanations why all aggressive carcinomas are not CD44 negative (33). This observation could also contribute to elucidation of the discrepancies regarding CD44 expression in UC (33). Although several studies have shown an altered expression pattern of CD44 in UC, some reports suggested that it has no association with clinico-pathological variables (14,35,36).

In our study, there was no association between CD44 expression and clinico-pathological variables similar to the reports of Muro et al. and Woodman et al. who observed an nonspecific increase in CD44v6 immunohistochemical expression in all 19 cases of UC of independent of grade, compared to the normal urothelium (14,37).

However this retrospective study conducted a limited number of cases, does not allow definite conclusions. Large prospective clinico-pathological studies to validate the prognostic value of E-cadherin, β-catenin and CD44 immunoreactivity should be performed. Our findings suggest that E-cadherin and, to some extend, β-catenin can be used as a prognostic indicators in the future.

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Large impacted bladder calculus causing renal failure: A case report

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Abstract

A 66 years old male presented with bladder stone which adversely resulted in chronic renal failure. Routine imaging with plain radiography clearly depicts a bladder stone and ultrasonography reveals bilateral hydronephrotic kidneys. A preliminary urethral catheterization failed due to impaction of the stone. Surgical removal of the stone confirmed an impacted bladder stone which assumed the shape of the lower bladder rendering a difficult removal. Bladder calculi which is relatively common, exhibits its peculiarity in present case with its shape and impaction to the bladder trigone. The ultimate effect is renal failure, while management is normal but follow up should be for long period of time.

Key words: Impacted; Bladder Stone; Renal Failure.

Introduction

Bladder stone disease is as old as mankind and the incidence of urolithiasis has increased in European countries, while the situation is variable in the United States, while the Asian counterparts stand at a higher incidence as high as 50%.[1,2] In African country like Nigeria has a higher incidence of stone disease.[3] In Malaysia the reported incidence was 442.7/100,000, and Male Chinese were affected higher.[4]

Urinary Bladder stones is a common disease, which generally recognized with clinical presentation of irritative urinary as well as obstructive symptoms. Bladder calaculus become so large as to cause bilateral hydronephrosis, which is a rare condition.[5] Impacted bladder stone can result in obstructive uropathy and renal failure.[6]

The aetiology and pathogenesis of bladder stones remain obscure, while diagnosis can be made easily with simple radiological investigations such plain X ray KUB and ultrasonography to enable early diagnosis and prompt interventions.[7] Presenting case was refered to tertiary level hospital with a huge bladder calculus causing acute renal failure.

Case Presentation

A 66 year old male presented to the urology department with a long history lower abdominal discomfort, which was characterized as dragging in nature. Pain was associated with incomplete voiding and frequency in the last few months before presentation. Patient had occasionally noticed lightly blood stained urine. Furthermore, complained of loss of appetite, nausea, generalized weakness and puffy of face. Patient was found to be pale, normotensive, afebrile and was not tachycardic. Abdominal examination revealed tenderness in the suprapubic region. Digital rectal examination did not show any prostatic enlargement. Baseline blood investigations showed a hemoglobin count of 7gm/dL, an elevated white cell count of 15000 mm^3, with a normal platelet count. Renal functions were grossly deranged with elevated urea of 28mmol/L and creatinine of 800mmol/L. Patient was acidotic however no hyperkalemia was noted, while preliminary KUB radiograph revealed a bladder stone of 7 x 4cm, as shown in figure 1.

An immediate ultrasonogram (figure 2) followed which confirmed a bladder stone complicated with bilateral hydronephrosis with hydroureter. An attempt to pass urethral catheter failed possibly due the large stone. Patient was soon referred to the nephrologist for a consult and emergency hemodialysis thereafter ensued. An emergency cystoscopic showed an impacted stone. A cystoscopic guided suprapubic punch catherization was successfully done following failed uret-

hral catheterization. The renal functions improved following multiple dialysis. After stabilization, it was planned for an open vesicolithotomy.

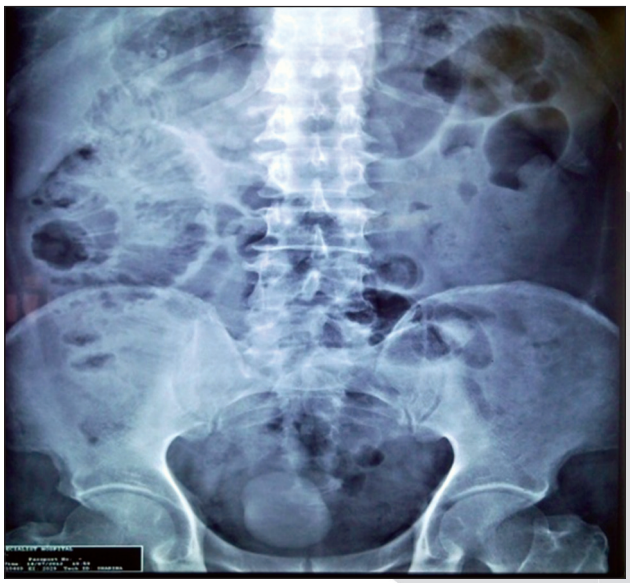

Figure 1. Plain Radiography showing bladder stone

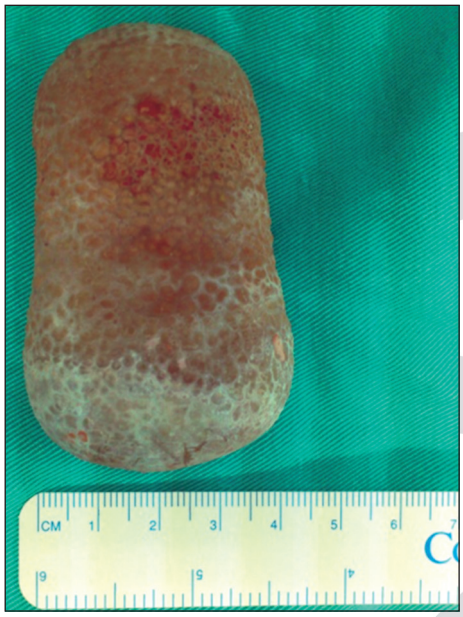

Figure 2. Ultrasonography of the bladder containing the stone

The peculiar shape of the stone which was shaped to the contour of the bladder base and neck caught our attention (figure 3a & 3b). He remained on a urethral catheter for ten days post surgery. Patient recovered well and serum urea and creatinine improved and is on regular follow up with the nephrologist.

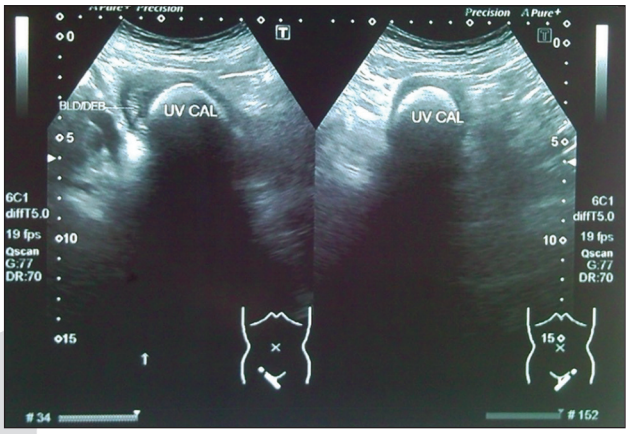

Figure 3a. Retrieved bladder stone

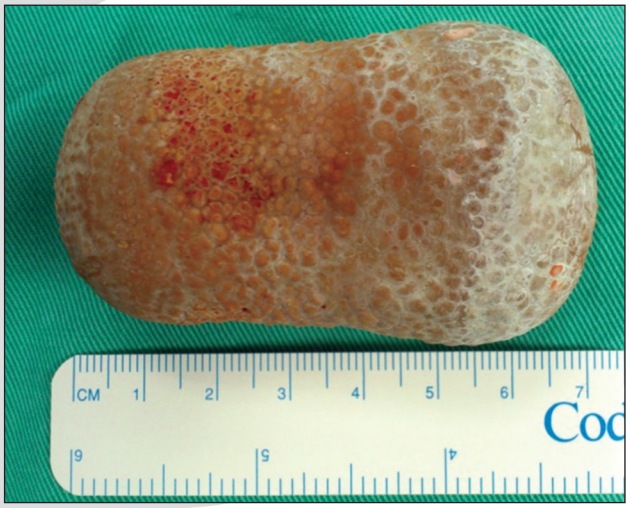

Figure 3b. Retrieved bladder stone

Discussion

Risk factors for the formation of bladder calculus are chronic urinary stasis, recurrent urinary tract infections and foreign body in bladder, whereas malnutrition is also reported from developing countries.[6] Furthermore, prolonged catheterization and stones associated with bladder augmentation and diversion have reported as risk factors.[5, 7-9]

Patients with bladder stones present differently according to type of stone they possess. Stone in the bladder are classified as primary and secondary. Though majority of reported cases of bladder tend to be large and secondary to foreign bodies, there are very few reported cases of impacted bladder stones resulting in renal failure as seen in present case. Majority of patients who present with chronic bladder outlet obstruction (BOO) are at risk of developing bladder stone. The commonest cause of bladder outlet obstruction leading to bladder stone is benign

prostatic hyperplasia.[2,10] The patient in current report did not have an enlarged prostate and attribute the development of the stone to be dietary in origin. Several large studies were done looking into the composition and causes of stone formation. Takasaki and colleagues found that calcium stones predominated while magnesium ammonium phosphate made up for the remaining of the stone composition.[11]

Patients with associated BOO typically have long history of obstructive symptoms while patients with endemic bladder stones present with non- specific symptoms such as vague abdominal discomfort and may be associated with irritative bladder symptoms.[10] When associated with renal failure, it is common for patients to present with only uraemic symptoms. Majority of these stones can be detected by plain radiography and ultrasonography can be done to detect associated renal pathology and complications such as hydronephrosis and hydroureter.[2] The use of CT Urography is highly accurate and superior to other modalities.[12]

Cystoscopy is generally not done routinely, however in our case where blind urethral catheterization failed, suprapubic punch catheterization was successful following cystoscopic guidance. Open vesicolithotomy is generally reserved for stones larger than 4cm. Majority of these stones though large, are usually mobile.[13] In current scenario, difficulty was encountered while dislodging the stone which was impacted in the lower segment of the bladder occluding both the ureteric orifice. Following surgery patients are usually catheterized for an average of ten days. A post operative cystogram is usually not a prerequisite for removal of catheter. A combined disciplinary approach with the nephrologist is required until the renal failure resolves and continued follow up is usually advised as recurrences are common.[13]

Conclusion

Bladder stones are common occurrences in urology however large impacted stones leading to renal failure are rare. Even though these stones are large and impacted, they are treated the same way as other large stones. A prompt and multidisciplinary treatment is usually required. Special attention should also be placed in identifying and treating the underlying cause.

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Seasonal variation in hospital admissions and in-hospital mortality among elderly patients with acute myocardial infarction: A single centre retrospective analysis in the European part of Turkey

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Abstract

Background: Majority of the previous studies have demonstrated a winter peak in the incidence and mortality of acute myocardial infarction (AMI) particularly among the elderly population. Accordingly, the present single-centre retrospective analysis was devised to investigate the potential seasonal variation in hospital admissions and patient characteristics including in-hospital mortality, etc. among elderly patients (65 years and older) with an acute ST segment elevation myocardial infarction (STEMI) in the European part of Turkey.

Methods: The study population was selected among 6103 coronary care unit (CCU) admissions in Thrace University Hospital during the particular 5-year period between January 1, 2006 and January 1, 2011. A total of eligible 521 elderly STEMI admissions were categorized into four groups according to the season of admission: spring, summer, autumn and winter groups. Thereafter, season groups were compared with each other in terms of number of admissions, demographic and clinical features including in-hospital mortality, etc.

Results: Overall admissions (n=521) were characterized by a particular spring peak and a summer-autumn trough (spring: 152, summer: 118, autumn: 118 and winter: 133 admissions) leading to a statistically significant seasonal variation ($p<0.001$) along with a similar trend in gender and age (elderly, very elderly) subgroups ($p<0.001$ for all). More importantly, very elderly STEMI patients (80 years and older) were found to have a statistically significant seasonal variation in in-hospital cardiovascular mortality (percents within season; spring: 14.6%, summer: 30%, autumn: 34.3 %, winter: 40.5 %, p=0.04). In this subgroup, logistic regression analysis demonstrated significant effects of winter (OR: 6.42, 95%: 1.93-21.38, p= 0.002) and autumn (odds ratio (OR): 3.65, 95% confidence interval (CI) : 1.04 -12.72, p=0.042) on cardiovascular mortality in comparison to spring after adjustment for demographic and clinical parameters.

Conclusion: In contrast with the majority of the literature (favoring a classical winter peak), the present analysis has demonstrated a spring peak in acute STEMI admissions among the overall elderly population along with a winter peak in in-hospital cardiovascular mortality exclusively confined to the very elderly population with STEMI. These findings may suggest important preventive, therapeutic and prognostic implications for the elderly AMI admissions during particular seasons in the European part of Turkey.

Key words: Acute myocardial infarction, seasonal variation, in-hospital mortality, elderly subjects.

Introduction

Cardiovascular diseases including AMI have been the leading cause of hospitalizations and mortality worldwide (1,2). Acute cardiovascular diseases are well known to exhibit circadian, weekly and seasonal patterns (3). Previous studies have demonstrated the presence of a morning peak in the onset of a variety of cardiovascular diseases including AMI (4), cardiovascular arrest (5) and cerebrovascular disease (6), etc. Similarly, seasonal variation in AMI with winter and spring peaks has also been reported previously (7-9). Moreover, a couple of studies have also demonstrated the presence of a seasonal variation in mortality rates of AMI (10,11).

It is well known that the incidence of coronary artery disease (CAD) increases with age. Elderly

patients (65 years and older) with ACSs represent a spesific subgroup that warrants particular attention in diagnostic and therapeutic approaches. AMI complications including post-AMI heart failure and side-effects of therapeutic regimens may be encountered more frequently among these patients (1). Moreover, elderly patients with AMI may have an increased risk of death suggesting advanced age as one of the strongest predictors of mortality in the setting of AMI (12). On top of clinical risk stratifiers, evaluation of seasonal pattern of AMI admissions and characteristics in this already vulnerable group may be of greater clinical value suggesting stronger clinical implications compared with younger subjects. Accordingly, the present retrospective study was devised to investigate the potential seasonal variation in hospital admissions and patient characteristics including in-hospital mortality, etc. among elderly patients with an acute STEMI in the European part of Turkey.

Methods

Study population

In this single centre retrospective analysis, the study population (elderly patients with acute STEMI) was selected among a total of 6103 CCU (serving as a general cardiology intensive care unit) admissions in Thrace University Hospital during the 5-year period between January 1, 2006 and January 1, 2011. The University Hospital is located in Edirne, an ancient city of the European part of Turkey with a population of about 140.000 and with a continental climate characterized by hot summers and cold winters. Due to the significant number of admissions from the neighboring cities, villages,etc., CCU patients not only represent local residents in Edirne but a larger population in the European part of Turkey as well.

Data collection

As an initial analysis, CCU admission records (official large-volume books (comprising consecutive admissions with names, protocol number, date and diagnosis on admission,etc.) and computerized patient record system) within the particular 5-years period were retrospectively reviewed. Thereafter, patient files and/or hospital discharge sheets of elderly STEMI admissions were obtained from the hospital archive and/or computerized patient record system of the hospital. The STEMI diagnosis was made by the attending physician at the time of admission using electrocardiographic (ECG) (ST segment elevation in at least two contiguous ECG leads, acute left bundle branch block (LBBB)) and clinical (persistan angina or angina equivalent including dyspnea,etc.) and enzymatic (creatine kinase MB isoenzyme and troponins) criteria. The study protocol was approved by the institutional local ethics committee.

Presence of a STEMI in a non-elderly (< 65 years of age on admission) patient or any important missing information necessary for the study protocol (for ins; date of admission), presence of subacute (or chronic) or equivocal acute STEMI admissions, presence of ACSs other than STEMI or in-hospital STEMI (during hospital stay for any reason) and presence of sudden cardiac death (SCD) (on admission or after successful out of - hospital cardiopulmonary resuscitation (CPR)) were accepted as exclusion criteria.

Data analysis

After careful review of admission records (n=6103) and patient documents, a total of 521 elderly STEMI admissions were found to be eligible for the analysis according to the exclusion criteria. Based on hospital discharge sheets, these patients were categorized into four groups according to the season of admission: spring (March to May), summer (June to August), autumn (September to November) and winter (December to February). Thereafter, patients in each group were assessed for their demographic features including age, age group (elderly, very elderly), gender and for their clinical features including symptoms on admission, time from symptom onset to admission, history of congestive heart failure (CHF), history of diabetes mellitus (DM), hypertension (HT) and smoking, history or family history of ACS, presence of pre-existing chronic illness, STEMI localisation, reperfusion strategy, in-hospital mortality (including cardiovascular and total mortality). Finally, the four groups were compared with each other in terms of number of admissions, demographic and clinical features (particularly in-hospital mortality) as described above.

The terms ‘elderly’ and ‘very elderly’ were defined as ages 65 to 79 years and 80 years and over,

respectively. Symptoms on admission were categorized into two goups according to the presence or absence of angina pectoris on admission: persistent angina on admission (with or without other symptoms including dyspnea, nausea, etc.) and symptoms other than angina (angina equivalent) on admission. Patients were also categorized into 3 subgroups according to the time from symptom onset to hospital admission: early presenters (0-2 hours), medium presenters (2-12 hours) and late presenters (more than 12 hours). DM was defined by the patient's self report of such history or use of any antidiabetic agent (insulin, oral hypoglycaemic). HT was defined by the patient's self report of such history or use of any antihypertensive agent. Smoking was defined as an active use within the last 12 months before admission. Chronic illness was defined as any kind of pre-existing systemic disease including active malignancy, chronic pulmonary, renal and hepatic diseases, endocrinological (other than DM), chronic infectious (tuberculosis,etc.) and collagen vascular diseases. STEMI localisation was categorised into 4 subgroups: STEMI involving anterior wall of the left ventricle (LV) (anterior, anteroseptal,etc.), STEMI involving inferior and/or posterior walls of the LV, STEMI involving the right ventricle (RV) (with or without involvement of inferior and/or posterior walls,etc.) and STEMI of any other localisation (lateral, etc.). History of congestive heart failure (CHF) was defined as the presence of documented systolic and/or diastolic heart failure prior to admission (excluding physiological diastolic dysfunction of the elderly). In-hospital cardiovascular mortality of a STEMI patient was defined as any kind of death resulting from any arrhythmic or mechanical complication, worsening heart failure, cardiogenic shock, stroke or resulting from any complication of the revascularisation procedure during the hospital stay. Total mortality was defined as any kind of death due to cardiovascular or non-cardiovascular causes (hepatic, renal, pulmonary, gastrointestinal, sepsis, massive extracranial haemorrhage,etc) or death of any undetermined etiology during the hospital stay.

Statistical analysis

One sample Kolmogorov Smirnov Test was used to evaluate the seasonal distribution of hospital admissions. Categorical variables were compared using Chi square test. The potential effects of seasons on mortality were tested using logistic regression analysis. A p value of < 0.05 was considered statistically significant. Statistical analyses were performed by using SPSS 20.0 Statistical Package Programme.

Results

Regarding the overall 521 admissions, there was a spring peak and a summer-autumn trough (spring: 152, summer 118, autumn: 118 and winter: 133) with a statistically significant seasonal variation ($p<0.001$). Figure 1 demonstrates the seasonal distribution of acute STEMI admissions among the overall elderly population (65 years and older). When each age group was analysed separately, there were 376 admissions in the elderly group (65 to 79 years) with a spring peak and an autumn trough (spring: 104, summer 98, autumn: 83 and winter: 91) ($p<0.001$), and 145 admissions in the very elderly group (80 years and older) with a spring peak and a summer trough (spring: 48, summer 20, autumn: 35 and winter 42) ($p<0.001$). A similar trend with a spring peak was also observed in male (n=335) (spring: 95, summer 76, autumn: 76 and winter: 88) and female (n=186) (spring: 57, summer 42, autumn: 42 and winter: 45) subgroups ($p<0.001$ for both).

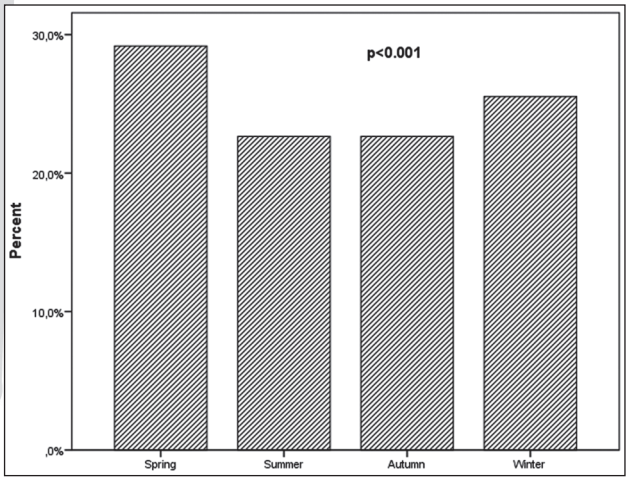

Figure 1. Seasonal distribution of acute STEMI admissions among the overall elderly population (65 years and older)

Table 1 demonstrates an overall comparison of demographic and clinical features among season groups. Unfortunately, the absolute time from

symptom onset to admission (in hours) were not clearly available for all admissions. However, analysis of the available values (n=285) did not demonstrate any significant seasonal variation in this variable (p=0.748), and signified a predominance of medium presenters (2 to 12 hr) (75.8 % of the available values). Regarding overall cardiovascular and total mortality, there was no statistically significant seasonal variation in cardiovascular and total in-hospital mortality (p=0.498 and 0.62, respectively) (Table-1). When each gender was analysed separately, there was again no statistically significant seasonal variation in total and cardiovascular mortality in females (n=186) (p=0.76, p=0.64, respectively) and males (n=335) (p=0.64, p=0.55, respectively). When each age subgroup was analysed separately, elderly patients (n=376) did not demonstrate any statistically significant seasonal variation in total and cardiovascular mortality (p=0.67, p=0.49, respectively). In the very elderly group (n=145), seasonal variation in total mortality was also insignificant, but had a trend towards a statistical significance (p=0.07) More importantly, very elderly patients were found to have a statistically significant seasonal variation in cardiovascular mortality characterized by a winter peak (percents within season; spring: 14.6%, summer: 30%, autumn: 34.3 %, winter: 40.5 %, p=0.04).

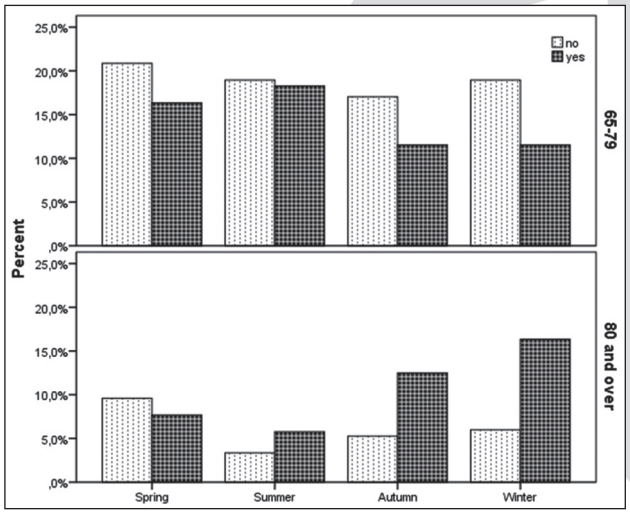

Figure 2. Seasonal distribution of in-hospital cardiovascular mortality according to age subgroups

Figure 2 demonstrates the seasonal distribution of in-hospital cardiovascular mortality according to age subgroups. In the very elderly subgroup, logistic regression analysis demonstrated significant effects of autumn (OR: 3.65, 95% CI : 1.04 -12.72, p=0.042) and winter (OR: 6.42, 95%: 1.93-21.38, p= 0.002) on cardiovascular mortality in comparison to spring after adjustment for demographic and clinical parameters including gender, risk factors for atherosclerosis, previous histories of ACS, CHF, pre-existing chronic systemic disease, STEMI localisation and reperfusion strategy.

Discussion

In the present study, there are two major findings that might be of clinical relevance: first; this retrospective analysis clearly demonstrated the presence of a significant seasonal variation in acute STEMI admissions characterized by a spring peak and a summer-autumn trough among the overall elderly subjects. Second; there was a significant seasonal variation in in-hospital cardiovascular mortality with a winter peak and a spring trough exclusively among the very elderly STEMI patients (80 years and older).

Current literature generally substantiates the seasonal pattern of AMI occurence with a predominance of winter peak in a variety of studies (7-9,13,14) suggesting potential effects of ambient factors on the onset of coronary events (15). Blood pressure (BP) (16), incidence of acute infections (13,17), levels of inflammation and coagulation markers (17-19) were all shown to be higher in winter compared with other seasons indicating the potential link between cold temperature and the incidence of ACSs (11). Moreover, elderly subjects were suggested to be more vulnerable to mechanisms leading to increased admissions for AMI in winter (14,17). On the other hand, previous studies in young subjects have demonstrated spring (20,21) and summer (15) peaks in coronary events that might be associated with an androgenic risk factor particularly in young males (20). Based on these diverse results, seasonal pattern of AMI admissions may, in part, be regarded as an age-dependent phenomenon (21). Interestingly, in our study, STEMI admissions have typically demonstrated a spring peak rather than a winter peak among the overall elderly subjects (and also in gender and age subgroups). This finding is in contrast with the majority of the current literature favoring a classical winter peak among the elderly population. Unknown humoral changes, spring-spesific local respiratory infections or aller-

Table 1. General comparison of demographic and clinical features among season groups

Elderly STEMI admissions (n=521)	Spring (n=152)	Summer (n=118)	Autumn (n=118)	Winter (n=133)	p value
Age group (n) (elderly vs. very elderly)	104/48 (68.4 / 31.6 %)	98/20 (83.1 / 16.9 %)	83/35 (70.3 / 29.7 %)	91/42 (68.4 / 31.6 %)	0.027
Mean age	76.0 ± 7.0	73.4 ± 6.6	75.8 ± 7.0	75.8 ± 6.7	0.009
Gender (n) (male vs. female)	95/57 (62.5 / 37.5 %)	76/42 (64.4 / 35.6 %)	76/42 (64.4 / 35.6 %)	88/45 (66.2 / 33.8 %)	0.93
Admission symptom (n) (angina vs. angina equivalent)	139/13 (91.4 / 8.6 %)	110/8 (93.2 / 6.8 %)	104/14 (88.1 / 11.9 %)	118/15 (88.7 / 11.3 %)	0.49
DM (n)	41 (27 %)	30 (25.4 %)	29 (24.6 %)	23 (17.3 %)	0.24
HT (n)	97 (63.8 %)	72 (61.0 %)	86 (72.9 %)	75 (56.4 %)	0.053
Smoking (n)	44 (28.9%)	42 (35.6%)	34 (28.8 %)	39 (29.3 %)	0.60
Family history of premature CAD (n)	11 (7.2 %)	4 (3.4 %)	15 (12.7%)	9 (6.8 %)	0.054
Previous ACS (n)	25 (16.4%)	18 (15.3 %)	23 (19.5 %)	23 (17.3 %)	0.84
CHF history (n)	4 (2.6 %)	1 (0.8 %)	9 (7.6 %)	3 (2.3 %)	0.01
Pre-existing chronic systemic disease (n)	13 (8.6 %)	9 (7.6 %)	18 (15.3%)	11 (8.3 %)	0.15
STEMI localisation (n) -Anterior wall -Inferior and/or posterior -RV -Other	 63 (41.4 %) 40 (26.3 %) 35 (23 %) 14 (9.2 %)	 54 (45.8 %) 26 (22.0 %) 32 (27.1 %) 6 (5.1 %)	 57 (48.3 %) 29 (24.6 %) 24 (20.3 %) 8 (6.8 %)	 55 (41.4 %) 27 (20.3 %) 37 (27.8 %) 14 (10.5 %)	0.64
Reperfusion strategy (n) -Streptokinase -TPA -Primary PTCA -Rescue PTCA -Spontaneous reperfusion -No attempt for reperfusion	 81 (53.3 %) 33 (21.7 %) 9 (5.9 %) 5 (3.3 %) 6 (3.9 %) 18 (11.8%)	 48 (40.7 %) 44 (37.3 %) 9 (7.6 %) 0 (0.0 %) 2 (1.7 %) 15 (12.7 %)	 50 (42.4 %) 33 (28.0 %) 12 (10.2 %) 0 (0.0 %) 11 (9.3 %) 12 (10.2 %)	 62 (46.6%) 32 (24.1 %) 19 (14.3 %) 4 (3.0%) 6 (4.5 %) 10 (7.5 %)	0.006
In-hospital cardiovascular mortality (n)	23 (15.1 %)	23 (19.5 %)	20 (16.9 %)	29 (21.8 %)	0.49
In-hospital total mortality (n)	25 (16.4 %)	25 (21.2 %)	25 (21.2 %)	29 (21.8 %)	0.64

STEMI*; ST segment elevation myocardial infarction,* **DM***; diabetes mellitus,* **HT***; hypertension,* **CAD***; coronary artery disease,* **ACS***; acute coronary syndrome,* **CHF***; congestive heart failure,* **RV;** *right ventricle,* **TPA***; tissue plasminogen activator,* **PTCA***; percutaneous transluminal coronary angioplasty.*

gens and altered atmospheric pressure etc. might have accounted for this spring peak in elderly STEMI admissions. Moreover, in a similar manner with a previous report (22), intrinsic or extrinsic over-adaptive (behavioural,etc.) changes against winter effects might have also blunted the typical winter peak pattern in this geographic area characterized by a harsh continental climate.

More importantly, seasonal variation in in-hospital cardiovascular mortality (characterized by a winter peak) was exclusively confined to the very elderly STEMI patients in our study suggesting a potential age threshold for this variation. Current literature (10,11,20,23) also substantiates the presence of a winter peak in AMI mortality, particularly with increasing age (11), but has not indicated a clear-cut age range or threshold regarding seasonal mortality of AMI. Cold stress may be associated with AMI mortality through a variety of potential mechanisms: it was previously suggested that hypercoagulation and enhanced inflammatory response might appear to have a role in the genesis of malign ventricular arrhythmias and SCD during or after an AMI (24-27). Therefore, increased co-

agulation and inflammation during winter (17-19) may suggest a potential association between cold temperature and arrhythmic mortality in STEMI patients. Moreover, cold temperature was previously suggested to be associated with sympathetic hyperactivity (28) that might trigger malign ventricular arrhythmias in arrhythmia-prone subjects (29,30), and might also be associated with increased infarct size, etc. in the setting of AMI. However, this temperature-related autonomic dysfunction may exhibit significant variations across different age groups (as previously demonstrated in levels of BP , an index of sympathetic system (28)). Taken together, seasonal variation in AMI mortality may also be regarded as an age dependent phenomenon as in the event of AMI admissions. Accordingly, in a previous large scale study, seasonal variation in AMI mortality (with a winter peak and a summer trough) was reported to be more striking with increasing age in a graded manner (11). However, in our study, seasonal variation in mortality was exclusively observed in the very elderly STEMI patients (not in the elderly) indicating the particular presence of a disrupted homeostatic mechanism against cold stress in this subgroup. In this fragile age group, over-adaptive changes (behavioural, etc.) against winter-autumn effects, that might have reduced hospital admissions during these seasons (and led to a spring peak), might not have been strong enough to blunt the winter peak in cardiovascular mortality. On the other hand, absence of seasonal variation in mortality in the elderly subgroup (65 to 79 years) remains unclear. Elderly patients might have had stronger protective and over-adaptive mechanisms against winter effects compared with the very elderly. Moreover, elderly people in this geographic region are generally more prone to adopt a healthier life-style due to the greater public awareness compared with other regions of Turkey. Therefore; the neutral effects of cold seasons on cardiovascular mortality in the elderly subgroup might also be due to the less pronounced dependence of these patients on environmental factors including external temperature, etc. (due to relative robustness and delayed phsiological ageing, etc.).

Studies regarding the chronobiology of ACSs in Turkish population have been quite scarce. According to the major scientific indexes (in English language), to the best of our knowledge, this is probably the second study across the whole country, and the first study in the European part specifically aiming to investigate the seasonality of AMI characteristics. In Turkey, the first study on this issue was from the the Asian part (Western Anatolia), and demonstrated the absence of a seasonal variation in AMI cases (despite higher frequency of AMI in winter) in a selected Turkish population (31). The results of our study may confer a variety of clinical implications regarding overall elderly patients with AMI: first; medical facilities regarding ACS admissions (beds, medical staff, tools, etc.) should be optimized according to geriatric needs particularly in spring months in this geographic region. Second; admission in wintertime may serve as a strong prognostic criterion in the very elderly subjects with AMI suggesting agressive preventive and therapeutic strategies aiming to reduce in-hospital mortality during this particular period.

Conclusion

The present retrospective analysis has demonstrated the presence of a spring peak in STEMI admissions among the overall elderly subjects (65 years and older) along with similar trends in gender and age subgroups in a spesific region of Turkey. This finding is in contradiction with the majority of the literature that demonstrates a classical winter peak in AMI admissions particularly among the elderly population. On the other hand, increased winter-related cardiovascular mortality during the hospital stay for a STEMI was found to be exclusively confined to the very elderly population (80 years and older) suggesting that envorinmental factors including cold temperature,etc. might exert their adverse effects only in the already vulnerable and fragile subjects in the setting of an acute STEMI. Future studies are still warranted to further analyse the seasonal characteristics of AMI in Turkish population.

Study limitations

It is well known that hospital admissions for a given disease generally comprise a population of symptomatic patients seeking medical care. Therefore, the main limitation of the study is that

it might be representative of hospital admissions rather than the whole elderly STEMI population in this geographic region.

There are also a couple of limitations regarding clinical variables: parameters of left ventricular (LV) function including LV ejection fraction (LVEF) (a potential predictor of mortality) measured echocardiographically during hospital stay were not available for all subjects (particularly patients with early mortality), and thus were not taken into analysis. However, history of ACS, CHF and localisation of STEMI that might, in part, be informative about the status of LV functions were taken into analysis. Therapeutic regimens during the hospital stay (other than reperfusion therapy) were not also compared between the groups as types, dosages or duration of these regimens were unavailable or unclear in a portion of patients. Finally, time from symptom onset to hospital admission, an important clinical variable usually reported by the patient, were not available for all patients probably due to the cognitive failure of the elderly population about the exact time of symptom onset. However, comparison of the available values among groups did not demonstrate any seasonal variation in this parameter.

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Synchronous renal cell carcinoma and Bellini duct carcinoma: A case report and review of the literature

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Abstract

The Bellini collecting duct carcinoma (CDC) is a very rare form of renal cell carcinoma (1%) associated with an extremely poor prognosis. Synchronous renal cell carcinoma and Bellini duct carcinoma are an aggressive variety of kidney neoplasm and two cases reports in the literature. That is often associated with nodal and visceral metastases at presentation. It is associated with poor prognosis. For the majority of patients surgical treatment will not result in a cure. Our case was third case in the literature.

Key words: Renal cell carcinoma; Bellini collecting duct; metastasis; nephrectomy; poor prognosis.

Introduction

The coexistence of multiple and synchronous primary neoplasms in the same organ (including kidney) has only rarely been described in the literature. (1) Bellini's collecting duct carcinoma (CDC) is an unusual renal neoplasm with a poor prognosis. Its origin is not entirely known, although it is thought to arise from the distal collecting duct system, whereas clear cell renal carcinoma (RCC) may originate from the proximal tubular epithelium. The coexistence of multiple and synchronous primary neoplasms in the same organ (including kidney) has only rarely been described in the literature. Some cases of rare synchronous occurrence of transitional cell carcinoma (TCC) and RCC have been reported. CDC was found to be associated with TCC (2 cases) and with RCC (3 cases). Recently, we had the opportunity to examine one such case of synchronous CDC combined with RCC: the clinical radiological and histopathological findings are reported. The clinical outcome was extremely favorable.

Case Report

A 66-year-old woman presented with loss of weight and stomach pain lasting for four months. Clinic examination and renal function were normal. CT scan (Figure 1) showed a left 6-cm-large renal mass developing towards and infiltrating the lower pole of kidney with evidence of tumor extension into the renal hilum. Intra-abdominal multiple lenfadenopaties were seen.

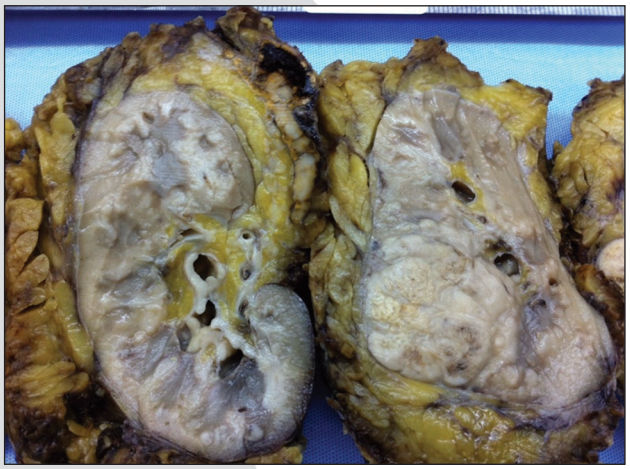

Figure 1. Macroscopic view of kidney with synchchoronous tumours.

Radical nephrectomy and adrenelecomy were performed, without ureterectomy. Histopathological examination demonstrated two tumors: one, centered on the renal medulla, grossly infiltrating the major calyx of lower and upper poles (collecting duct carcinoma), and another involving the lower renal poles and extending to the capsule (papillary renal cell carcinoma).

Materials and Methods

The left nephrectomy specimen was examined grossly and representative tissue blocks were taken

from the tumors and the adjacent renal parenchyma. They were fixed in 10% buffered formalin and embedded in paraffin. Four micrometer sections were cut and stained with hematoxylin and eosin. Immunohistochemical studies using a panel of antibodies (CK 7(Low molecular weight cytokeratin), CK 20 (Low molecular weight cytokeratin), UEA-1(Ulex Europeus Agglutinin-1) HMWCK (High molecular weight cytokeratin), and cerb B2(Antibody for oncogene)) were carried out.

Results

Kidney size was 11*6*6 cm and weight 540 gram. Tumour sited lower pole measuring 4 cm in maximum diameter. (Renal cell carcinoma) On gross examination; out of the tumour area showed 0, 1-0, 7 cm different multiple tumoural structure heterogeneous white and greyish growth, lots of calisial deformity, some necrotic areas on the medulla and cortex. It infiltrated the lower pole and infundibulum. Microscopically; the feature of synchoronous collecting duct carcinoma and renal cell carcinoma penetrating the capsule of the kidney, peri-nefric and hilar fat, Gerota fascia, renal artery, renal vein and adrenal gland metastasis were found. The adjacent renal parenchyma was invased. The patient developed lung metastases and died two months later after surgery.

Discussion

The coexistence of multiple synchronous primary neoplasms in the same kidney has been rarely described in the literature. The most frequent combination is RCC and TCC. Hart et al. reviewed 22 cases reported in the English Literature (2). CDC was found to be associated with TCC (2 cases) (3) and with RCC (1 case).

Bellini CDC represents a subgroup within renal carcinoma with an incidence of 0. 4-2% (2) of all renal tumors. It is a rare neoplasm with less than 150 cases described in the literature to date (4-5).

CDC is a highly aggressive renal tumor, with a poor prognosis (mean survival 11. 5 months (2). Its histogenesis is still a matter of debate although a putative origin from collecting ducts has been proposed (6). The collecting ducts share their embryological origin in Wolf's duct with the calyces, renal pelvis and ureter (7)This common embryological origin could justify its synchronous or metachronous association with in situ or papillary TCC in the adjacent renal pelvis, but not with RCC. In fact, while CDC originates in the medullar collecting duct, a mesonephric structure, RCC originates from tissues having metanephric precursors.

Reviewing the literature, we found only two other reference reporting the coexistence of CDC with RCC (1-8). Ours seems to be the third case with coexisting RCC and CDC.

CDC spreads aggressively and many patients have metastases at the time of presentation. Bone and lung metastasis and also extensive regional node involvement were reported (9). A fatal CDC, presenting with pleural metastases, arising from the right kidney in an 8-year-old child, was reported by Craver (10), while direct invasion of the liver was reported by Sue et al. (11), thus requiring radical nephrectomy and segmental hepatectomy. One case of tumor extension into the inferior vena cava was also described (12).

The majority of reported patients were treated by radical nephrectomy and regional lymph node dissection. Immunoactive agent such as interferon, interleukin and combination chemotherapy such as (paclitaxel+doxorubisin; gemcitabin+cisplatin) for relapsed disease or metastatic lesions usually seem to be ineffective. (13)

Conclusion

Collecting duct carcinoma is a rare renal cancer subtype; its association with other renal tumors is found exceptionally. In most of the cases, it has aggressive course and poor prognosis. In recent literature reviews, some cases with good outcome were, however, described. Current Standard chemotherapy or immunotherapy treatment regimens have been found to be ineffective. Moreover, in case of metastatic CDC, even nephrectomy seems not to be useful except for palliative reasons.

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Investigation of the relationship between postpartum depression and social support: A hospital-based cross-sectional study

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Abstract

Lack of social support is one of important factors in the occurrence of postpartum depression. This study has been designed to investigate the relationship between postpartum depression and social support in Turkey. Descriptive and hospital-based cross-sectional research design was used. Participant Information Forms, Edinburgh Postnatal Depression Scale (EPDS) and Multidimensional Scale of Perceived Social Support (MSPSS) were applied to 188 women in postpartum period. An important relationship between EPDS/MSPSS scores and education, economic status, employment status was found. In addition, a negative relationship between social support scores and postpartum depression (r=-0.29, p=0.003) has been recorded. As a result, social support was found to be an important factor in the management of postpartum depression. This information is believed to contribute to health care applications.

Key words: Depression, postpartum, social support.

Introduction

"Postpartum depression" is the most common mood disorder in the postpartum period; it is a condition usually seen within the first 6 weeks after childbirth and extends up to 1 year [1]. The studies carried out in Turkey using self-report scales showed that between 14 and 41% of mothers had postpartum depression symptoms [2,3]. UNFPA and WHO 2007 data, India, Vietnam and Turkey stated as 25% of postpartum depression [4].

UNFPA and WHO data, in 2008 the rate of postpartum depression, medium and low-income countries varies between 14% and 50% [5].

There are many reasons that increase mood disorders in mothers in the postpartum period. These reasons include stresses involved in child care, hormonal changes, past mental problems, unwanted and risky pregnancies, a difficult delivery, adolescent pregnancies, inter-family conflicts, financial difficulties, lack of support from the healthcare professionals during the delivery, a stressful life, low self-esteem and lack of social support [6,7,8]. Social support can be defined as the social and psychological support an individual receives from his/her associates [9]. A large number of studies have been conducted showing that social support has a positive relationship with mental and physical health [7,8].A supporting relationship is widely agreed to play an important role in strengthening the efforts for improvement of health, prevention of health problems, and protection against and coping with the impacts of stress. The support received from significant people in a social environment has a positive effect on maternal experience. Lack of support, on the other hand, may affect this experience negatively [10].

Nurses/midwives have important responsibilities in prevention, early diagnosis, referral, treatment and care of postpartum depression which may produce serious consequences. In the studies made, a significant decrease in depressive symptoms, an improvement in mother-child interaction, and an increase in the quality of life of women have been observed as a result of the education given by healthcare nurses/midwives to the women with postpartum depression during home visits [11,12]. Depression seen in the postpartum period requires a special attention as it will have unfavorable effects not only on the mother but also on the baby and the whole family [13]. Since many wo-

men hide the symptoms because they feel guilty for having depressive feelings in a period in which they believe they should be happy, the condition of postpartum depression can easily go unnoticed [14]. Therefore, postpartum depression is a disease that should be taken into consideration both for timely identifying those pregnant women in the risk group and for employing the appropriate approach at the right time [2].

In this study, it is aimed to investigation of the relationship between postpartum depression and social support and draw nurses/midwives' attention to the importance of social support in management of postpartum depression.

Materials and Methods

Sample and data collection

This study was performed between August and December 2009 as a descriptive and hospital-based cross-sectional study. The population of the study consisted of a total of 768 women who had a normal delivery without any postpartum complications or a caesarean section without any postoperative complications in Turgut Ozal Medical Center of Inonu University when the study was underway. One hundred and eighty eight women who had no pharmacological or psychotherapeutical treatment and agreed to take part in the study were included in the study. Home addresses and phone numbers of 188 eligible women were obtained, and they were contacted on the phone after the 42nd day and data was collected by making home visits when they were available.

In collecting the data, an information form developed by the investigators which consisted of 20 questions about the descriptive characteristics of the women, the Edinburg Postnatal Depression Scale (EPDS) and the Multidimensional Scale of Perceived Social Support (MSPSS) were administered.

Information form

This form consisted of a total of 20 questions for identifying the sociodemographic characteristics of the women and the factors that were likely to affect their postpartum depression (marriage age, number of deliveries and children, planned pregnancy, evaluation of pregnancy, metod of delivery and receiving information after the delivery).

Edinburg postnatal depression scale (EPDS)

This scale, which was developed by Cox and associates (1987) and whose validity and reliability were assessed by Engindeniz and associates (1996), identifies any risk of depression in the postpartum period. The scale is a self-report scale of a quadruple Likert-type and consists of 10 items. Items 3, 5, 6, 7, 8, 9 and 10 of the scale indicate increasingly declining severity and they are scored as 3, 2, 1 and 0 whereas Items 1, 2 and 4 are scored as 0, 1, 2 and 3. The total score of the scale is found by adding the scores of each item and the lowest score that can be obtained is 0 while the highest 30. The cut-off point of the scale was determined as 12/13 and its cronbach's reliability coefficient alpha as 0.79 [15]. The cronbach's reliability coefficient alpha of the scale was also found to be 0.78 in our study.

Multidimensional scale of perceived social support (MSPSS)

This scale, which was developed by Zimmet (1988) and whose validity and reliability were assessed by Eker and Arkar (1995), subjectively assesses the adequacy of social support received from three different sources. Three different support sources, family (Items 3, 4, 8 and 11), friends (Items 6, 7, 9 and 12) and a significant other (Items 1, 2, 5 and 10) can be assessed using this scale with twelve expressions. The total score of the scale can also be found by adding the subscale scores. Each item is rated by using a 7-point measure with a Likert-type scoring. The scores of the sub-dimensions in the scale range between 4 and 28 and the total score of the scale between 12 and 84. A high score received indicates a high level of perceived social support. The Cronbach's coefficient alphas of subscales were found to be between 0.77 and 0.92 [16]. In our study, the cronbach's reliability coefficient alpha value of the scale was observed to be 0.82 for the entire scale, 0.90 for the friend and significant other sub-dimensions and 0.87 for the family sub-dimension.

Statistical evaluation

The percentage distribution, ANOVA test, Mann Whitney U-test, Student's t-test and correlation analysis were used in statistical evaluation of the data. The level of significance was accepted to be $p<0.05$ in the study.

Ethical principles

Institutional review board approval was obtained from the research ethics committee of Inonu University. A written permission was obtained from the relevant agency to collect the data. Before administering the forms, the purpose of the study was explained to the women and their verbal approvals were obtained; their privacy was respected. After completion of the study, the mothers included in the study were visited by the investigators at their homes to educate them about postpartum depression.

Results

In the study, 59% of women were in the age group of 26-34, %57.4 of women was primary school graduates and all of them were married. The age of marriage of the women was minimum 15 and maximum 34 years with a mean age of 22.17±22.0. 46.8% of the women perceived their economic status as poor; they had minimum 1 and maximum 4 children and the mean number of children was 2.04±2.0; only 8.5% of them were working and the other 91.5% were housewives; 82.4% of them said that they had planned their pregnancies.

A significant relation was found ($p<0.05$) between their EPDS scores and their education status, economic status, employment status and family type (Table 1), but no significant relation was found between their EPDS scores and their age and husband's education status ($p>0.05$) (Table 1). A significant relation was found ($p<0.05$) between their MSPSS scores and their age groups, education status, economic status and employment status (Table 1), but no significant relation was found between their MSPSS scores and their husband education status and family type ($p>0.05$) (Table 1).

In Table 2 present the distribution of relations between mean scores of EPDS and MSPSS and some fertility data of women. The mean score of EPDS was found 8.70± 4.3 in women marriage age the age group of ≤ 18. The difference was found statistically significant ($p<0.05$). No statis-

Table 1. A comparison between mean scores of EPDS and MSPSS and some demographic data of women

Factors	*n*	*(%)*	EPDS *(Mean SD)*	*Significance*	MSPSS *(Mean SD)*	*Significance*
Age groups						
17-25	38	(20.2)	7.30 (4.6)	0.786	58.13 (15.0)	3.197
26-34	111	(59.0)	6.83 (5.4)	p=0.457 [a]	57.37 (16.7)	**p=0.043** [a]
35 or more	39	(20.7)	5.89 (4.5)		64.84 (14.9)	
Education						
Primary education	108	(57.4)	9.12 (7.6)	4.235	55.46 (17.4)	7.901
High school	49	(26.1)	6.38 (4.6)	**p=0.016** [a]	61. 83 (13.0)	**p=0.001** [a]
University	31	(16.5)	6.22 (4.1)		67.32 (12.4)	
Husband education						
Primary education	74	(39.4)	7.06 (6.7)	1.512	59.05 (18.2)	1.807
High school	69	(36.7)	7.37 (4.6)	p=0.223 [a]	56.76 (15.7)	p=0.167 [a]
University	45	(23.9)	5.95 (4.2)		62.66 (12.8)	
Economic status						
Low	88	(46.8)	8.23 (6.6)	3.569	57.34 (16.5)	9.940
Middle	49	(26.1)	6.75 (4.9)	**p=0.030** [a]	58.30 (17.9)	**p=0.019** [a]
High	51	(27.1)	5.87 (3.7)		62.82 (13.5)	
Employment status						
Housewife	172	(91.5)	6.08 (4.1)	-3.930	58.55 (16.5)	-3.930
Employed	16	(8.5)	3.87 (7.9)	**p=0.001** [b]	64.68 (12.4)	**p=0.001** [b]
Family type						
Nuclear	152	(80.9)	7.17 (5.2)	2.781	56.05 (15.3)	0.216
Large	36	(19.1)	4.94 (4.0)	**p=0.007** [c]	59.79 (16.4)	p=0.200 [c]

[a]*ANOVA test,* [b]*Mann-Whitney U test,* [c]*Student's t-test*

tically significant difference was detected between the other fertility data and between mean scores of EPDS and MSPSS (p>0.05) (Table 2).

The lowest score that can be obtained from the scale is 0 and the highest is 30 points; the mean EPDS score was 6.74±5.0. The MSPSS scores are between 12 as the lowest and 84 as the highest; the mean score was found to be 59.07±16.2 (Table 3). The prevalence of postpartum depression in the women in this study was found to be 15.4% (scores of ≥ 13). A negative correlation was found between their EPDS and MSPSS scores (r=-0.29, p=0.003) (Table 3).

Discussion

When the mean scores of EPDS and MSPSS of the women in postpartum period are and compared with their some demographic data, a significant relation was found between their mean scores of EPDS and MSPSS and their education status, economic status and employment status (Table 1). In addition, with the increase in age, education, husband's education, economic status and employment status level, postpartum depression scores decreased and increased levels of perceived social support. We can relate low PPD scores to the fact that when women come to a better position in terms of their education and economic statuses

Table 2. A comparison between mean scores of EPDS and MSPSS and some fertility data of women

Factors	*n*	*(%)*	EPDS *(Mean SD)*	*Significance*	MSPSS *(Mean SD)*	*Significance*
Marriage age						
≤ 18	44	(23.4)	8.70 (4.3)	5.372	56.86 (19.0)	0.549
19-21	48	(25.5)	5.35 (4.3)	**p=0.005**[a]	59.39 (14.8)	p=0.579[a]
22 or more	96	(51.5)	6.54 (5.4)		59.93 (15.6)	
Number of children						
1 child	71	(37.8)	6.61 (5.2)		59.01 (14.5)	
2 children	62	(33.0)	6.85 (5.5)	0.258	57.61 (17.0)	0.406
3 children	34	(18.1)	7.23 (4.1)	p=0.856[a]	61.35 (16.6)	p=0.749[a]
4 children	21	(11.1)	6.04 (4.8)		59.95 (19.2)	
Planned pregnancy						
Yes	155	(82.4)	5.90 (4.9)	t=1.039	60.00 (15.8)	t= 1.690
No	33	(17.6)	6.92 (7.5)	p=0.295[b]	54.75 (17.8)	p= 0.093[b]
Evaluation of pregnancy						
Good	49	(26.6)	7.16 (6.1)	0.558	59.30 (18.5)	0.960
Middle	70	(37.2)	6.94 (4.6)	p=0.573[a]	60.07 (15.7)	p=0.385[a]
Bad	69	(36.2)	6.23 (4.6)		56.33 (15.9)	
Metod of delivery						
Vaginal delivery	49	(26.1)	7.51 (6.3)	t= 0.136	58.59 (11.5)	t= -0.244
Cesarean	139	(73.9)	6.47 (4.5)	p=0.300[b]	59.25 (16.8)	p= 0.808[b]
Receiving information after the delivery						
Yes	30	(16.0)	6.36 (4.1)	t= -0.517	63.40 (13.7)	t= 1.775
No	158	(84.0)	6.81 (5.2)	p=0.608[b]	57.56 (16.9)	p= 0.078[b]

[a]*ANOVA test,* [b]*Student's t-test*

Table 3. The distribution of relations between mean scores of EPDS and MSPSS

Scales	Mean (SD)	Correlation	Depressed (scored ≥ 13) n (%)	Not depressed (scored<13) n (%)
EPDS	6.74 (5.0)	r= -0.29		
MSPSS	59.07 (16.2)	**p=0.003**[a]	29 (15.4)	159 (84.6)

[a]*Correlation analysis*

and when they get older, these facilitate their adaptation to the role of motherhood. It was reported in the studies made in other countries and in other provinces of in Turkey that a low level of education and economic status in women increased the risk of postpartum depression. İnandı et al. (2002), Mayberry et al. (2007), Ege et al. (2008), Özdemir et al. (2008) and Yildirim et al. (2011) reached similar results in their studies [2,14,17,18,19]. A significant relation was found between the family type and the PPD scores. Women who had nuclear-type families appeared to have higher PPD scores. It was reported in a previous study that having a broad family increased the risk of depression in women [20]. Women having nucleus families was found to have higher PPD scores in the study conducted by Kirpinar et al., which is in line with this study [21].

The relation between the age of marriage and postpartum depression was found to be significant (Table 2). Adolescent women whose age of marriage was below 18 years had higher mean score of EPDS and were lower than the mean score of MSPSS. Bingöl et al. reported in their study that as the age of marriage increased, the level of social support also increased and the level of postpartum depression decreased [22]. Those who were adolescent at marriage have a higher risk of exhaustion and postpartum depression in their progressing years due to a fast physical and psychological development along with difficulties in adapting to the role of a new spouse and a mother [23,24]. When we compared some fertility data of women with their mean scores of EPDS and MSPSS, no significant relation was found in terms of number of children, planned pregnancy, evaluation of pregnancy**,** method of delivery and the receiving information after the delivery.

The prevalence of postpartum depression in the women in this study was found to be 15.4% (scores of $\geq$ 13) (Table 3). The other studies made in Turkey revealed that this ratio ranged between 14.0 and 34.6 [18,25,26]. When we look at the results of the postpartum depression studies carried out by using the EPDS in other countries, the prevalence was found to be 22.6% in a study made in Israel [27] on 288 women in their 6th postpartum weeks; 12.5% in week 8 and 8.3% in week 12 in a study made in Sweden [28] on 1584 women in their 8th and 12th postpartum weeks; 36.7% in a study made in Santiago [29]; and 13.1% in a study made on 352 women at a city center in Portugal [30]. O'Hara and Swain reported in their 12-month study a prevalence of PPD ranging between 10 and 20% [31].

Social support is said to prevent depression by increasing the sense of adequacy with respect to the role of motherhood in women in the period after the end of a delivery [32]. A significant difference was found between Postpartum Depression and the Multidimensional Scale of Perceived Social Support scores in a negative way (r=0.29, p=0.003) (Table 3). In other words, as the mean scores of Multidimensional Scale of Perceived Social Support increased, the scores of a postpartum depression risk decreased. Inadequate social support was found to be an important risk factor for postpartum depression in a study made in Israel [33]. Studies showed that the social support perceived from the family and close associates increased the quality of life and well-being of women and the social support received from the family, husband and healthcare professionals in particular had a significant role in preventing postpartum depression [19,34,35,36,37]. Nurses/midwives must communicate together with the individuals closely associated with the mother, and should act as a bridge to provide social support. The nurses/midwives may help the patient to establish positive interpersonal relationships, develop a positive personality concept and a sense of well-being by enabling her to use her power for her own benefit [38].

Conclusion and Suggestions

These results show that there is a significant relation between postpartum depression and social support, and social support is an important factor in management of postpartum depression. In addition, with the increase in age, education, husband's education, economic status and employment status level, postpartum depression scores decreased and increased levels of perceived social support. Early diagnosis of risk factors for postpartum depression is important for ensuring the safety of both the mother and the new baby or the other children. Therefore, the awareness levels of nurses/midwives should be increased to identify postpartum depression at an early stage and to make the appropriate interventions. In this way, nurses/midwives

can make positive contributions to the community health by raising the quality of life for the women in postpartum period and their families.

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Instructions for the authors

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

Table 1. Page layout description

Paper size	A4
Top margin	20 mm
Bottom margin	20 mm
Left margin	20 mm
Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

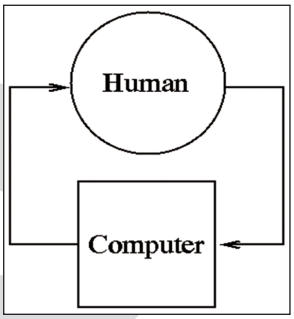

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

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