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# Prediction of preterm birth using vaginal polymorphonuclear count and its ratio to epithelial cells in Iranian women

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## Abstract

**Objectives:** Prediction of spontaneous preterm delivery in Iranian women by comparing the vaginal polymorphonuclear cell (PMN) counts on Gram-stain preparations and the ratios of PMN to epithelial cells (PMN/EPI) at second trimester of gestation.

**Methods:** In a prospective case-control study, 110 cases with spontaneous preterm delivery at gestational age of less than 37 weeks were compared with control group of 110 women at term. Vaginal Gram-stain preparations were collected at the first visit in 23-24 weeks of gestation and the cell counts were performed under oil-immersed microscope. To correct the intra-slide variations in cellular density, PMN/EPI ratios was calculated for each slide field.

**Results:** Mean delivery gestational age were  $34.5 \pm 3.5$  and  $39.4 \pm 1.2$  weeks for the cases and the controls, respectively. There was a significant difference in PMN counts between the two groups ( $P = 0.01$ ). The mean PMN/EPI ratio, however, was significantly higher in case group ( $P = 0.01$ ). No significant difference was found in gravidity between the two groups.

**Conclusion:** Different vaginal PMN count at second trimester were significantly associated with subsequent spontaneous preterm birth. However, the ratio of PMN to EPI count, was significantly higher in women with preterm birth at less than 37 weeks gestation.

**Key words:** Preterm birth, Vaginal Polymorphonuclear cells, Vaginal Epithelial cells.

## 1. Introduction

The incidence of preterm birth in the advanced countries during last 2 decades has remained constant around 5-10%, and despite improvements in perinatal care, it has not decreased over the past 40

years. In the United States in 2000, this rate was 11.6% increasing to 12.5% in 2004 (1, 2).

Preterm birth is the most common cause of infant mortality that is considered as an indicator for health care system internationally. Preterm Infants are at greater risk than infants born at term for mortality and a variety of health and developmental problems, including acute respiratory, gastrointestinal, immunologic, central nervous system, cognitive, visual, hearing, behavioral and growth problems (2).

In the United States, the annual economic burden associated with preterm birth is estimated to include a significant proportion of the health care costs that also includes all costs for long-term consequences of neonatal developmental defects (3).

Early diagnosis is the first step to prevent preterm birth, which is also difficult to achieve. Preterm delivery in women at high risk has been extensively investigated. It has been shown that early symptoms of delivery, including regular uterine contractions and cervical dilatation are not precise indicators to predict preterm birth (4). Implication of a rapid and inexpensive method to predict preterm birth is very important (2). Studies to determine more sensitive and specific new indicators to predict preterm deliveries have been led to introduction of the biochemical and biological markers, including 17-beta-estradiol, progesterone, CRP, saliva estriol and fetal fibronectin (5).

In a study performed during 2004-5 at Iran, investigating a set of variables, including the length of cervical canal, status of employment, education, gestation and delivery, only the cervical canal length was found to be significantly associated with preterm birth (not published data). In another study in Iran to investigate the use of HCG levels in cervicovaginal secretions as a biomarker to predict preterm labor, it was shown that the cervical

HCG levels were significantly higher at women with term delivery, compared with preterm delivery group, with a diagnostic accuracy of 90% (6).

In a study by Ramsey et al. (2005) investigating the PMN/EPI ratio in 83 women with preterm birth and 108 women with term birth, a significant difference was found, with an mean of  $1.8 \pm 2.4$  and  $3.4 \pm 6.0$ , respectively ( $P = 0.01$ ). In this study, the  $\text{PMN/EPI} > 7$  was considered to be significantly associated with premature delivery ( $\text{OR} = 3.8$ ,  $P = 0.01$ ) (7).

In the current study, we aimed to investigate the association of polymorphonuclear cell count (PMN) and PMN cells to epithelial cells ration (PMN/EPI) in vaginal Gram-stained preparations of Iranian women with preterm and term deliveries to better understand their usefulness for further implications and/or studies.

## 2. Materials and Methods

**Patients:** In this case control study, the specimens were collected from the pregnant women with gestational age of 20-25 weeks, based on the date of the last menstruation or pregnancy ultrasound performed at first trimester.

**Non-probable and Convenience sampling:** In this study, 250 women were selected in each case and control groups who had no exclusion criteria, including bleeding, nasal or vaginal discharge, urinary irritation, fever, symptoms of upper respiratory tract infection, diarrhea, symptoms of systemic infection, multiple pregnancies, abnormal ultrasound, polyhydramnios, uterine anomaly (based on HSG levels), had no intercourse at least at night before examination, had not apply any vaginal cream and only visit the doctor for a routine prenatal care. In addition, patients should not be taken antibiotics for any systemic disease during past 10 days.

Samples from upper posterior fornix of the vagina were collected in the lithotomy position using classic cotton applicator and then the slides were stained using Gimsa method for investigation by an independent pathologist who was not aware of patient's record. Twenty weeks later, the patient were contacted to get the required information about their gestational and delivery status. Based on inclusion criteria, 110 patients were selected in each group and their samples were analysed.

The ratios of PMN to epithelial cells in each field of slides were analysed to correct the inter-slide variations as described previously (7).

During the follow-up, the patients who had spontaneous preterm delivery due to severe pre-eclampsia, hemorrhage or properia detachment were also excluded from this study.

## Data analysis

The collected data were analysed using SPSS v18.00 software. For descriptive analysis, the frequency, mode, mean, standard deviation (SD) and standard were determined. For statistical analysis of data, the *t*-test, Levene, chi square tests were used.

## 3. Results

In the current study, 220 pregnant women (110 in each term and preterm deliveries group) attending prenatal clinics operated by Iran university of medical sciences, were investigated.

The mean age of the pregnant women was  $28.7 \pm 6.8$  years (ranging from 16 to 44 years). The mean age of pregnancy in women with preterm and term deliveries were  $34.2 \pm 1.3$  weeks and  $39.4 \pm 1$  weeks, respectively. Forty percent of preterm infants were male and 60% were female. From those with preterm labor, 47% had vaginal delivery and 53% were born by cesarean section. These figures for women with term delivery were 56.4% and 43.6%, respectively. In women with term delivery, 42% of infants were female and 58% were male.

The average gravidity was  $2.1 \pm 0.14$ , ranging from 1 to 6. The average parity was  $1 \pm 0.1$ .

The average number of children was  $0.9 \pm 0.12$ , ranging from 0 to 5 persons.

In this study, 87.5% of participants were Iranian and 12.5% had an Afghan nationality. No significant association was found between the nationality and the preterm delivery ( $P=0.02$ ).

As shown in Table 2, the mean PMN counts were  $42.52 \pm 10$  and  $30.24 \pm 4.76$  in preterm and term delivery groups, respectively, that was significantly different ( $P=0.01$ ). The PMN/EPI ratio was 1.09 in term delivery group, compared to 1.49 in the preterm delivery group, indicating a significant association between the higher PMN/EPI ratio and preterm deliveries.

Table 1. Demographic characters of the women in both groups

	Term delivery, % (n=110)	Preterm delivery, % (n=110)	P value
Mother age, year	6.3 ± 28.97	5.9 ± 28.32	0.58
Age at delivery time, year	1 ± 39.4	1.3 ± 34.2	0.01
Nationality			
Afghan	15 (%14)	12 (%11)	0.37
Iranian	95 (%86)	98 (%89)	
Gravidity	1.12 ± 2.09	1.16 ± 2.05	0.44
History of previous preterm delivery	11 (%10)	13(%12)	0.48
History of previous C-section	48 (%43.6)	58 (%53)	0.1

Table 2. PMN count and PMN/EPI ratio in both groups

	Term delivery, % (n=110)	Preterm delivery, % (n=110)	P value
PMN cells counts	29.35±4.5	45.22±15.3	0.01
Epithelial cells count	1.1 29.25±	32.71±2.5	0.23
PMN/EPI ratio	1.09±0.16	1.49±0.52	0.01

#### 4. Discussion

In the current study we investigated the PMN counts and PMN/EPI ratios in two group of women with term and preterm deliveries.

There is a considerable evidence indicating a link between the upper and lower genital tract infections and the spontaneous preterm deliveries. Polymorphonuclear cells are a part of innate immunity against infectious organisms. These cells can play an important role in stimulating inflammation and create a cascade of events that ultimately led to preterm birth. Several studies have shown a strong correlation between the number of vaginal PMN cells and the preterm labor. Because of the methods used, it is difficult to compare the results between these studies. Some of the limits in these studies are the methods of sampling, such as using smear vs vaginal lavage, and different microscope magnifications used to analyse the samples. Moreover, the PMN counting using Gram stain may introduce errors due to artifacts and variations in the thickness of the samples on the slides that affect the reliability and reproducibility of the counting. In the current study, to obviate these limitations, we used stained smear technique as the gold standard method.

Our study show a significant relationship between the PMN count and the preterm deliveries

( $P = 0.01$ ). AS explained earlier, The ratios of PMN to epithelial cells was used as a standard tool to correct the intra-slide variations.

The PMN/EPI ratios from our study were found to be significantly associated with the preterm deliveries ( $P = 0.01$ ). Previously, similar association was shown by Yamada et al (8) and Simhan et al (9). However, Ramsey and his colleagues found that second trimester vaginal PMN counts on Gram-stain preparations were not significantly associated with subsequent spontaneous preterm birth, despite the significantly higher ratios of PMN/EPI counts in women with subsequent spontaneous preterm birth at less than 35 weeks (7).

It has been also shown that the levels of IL-8 in the vaginal discharge liquid and ammonia are higher in the women with higher PMN count, suggesting a prognostic value for PMN cell number during investigating the preterm labor and infections in upper and lower genital tract (10).

As the PMN/EPI ratio method to predict the preterm birth is very cost effective it can be considered as a useful tool in most part of the world.



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# Analysis of physical and anthropometric maturity in young handball practitioners

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## Abstract

In modern sports, international competition has become fierce, involving increasingly younger athletes. Evaluation of the performance of these athletes is principally based on anthropometric, physical and maturational parameters which give a clear picture of the quality of the athletes.

**Objectives:** to evaluate the anthropometric, physical and maturational characteristics between male and female handball athletes.

**Methods:** the study consisted of 100 adolescents, 50 male and 50 female, aged from 10 to 14 years, participants in an initiation sports project in handball. The anthropometric variables analyzed were: age, height, weight, BMI, skinfolds, the physical variables: speed, power, strength and agility and the maturational variables: bone age and maturational stage according to the Cabral prediction equation (2011).

**Results:** The results presented statistical significance between the anthropometric, physical and maturational variables in both sexes, and the anthropometrical and physical variables with the maturational stage and bone age.

**Conclusion:** From the results of this study it is concluded that a correlation exists between the physical and anthropometric variables and the bone age and maturational stage of the athletes, providing clear evidence of this as a reliable indicator of maturity and demonstrating its importance as a further means of selection and promotion of sporting talent.

**Key words:** Maturation, Anthropometry, Physical qualities, Handball.

## Introduction

Handball is played by about 19 million players, distributed between 800,000 teams around the world. When we talk about sports performance, handball is considered a complex, intermittent sport, requiring maximum intensity efforts in a short period of time, where players must have developed aerobic and anaerobic capacities (1). Various motor skills are considered to be important aspects of the game and contribute to the high performance of the team, such as: speed, strength, explosive power and flexibility (1, 2). In addition, in the model of a modern handball player, specific anthropometric characteristics play a supporting role in helping athletes achieve their best performance under real competitive conditions (3, 4).

In modern sports, international competition has become fierce, involving increasingly younger athletes. Evaluation of the performance of these athletes is principally based on anthropometric, physical and maturational parameters which give a clear picture of the quality of the athletes and form the criteria for their promotion to a higher level. Thus, it is necessary to determine in which period of the human development process the children and adolescents are (5), in addition to seeking new evaluation approaches, using a range of methodological procedures which may support more complete training of each athlete(6).

Previous research has indicated that certain physical characteristics are related to high-level handball performance. In particular, high body weight and height is common among players (7), and evidence suggests that body weight and height



have increased in the junior and senior categories in recent years (8).

It is also known that elite male handball players have higher values of maximum force and muscle power than inactive handball players or those from lower categories. In addition, the speed of the ball in the games is an important skill for success in the sport, since the faster the ball is thrown at the goal, the lower the chance of the defenders and goalkeeper saving the shot.

According to (9, 10) sports coaches, physical and anthropometric variables can no longer be used in isolation when dealing with team building. Maturation, together with bone and relative age should be evaluated and controlled when assessing the potential of an athlete for future progression in the sport as it is extremely important to differentiate those athletes that may have a great future, taking into consideration the increase in children practicing sports at competitive levels at increasingly younger ages.

Successful performance depends primarily on the disciplinary relationship existing between the biological and behavioral characteristics, which influence other variables such as physical, physiological, psychological and perceptual-cognitive behaviour and even the specificity of the sport in question (11). Corroborating this study (9, 12), claim that young people must go through constant anthropometric, maturational and physical fitness evaluations as the realization of physical assessment in sport presents itself as a vital tool in the long term training process.

The implementation of physical fitness tests is a way to evaluate athletic performance and identify whether an athlete is ready to compete, subsidizing the long and short term training programs and identifying the weaknesses of the athletes, among other functions (10). In turn, the measurement of anthropometric variables enables the analysis of aspects that refer to the biological development of young people and could be related to the likelihood of sports injuries.

In younger teams there is a tendency to select young people with precocious physical development to the detriment of those who present late or normal development, in the belief that this could be a competitive advantage. Some researchers have emphasized the fact that higher level

competitive athletes are, in most cases, late in their maturation (10) (13) because, in general, the late maturing athletes are excluded from the training processes as they are not ready to achieve competitive success at the same chronological age as those who mature early.

The truth is that there are few papers that assess the maturation, anthropometric profile and physical condition of handball players together. This lack of research is even more evident in women's elite handball where there are even fewer published studies.

Therefore, the aim of this study was to evaluate the anthropometric, physical and maturational characteristics of male and female handball athletes, in order to provide, in the literature, a better base of the sport in the global context.

## **Materials and methods**

### ***Participants***

The research is characterized as descriptive with a comparative and cross-sectional design. The sample consisted of 100 adolescents, 50 males and 50 females, aged from 10 to 14 years, participants in an initiation sports project in handball, from which they were chosen in a non-probabilistic intentional way.

After selection of the subjects, their parents or guardians were contacted. The Free and Informed Consent form was presented in order to clarify the objectives and procedures of the study and on acceptance and signing, data collection commenced.

In line with the ethical principles of human research, this study was submitted to and approved under protocol no. 866.900/2014 by the Research Ethics Committee of the Federal University of Rio Grande do Norte – RN - Brazil and complied with the international standards on human experimentation (Helsinki Declaration of 1975).

### ***Anthropometry***

For the anthropometric evaluations; weight was measured using a calibrated digital scale (Filizola) with an accuracy of 0.1 kg; height was measured using a stadiometer mounted on the digital scales with a scale of 0.5 cm; skinfolds were measured using a high precision scientific caliper (Harpenden) with a sensitivity of 0.1 mm, according to the

Pollock protocol (1984). The measurements were then submitted to the Prediction equation proposed by Cabral (14).

### ***Bone maturity***

Bone age was measured using the Cabral predictor equation (14), and the anthropometric measurements following the guidelines of the International Society for the Advancement of Kinanthropometry (ISAK) (15). The measurement was performed using the height, weight, corrected arm circumference (CAC), triceps skin fold (TR) and humerus (DU) and femur (DF) bone diameters, according to the Prediction equation of Bone Age, proposed by Cabral 2011:

$$\begin{aligned} \text{Bone age} = & -11.620 + 7.004(\text{height}) + \\ & + 1.226.D_{\text{sex}} + 0.749(\text{age}) - \\ & - 0.068(\text{TR}) + 0.214(\text{CAC}) - \\ & - 0.588(\text{DU}) + 0.388(\text{DF}) \end{aligned}$$

Where:

Dsex = 0 for males and 1 for females,  
Height (m),  
Age (years),  
TR = triceps skin fold (mm),  
CAC = corrected arm circumference (cm),  
DU = humerus diameter (cm),  
DF = femur diameter (cm).

The corrected arm circumference (CAC) was calculated using an anthropometric tape, Sanny® (precision 0.1 mm), to verify the arm circumference in centimeters, less the value of the triceps skin-fold thickness (TR), transformed into centimeters. The bone diameters were measured using a caliper, brand Cescorf® (precision 0.1 mm), to verify the biepicondylar femur diameter (DF) and biepicondylar humerus diameter (DU).

### ***Physical Tests***

The physical qualities evaluated were: muscle strength in the upper and lower limbs, agility, coordination and speed. To collect data on the physical qualities, the most valid physical tests for the sport were performed: 20 meter speed test, Tapping test and explosive power of both upper and lower limbs. The data collected were recorded in the individual records of each athlete (16).

**20 meter speed test:** A track of 20 meters was marked with three parallel lines on the ground as follows: the first (starting line); the second, 20m distant from the first and the third line, marked one meter from the second (finish line). The third line served as an arrival reference for the participant in an attempt to prevent them from starting to slow down before crossing the timing line. Two cones signaled the first and third lines. The athlete started upright, in a standing position, with their front foot immediately behind the first line, and was informed that they should cross the third line as quickly as possible. At a signal from the evaluator, the participant was required to move as quickly as possible toward the finish line. The timekeeper started the clock at the time the participant took the first step (touching the ground), crossing the starting line. When the athlete crossed the second line (20 meters) the timer was stopped. The timekeeper recorded the course time in seconds and hundredths of seconds (two places after the decimal point), according to Gaya and Silva e Silva (2007).

**Explosive Power of the lower limbs (EPLL):** To verify the explosive power of the lower limbs, an Ergojump Jump Pro 2.0-Brazil mat was used according to the Bosco protocol (17). The countermovement jump (CMJ) test was performed with the athlete starting in a standing position, then crouching with legs bent at 90° before jumping straight up. The hands remained on the hips throughout the jump.

**Explosive power of the upper limbs (EPUL):** To evaluate the explosive power of the upper limbs, the medicine ball throwing test was used, whereby the participant sat in a chair, bound by a rope at chest level to avoid rocking during the throw, holding the 2kg medicine ball with both hands against their chest, then, threw the ball as far as possible. Three consecutive attempts were performed and the best result was recorded. A metric tape measure was placed on the floor from the front legs of the chair, and the distance measured between the starting point and the first point of contact of the ball with the ground (Gaya and Silva, 2007).

**Tapping test:** The plate tapping test from the EUROFIT test battery was used to evaluate upper limb speed. Two 20 cm diameter rubber plates were fixed horizontally, 80 cm apart, to a table. A rubber rectangle (10 x 20cm) was fixed at the central point between the two circles. The parti-

participant stood in front of the table, adjusted to their height, with their non-preferred hand in the central rubber rectangle and their preferred hand in one of the circles. At a signal, 25 cycles were performed at maximum speed of the subject moving their preferred hand back and forth between the plates over the hand in the middle as quickly as possible, without moving the hand in the central rectangle. If one of the plates was not hit an additional cycle was added. The lowest execution time of two attempts was computed and two evaluators were used, one for counting cycles and another to time the attempt: Not reported, in the Eurofit test battery, so its validity is *expost factum* (16).

### Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 20.0, was used for the treatment of the data in this study, using descriptive statistics to obtain values of mean, median, maximum, minimum, and standard deviation. With the objective of performing inferential statistical analysis, it was necessary to evaluate the normality of the data collected. Thus, taking into account the biological nature of the measurements, an analysis was performed to verify the normality of the population using the *Kolmogorov-Smirnov* test and the t test for independent samples, considering a signi-

ficance level of  $p < 0.05$ , to reveal possible average differences between the athletes of each sex.

### Results

The descriptive statistics with mean, standard deviation, median, minimum and maximum values of anthropometric, physical and maturational variables of young handball athletes are presented in Table 1. The mean value of the mean age for male and female participants in the study was 13.2 (1.2) years.

Table 2 presents the data relating to inferential statistics of the t-test for independent samples, which were separated by gender, and relating to the presented level of significance.

### Discussion

Little information is available on the anthropometric, physical and maturational variables of athletes for male handball teams, and for female handball, the studies are even scarcer, making comparisons very difficult and almost non-existent (18, 19). In the present study, we identified anthropometric, maturational and physical differences in young handball players. It was also noticeable that the number of young athletes in late maturational stage was 59%, which is extremely useful for the selection of sporting talents.

Table 1. Descriptive statistics of variables

Variables	Mean (SD)	Median	Minimum	Maximum
<b>Anthropometry</b>				
Age	13.2(1.2)	13.8	10.0	14.8
Weight	55.80(7.28)	55.3	41.0	77.7
Height	1.66( 0.08)	1.67	1.5	1.9
Waist	63.8(6.0)	65	48	72
Brainstem height	64(5)	64	50	74
ΣSkinfold	28.91(4.59)	28	19.4	41.9
Bmi	20.3(2.4)	20	15.0	27.3
<b>Physical tests</b>				
20M speed	5.7(1.5)	5.2	3.6	8.5
Cmj	36.3(8.7)	37.0	20.0	53.7
Medicine ball	139.4(7.3)	141.0	121.0	155.0
Tapping test	14.5(1.1)	14.2	12.0	17.6
<b>Maturity</b>				
Bone age	12.7(1.7)	12.8	8.7	21.6
Maturation Category	0.24(1.10) Accelerated	0.0 Normal	-1.4 Late	8.4
Maturational stage	12%	29%	59%	



Table 2. Inferential statistics of the t-test for independent samples

Variables	Men Mean (SD)	Women Mean (SD)	P
<b>Anthropometry</b>			
Age	13.6	12.7	0.000
Weight	56.2	55.38	0.574
Height	1.68	1.62	0.000
Waist	63.98	63.56	0.729
Brainstem height	64.72	63.12	0.111
ΣSkinfold	21.23	26.59	0.000
Bmi	19.7	20.89	0.012
<b>Physical tests</b>			
20M speed	4.4	6.92	0.000
Cmj	43.14	29.46	0.000
Medicine ball	143.94	4.22	0.000
Tapping test	14.62	14.3	0.147
<b>Maturity</b>			
Bone age	12.16	13.18	0.003
Maturation stage	0.006	0.478	0.031

There were significant results when analyzing men and women in the variables studied. Regarding anthropometry we can highlight the age, height, Brainstem height (BH) and cutaneous skinfolds, in the fitness tests: 20m speed, CMJ and medicine ball throwing, and in the maturational context: both the bone age and the maturational stage presented significant results in this study. We can point to a significant relationship between the anthropometric and physical variables and the maturational stage and bone maturation, corroborating studies that claim that bone maturation has become a gold standard in the detection and selection of sports talent (5, 10, 20). Technicians are no longer selecting athletes only by chronological age, but by performing more comprehensive data collection that establishes not only a current profile of the athlete but also, importantly, for the future (10, 21).

For many sporting bodies, the use of the chronological age is facilitated for the selection and promotion of young athletes by the fact that, generally, the categories are guided by this, in addition to evidence of the correlation between chronological age and maturational stage and individual motor performance, as shown by other studies (5, 22).

In spite of this, technicians require more feasible ways to perform a more complete analysis of the athlete, with maturity evaluation through bone

age being one way to achieve this (5). We must not forget that the rate at which the skeleton of a child develops is influenced by environmental, genetic and nutritional factors, among others. The skeletal maturation time is an important way to evaluate the life history and can be used to identify children and adolescents in accelerated or late maturational stages in their bone development, signaling potential problems (23). In addition, forecasts of future maturation spurts are valuable in a setting that uses this variable as a means of selection and sports detection (24).

The talent identification and selection programs should recognize that athletes progress at different rates and personal trajectories, which means that if opportunities are given, high abilities could appear later in life (10, 24, 25). However, what often occurs, at an early age, is that selection is influenced by biological attributes, where athletes with faster maturation are more likely to be selected (9, 26).

However, it is important to consider that the biological advantages of athletes with accelerated maturation are reduced in adolescence and later in adulthood (10, 20, 27). What may seem like an advantage at the time of selection does not translate into effective results since many of these athletes are simply replaced by others who mature a little later (6, 23).

The tallest athletes are routinely accepted as essential for success in handball, but there are other important anthropometric characteristics that need special attention. In particular, a link was observed between age, height and the sum of skinfolds and BMI, which reinforces the results found in the literature reporting the reasonableness of the assumption that high fat can make it difficult for the athlete to run, jump and throw (28, 29). Therefore, control of excess body weight in boys and girls may be an important factor for improvement in sport performance.

With regard to the results of the physical tests we can see that men had the highest average age and the results demonstrated a superiority in speed, strength and power, the women being more agile as evidenced by the results of the Tapping Test and other studies in the literature (14, 28, 30).

Interestingly, physical fitness seems to be as important as height or body weight in the selection of athletes. Some studies indicate a strong trend with significant results ( $p < 0.01$ ) in young people with higher body weight and height, therefore, older and more mature individuals presented better results in performance tests, which agrees with the results shown in the tables above (14, 28, 31).

## Conclusion

From the results of this study it is concluded that there is a correlation between the physical and anthropometric variables and the bone age and maturational stage of the athletes, providing clear evidence of this as a reliable indicator of maturity and demonstrating its importance as a further means of selection and promotion of sporting talent. Such measurements should be considered through practical and easy to use tests, able to minimize the possibility of errors in the training and development of future athletes at different maturation states, thus minimizing errors in choosing them.

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# Correctness of milk and dairy products from small domestic producers, and their actual microbiological quality

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## Abstract

**Introduction and the goal:** Milk and dairy products with the meat represent one of the most important types of food in the human nutrition. The main milk ingredients are water, fat, protein, lactose (milk sugar) and minerals (salt). Therefore, milk is very suitable substrate for the development of all kinds of microorganisms. Milk can be contaminated at any point in the production process. Contamination of milk is manifested as: contamination of raw milk and heat-treated milk contamination. The goal of this study is to examine the accuracy and the microbiological quality of milk and dairy products from small local producers through research time.

**Methods:** With qualitative and quantitative methods of microbiological analysis to examined the presence and number of Salmonella species, sulfite-reducing clostridia, Escherichia coli, aerobic mesophilic bacteria, yeasts, molds, Enterobacteriaceae, Staphylococcus aureus on selective agar on 1 g or ml of a sample.

**Results:** Microbiological norms used in BiH did not satisfy only 0.9% of tested samples. The real microbiological quality of milk and dairy products we get by a comparison of test results with standards used in most developed countries and which are prescribed by the Codex Alimentarius organization according to which 19.2% of the samples did not meet the prescribed quality norms. The highest numbers of samples that do not meet the norms were produced in August.

**Conclusions:** Analysis of test results of dairy products of small local producers indicates a poor microbiological norms that are valid in Bosnia and Herzegovina, because the real microbiological si-

tuation shows that 19.2% of specimens tested product do not meet the microbiological quality, and gained 0.9% comparing with norms, satisfy only the products to be sold in the domestic market, but does not represent a real microbiological quality of the product.

**Key words:** milk and dairy products, small domestic producers, microbiological quality.

## Introduction

From the point of view of healthy and consistent nutrition, milk is in many ways a unique food. (1) The main milk ingredients are water, fat, protein, lactose (milk sugar) and minerals (salt). Therefore, milk is very suitable substrate for the development of all kinds of microorganisms as well as for production of various dairy products such as yogurt, sour cream, butter, cheese and other sorts of fermented products. (2,3)

In small amounts milk contains also other substances such as dyes, pigments, enzymes, vitamins and gases. In order to produce highly qualitative and hygienically proper milk, it is necessary to recognize the milk contamination sources.(4)

Milk can be contaminated at any point in the production process. It is on the manufacturer to detect those points, and to apply the control measures in order to protect the milk from contamination. (5) Contamination of milk is manifested as: contamination of raw milk and heat-treated milk contamination.

The most common sources of contamination of raw milk are coming from the accessories and milking equipment, diseased cows udder and other sources in the environment, and causes are the appearance of bacteria of the genera *Micrococcus*, *Streptococcus*, *Corynebacterium*, *Staphylococcus*,

*Enterococcus*, *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium* and *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Clostridium* spores.

The occurrence of these microorganisms in the finished dairy products is the cause of the contamination of heat-treated milk or errors in the procedures of conducting heat treatment of milk, because almost all of these microorganisms do not have the ability to survive heat treatments of milk.(6,7)

After a well spent cleaning process and heat treatment of milk, as possible sources of contamination of dairy products remain in the air of production space and packaging.(8)

Because of these data milk and dairy products even as highly perishable products are responsible for only 2-6% of foodborne outbreaks in many industrialized countries. (9)

The goal of this study is to examine the accuracy and the microbiological quality of milk and dairy products from small local producers through research time.

## Methods

Microbiological analysis of milk and dairy products were made by the Regulations for con-

ducting microbiological analysis and superanalysis of foodstuffs (Official RBH 2/92, 13 and 14/94) as follows: qualitative and quantitative methods of microbiological analysis examined by the presence and number of *Salmonella* species, sulfite-reducing clostridia, *Escherichia coli*, aerobic mesophilic bacteria, yeasts, molds, *Enterobacteriaceae*, *Staphylococcus aureus* on nutrient agar in 1 g or ml of sample. (10)

## Results

In the time period from June 1<sup>st</sup> 2011 till February 1<sup>st</sup> 2012, for research on microbiological analysis 323 samples of all kinds of milk products were taken from small producers in the area of Posušje. Only 0.9% of the samples did not meet the microbiological norms of Bosnia and Herzegovina required by Regulation on conditions for the microbiological safety of foods which must correspond to the sale terms (Sl. list RBiH 2/92), and regarding microbiological norms Code of hygienic practice for milk and milk products Codex Alimentarius CAC / RCP 57-2004 prescribed by the Codex organization Allimentarius applied in most European countries, 19.2% of the product did not meet the norms. Butter samples in 54.8%

Table 1. Microbiological reference criteria for dairy foods in Bosnia and Hercegovina

Product	Microorganism (group)	n	m	M	n	m	M
			BiH Legislation <sup>1</sup>				Codex Legislation <sup>2</sup>
UHT milk	Total plate count	5	-	100	5	-	1
Cultured dairy product	<i>Salmonella</i> spp.	5	-	0/25g	5	-	0/25g
	<i>E. coli</i>	5	-	0	5	0	0
	<i>Staphylococcus aureus</i>	5	-	0	-	0	0
	Molds	5	-	-	-	0	10
	Coliforms	-	-	-	5	0	5
	Yeasts	-	-	-	5	0	10
	Sulfit-reduc. Clostridia	5	-	0	-	-	-
Butter and butter product	<i>Salmonella</i> spp.	5	-	0/25g	5	-	0/25g
	<i>E. coli</i>	5	-	0	5	-	0
	Coliforms	-	-	-	5	1	10
	<i>Staphylococcus aureus</i>	5	-	0	5	-	0
	Sulfit-reduc. Clostridia	5	-	0	5	-	0

n: The number of sample units to be examined from a lot of dairy food; m: Expressed in CFU/g, it represents an acceptable level and values above it are marginally acceptable or unacceptable in terms of the sampling plan; M: Expressed in CFU/g unless otherwise stated, it is a microbiological criterion which separates marginally acceptable quality from defective quality; Sulfit-reduc. Clostridia: sulfite-reducing clostridia. <sup>1</sup>Regulation on conditions for the microbiological safety of foods which must correspond to the sale (Sl.list RBiH 2/92), <sup>2</sup>Code of hygienic practice for milk and milk products Codex Alimentarius CAC/RCP 57-2004. <sup>3</sup>Ultra high temperature Milk.

Table 2. Results of microbiological analysis of milk products

Product	n	non-acceptable BH Legislation <sup>1</sup>		non-acceptable Codex Legislation <sup>2</sup>	
		n	%	n	%
Yoghurt	94	0	0	11	12,1
Butter	31	3	9,7	17	54,8
UHT Milk <sup>3</sup>	55	0	0	0	0
Liquid yoghurt	101	0	0	26	25,7
Sour cream 12% fat	20	0	0	4	20
Sour ceam 20% fat	22	0	0	4	18,2
TOTAL	323	3	0,9	62	19,2

n: The number of samples; <sup>1</sup>Regulation on conditions for the microbiological safety of foods which must correspond to the sale (Sl.list RBiH 2/92), <sup>2</sup>Code of hygienic practice for milk and milk products Codex Alimentarius CAC/RCP 57-2004.

<sup>3</sup>Ultra high temperature Milk.

do not have a real satisfactory microbiological quality, while all samples of UHT milk meet the microbiological norms (11, 12).

There are significant differences in the appearances of the samples that did not meet the Codex of microbiological norms between different types of milk products  $\chi^2 = 44.674$ ,  $p < 0.05$ .

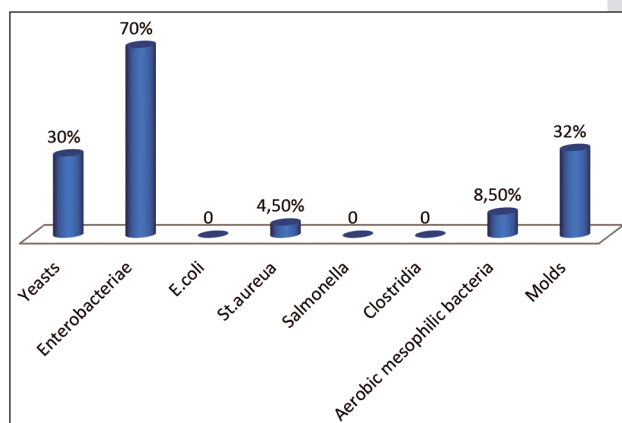


Figure 1. The isolated microorganisms in products that do not meet microbiological norms

In all milk product samples that do not meet microbiological normatives most commonly isolated organisms are species of the family *Enterobacteriaceae* in 70% of cases, while the least are from family of *Staphylococcus aureus*.

Average largest number of all pathogens has been detected in samples of butter. Samples of sour cream (12% and 20% fat) contained on average an equal number of species of the family *Enterobacteriaceae*, while liquid yogurt samples contained more on average of all types of microbes than yoghurt samples.

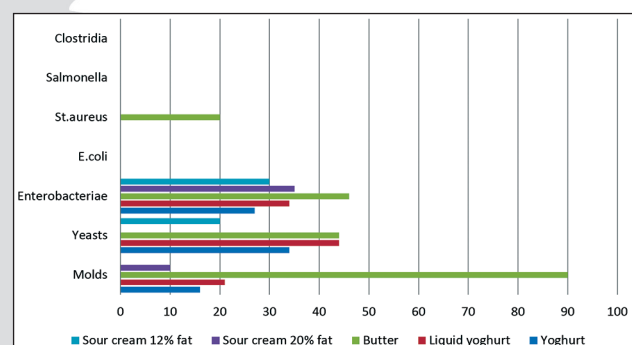


Figure 2. Average number of pathogens isolated in samples of products which don't meet microbiological norms

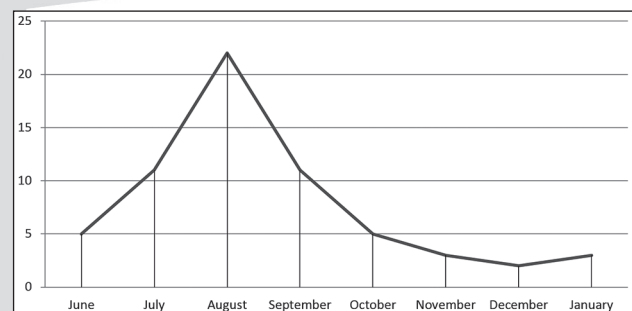


Figure 3. The emergence of samples that do not meet microbiological norms at the time of manufacture

Most dairy products that do not meet microbiological norms are produced during the warmer months (from July to September), as opposed to the products produced in the colder period of the year (from November to January) which makes a very small part of all products that did not meet the norms. The largest number of analyzed samples that did not meet the norms was produced in the August 22 samples, and the smallest in the December is only 2 samples.



A significant statistical difference was found in the appearance of milk products that do not meet microbiological norms by month through research time  $\chi^2 = 25.121$ ,  $p < 0.05$ .

## Discussion

All types of food safety are essential for the health of the consumer. Therefore, a strict control must be performed. A large number of studies in the world are carried out with the task to determine the microbiological quality of the dairy products. Milk and dairy products may be defective due to faulty materials, manufacturing defects or long-standing prior to use (13). From over 323 dairy products only 3 or slightly less than 1% of total products analyzed did not meet currently valid Bosnia and Herzegovina microbiological norms, while the actual situation shows that 19.2% of the samples do not meet the microbiological quality, which is slightly more than the products of small producers with neighboring areas Croatian (14), and much more of the analyzed products from manufacturers from Hungary (15). In samples that do not meet microbiological norms, *Enterobacteriaceae* are present in 70% of samples which is the highest percentage, while *Staphylococcus aureus* are detected in 4.5% of samples. By average largest number of species of microorganisms were isolated in butter samples. The highest number of samples that do not meet microbiological norms were produced in the period from July to September.

Microbiological testing of products during production and immediately after production is the best way of ensuring the microbiological quality of dairy products.

## Conclusions

In this research, we came to the conclusion that the legislation in Bosnia and Herzegovina which is outdated, gives a false picture of the quality of milk and dairy products, as 19.2% of the tested products do not have a satisfactory microbiological quality and only 0.9% do not meet current legislation. Significant impact on the microbiological quality of dairy products has a temperature environment in which they are produced, since most samples that did not meet the microbiological criteria were produced in the warm months from July to September.

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# Application of the todd's method of pubic symphysis in evaluating age skeletal remains in males in Bosnia and Herzegovina

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## Abstract

**Introduction:** For determining the age on a human skeletal remains it is necessary to analysis morphological characteristics of whole skeleton. Morphological changes on pubic symphysis show us a precise information about their age at the time of death. Today, there are many standard methods established on morphological characteristics of pubic symphysis. The first sistematic study about aging determining changes of pubic symphysis is made by anatomist Todd 1920 years. Todd was sorted the morphological changes of pubic symphysis in ten phases, where each phase has a specific span of ages.

**Aims:** The goal of this study was to examine practical application of morphological changes in pubic symphysis according to Todd's method in order to estimate the age of exhumed skeletal remains of male population in Bosnia and Herzegovina.

**Material & methods:** This study included 363 pairs of male pubic symphysis. Date of birth of all the persons was unknown during the study and it was determined additional during the process of identification. The youngest person was 15 years of age, while the oldest one was 75 years old. Morphological characteristics of each pair of pubic symphysis were compared and sorted in one of ten phases according to Todd's standard.

**Results:** For each phase of Todd's standard, an average age, standard deviation and corresponding 95% CI test were determined. Statistical analysis has shown that average values of age, according to Todd's standard, are lower in all phases as compared to average values of age according to original Todd's standard. In addition, standard deviation values were higher in all phases except for phase 1, and our study group had broader age difference as they should according to Todd's

standard. This span of years (in all phases) for our sample was not able to demonstrate the correct borderline between phases in comparison to Todd's standard and in practice, phase 1 which has span from 8 to 28 years that including 2, 3, 4 and 5 phase and phase 10 including 6,7,8 and 9 phase which made this method difficult to apply in practice. Inferent statistical methods (ANOVA i Scheff's test) used in this study also showed the significant difference in determining the age of persons in the study group from the Todd's standard ( $P < 0.0001$ ). Even though, the results obtained by regression analysis are somewhat better from the results obtained by descriptive statistics, nevertheless they still inplay that Todd's methods is not easily applied in practice.

**Conclusion:** This study showed that Todd's method of age evaluation in our population leads to undervaluing the age, but was also overvaluing the age in certain number of cases.

**Key words:** Pubic symphysis, age estimate, Todd's method, identification.

## Introduction

Of the entire human skeleton, morphological changes in the face of pubic symphysis provide the most precise information on the age of a person in the moment of death. Currently, there are several standardized methods based on morphological changes at pubic symphysis. The first pubic symphysis standards in determining the age were set by the US anatomist Todd in 1920, 1921. (1, 2). He presented the morphological changes on pubic symphysis through ten phases, from age 18/19 to age 50+. Afterwards, the other authors developed different modifications of Todd's method (3, 4). Acsadi and Nemeskeri in 1970 presented the morphological changes on pubic symphysis of the

Europeans in five phases of a certain age range (5). Todd's methods were modified by Suchey and Brooks in 1986 and 1990, who presented the age-related changes in the face of the pubic symphysis in six phases (6, 7).

While working on forensic-anthropology analyses of numerous skeletal remains, it was noticed that Todd's method does not provide sufficiently good results in determining the age of our population, in fact it leads to underestimation, and in some cases even to overestimation of age.

### Material and methods

In this study, the pubic symphysis of 363 male persons aged from 15 to 75 was used. During the period of this study, the author did not know the age of persons whose samples were tested, and the exact date and the year of birth were determined after seeing the birth certificate presented by the family during identification, while the date of death pertained to the war period from 1992 and 1995, and in two cases to 1993. The analysed sample comprised only the whole, undamaged pubic symphysis, which were naturally skeletized.

In this study, morphological features of each pubic symphysis pair (horizontal ridges and furrows, granulation, convexity and concavity of symphysis face, dorsal and ventral margin, upper and lower extremities, abdominal slope, symphysis frame, ossification of excrescences) were compared with clearly defined morphological features of pubic symphysis standard according to Todd, which

were defined by narrative description, presented on photos for all 10 (ten) age development phases. In further work, the age of each pubic symphysis pair was evaluated, and they were grouped to one of 10 (ten) age phases of pubic symphysis, which is by its morphological features the closest to the Todd's standard.

### Results

363 pubic symphysis pairs were grouped according to Todd phases and the medium age in the moment of death was determined for each phase, as well as standard deviation and related 95% confidence interval. Table 1.

The Table 1 shows that median values of the estimated age of our sample were smaller in all phases in comparison with Todd's standard. The value of standard deviations of tested sample increases with years from the second to the tenth phase, while in the first phase they total 4.76. It is evident that 95% CI of estimated age according to Todd in all phases have much wider range than original Todd's standard and that 95% CI in some classes includes the scale of several nearby phases per Todd, which makes this classification hardly applicable in practice. The results of study showed that in estimated age of our sample according to Todd there were individual cases of discrepancies in relation to real age. So, in the first phase, an exceptional overestimation of age in relation to actual age is noticed in the third, the fifth and the tenth phase at several cases, where the age was par-

*Table 1. Medium values, standard deviations and 95% CI according to Todd phases for our sample and Todd's standard*

Todd phases	N- number of cases	Xs -Todd	95% CI Todd	Xs $\pm$ SD BH Todd	95% CI BH Todd
1	28	18.5	18-19	17.75 $\pm$ 4.76	8-28
2	19	20.5	20-21	20.37 $\pm$ 0.50	19-22
3	21	23.0	22-24	22.24 $\pm$ 1.81	19-26
4	15	25.5	25-26	24.27 $\pm$ 1.83	20-28
5	16	28.5	27-30	26.50 $\pm$ 2.00	23-30
6	67	33.0	30-35	30.30 $\pm$ 5.71	19-42
7	34	37.5	35-39	35.00 $\pm$ 8.77	17-53
8	40	42	39-44	41.65 $\pm$ 6.44	29-55
9	47	47.5	45-50	45.53 $\pm$ 6.05	33-58
10	76	60.0	>50	55.69 $\pm$ 8.09	31-62

*Xs- Todd's phases medium values of age, SD – standard deviation, BH- our sample*



ticularly underestimated, while in the fourth, the sixth, the seventh, the eighth and the ninth phase there were several cases where the age was underestimated, but also overestimated. The Scheffé's test proved that there was statistically significant discrepancy between the forecasts according to Todd and actual life age ( $P < 0.0001$ ).

Median values of estimated age according to Todd obtained through regressive analysis were smaller in the first, the second, the eighth, the ninth and the tenth phase, and bigger in the third, the fifth, the sixth and the seventh phase in relation to the median value results obtained based on the descriptive statistics. Standard deviations obtained from regression analysis and those obtained from descriptive statistics are almost the same in the first five phases, while in the sixth, the seventh, the eighth, the ninth and the tenth phase the values of standard deviations are much smaller in regression analysis. It was determined that 95% CI obtained from regression analysis is somewhat bigger in the second, the third, the fourth and the fifth phase, while it is much narrower in the first, the sixth, the seventh, the eighth, the ninth and the tenth phase, in range from 11 to 24 years, in comparison with the results obtained from descriptive statistics. It is evident that 95% CI obtained from the regression analysis in all phases has much wider range than 95% CI of original Todd's standard and that 95% CI includes the scale of several surrounding phases per Todd, which makes this classification hardly applicable in practice. Table 2.

*Table 2. Age in the moment of death according to Todd's classification for our tested sample obtained from regression analysis*

Todd phases	$\bar{X}_s \pm SD$ regression analysis	95% CI
1	$16.10 \pm 1.64$	12-20
2	$19.50 \pm 1.70$	15-24
3	$22.90 \pm 1.80$	18-27
4	$26.20 \pm 1.93$	25-31
5	$29.60 \pm 2.10$	24-35
6	$33.00 \pm 2.27$	27-39
7	$36.30 \pm 2.46$	30-42
8	$39.80 \pm 2.67$	33-46
9	$43.10 \pm 2.90$	36-50
10	$46.50 \pm 3.11$	39-54

$\bar{X}_s$  - medium values,  $SD$  – standard deviation

## Discussion

Results of descriptive statistics of the estimated age of tested pubic symphysis sample according to Todd's method showed that median values of years are lower in all phases in relation to median values of years from the original Todd's standard. So, the median value of years of the estimated age of our sample in the phases 1-9 is smaller to 2.7 years, while in the phase 10 the median value of estimated years of our sample is smaller for 4.3 years. The values of standard deviations at estimated age according to Todd show the growing trend with years, except in the phase 1, which for our sample totals  $\pm 4.76$ , while in the seventh phase the standard deviation is the biggest and it totals 8.77.

The results clearly show that in the phase 1 there is a case of age overestimation, in the phases 3 and 5 there are several such cases, and in the phase 10 there are many cases of age overestimation, while in the phases 4, 6, 7, 8 and 9 there are the cases of age underestimation and overestimation.

Comparing the values of years range for each phase from the Todd's standard and values of years age for our sample obtained from the estimates according to Todd, it is evident that those ranges are much bigger for our sample and do not show clear limitations of years range between the phases, as it is the case in Todd's method. The table 1 shows that for our sample according to Todd the years range of one phase includes several surrounding phases, for example the phase 1 which ranges from 8 to 28 years includes the phases 2, 3, 4 and 5, while the phase 10 from our sample includes with its range the phases 6, 7, 8 and 9, which makes this method hardly applicable in practice.

Although the results from the regression analysis are somewhat better than those from descriptive statistics, e.g. the values 95% CI obtained from regression analysis are generally smaller, but still insufficiently to make the limitations in age ranges between the phases clearer as in the original Todd's standard, the results of the regression analysis in the process of life age evaluation on bosnian-herzegovinian population are hardly applicable in practice.

Analysis of the results from our study clearly indicates the problem of applicability of Todd's method of pubic symphysis in determining the

life age of human skeletal remains at our population. Therefore, it is necessary to conduct additional research which would result with the pubic symphysis model applicable to our population and whose results would be more precise.

### Conclusions

Todd's method of life age evaluation at our sample leads to underestimation and overestimation of age.

Values of year range (95% CI) for our sample and for each phase according to Todd are much higher than original Todd's method. Results 95% CI for our sample do not show proper limitation of year range among the phases, as it is the case in Todd's standard. Year range of one phase according to Todd is too high for our sample, so that they include the scale of several surrounding phases, which makes this method hardly applicable in practice.

Results of regression analysis in evaluation of life age of our population according to Todd generally show narrower 95% confidence interval (CI) in relation to the results obtained from descriptive statistics, but their range still includes several surrounding phases.

Numerous contemporary studies based on modern well-documented pubic symphysis samples indicate the existence of significant differences in human skeletal morphology among one population, as well as racial differences, which is practically shown by our study as well. It indicates that precise evaluation of human skeletal age is possible only with development of specific models for certain population.

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# Effect of Propolis on Blood Glucose Levels in Diabetes Studies with Rats: A meta-analysis study

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## Abstract

In the studies on diabetic organisms, it was shown that propolis has a potential to decrease blood glucose levels of diabetic organisms. However the current findings represent conflicting results. For making the situation clearer, there is a need to make an analysis of the findings on the effect of propolis on blood glucose levels of diabetic organisms. Therefore the purpose of this study is to make a meta-analysis of the studies on the effect of propolis on blood glucose levels in diabetic organisms. In the study 10 effect sizes (7 studies) about levels of blood glucose in the literature were analyzed by calculating individual effect sizes. The effect sizes were changed from -0.01 to +8.5 and mean effect size value (-1.5) showed a large effect that propolis was an effective agent to decrease blood glucose levels of diabetic organisms. The findings showed that blood glucose levels of the subjects before propolis application are higher than blood glucose levels after propolis application to diabetic subjects. In conclusion, propolis is an important agent for decreasing blood glucose levels of diabetic subjects.

**Key Words:** Propolis, diabetes mellitus, blood glucose level, meta-analysis.

## Background of the Study

Diabetes is a common disease that has prevalence of 382 million people in the world (Shi & Hu, 2014). Diabetes involves lack of sufficient secretion of insulin or lower rate of insulin activity (Ayoub, 2009; Delibaş & Kılınç, 2003). Diabetes is a serious illness which might lead to neuropathy, cardiovascular diseases, retinopathy, ulcers and amputations (Ciechanowski, Katon, Russo & Walker, 2001; WRIGHT, Piroli, Grillo & Reagan, 2009). To find solutions on the problems regarding diabetes, different animal models and treatment agents were used in previous studies (Lenzen, 2008; King, 2012;

Roumie, Greevy & Griffin, 2014; Usman, 2014). Among treatment agents propolis is frequently emphasized in the current studies (Usman, 2014; Aral, Kesim, Greenwell, Kara, Çetin & Birkan, 2015; Henshaw et. al., 2014). Propolis is a resinous organic matter involving Zn, Ca, Mg, K, Na, Fe, Cu, Mn minerals and herbal matters, it is produced by honeybees (Fuliang, Hepburn, Xuan, Chen, Daya, & Radloff, 2005; Chen, Long, Han, Wang, Chen, & Wang, 2008; Marcucci, 1995). Role of propolis in diabetes treatment is to prevent harm of  $\beta$  cells which are units of insulin secretion in pancreas tissue (Fuliang, Hepburn, Xuan, Chen, Daya, & Radloff, 2005). By this effect propolis might balance blood glucose levels of the patients having high blood glucose levels. However the studies measuring blood glucose levels of diabetic organisms such as rats and humans before and after propolis application show contradictory findings (Hu, Li, Chen & Xuan, 2012; Aoi, et. al., 2013). Some of them indicate lower levels of blood glucose than non-application stage levels after propolis application while the others show higher levels of blood glucose than non-application stage levels after propolis application (Chung, Cho, Ha & Kim, 2010; Hu, Li, Chen & Xuan, 2012). For making the effect of propolis on blood glucose levels in diabetic organisms, there is a need to make a meta-analysis. Based on this idea, the purpose of this study is to make a meta-analysis of the studies on the effect of propolis on blood glucose levels in diabetic organisms.

## Materials and Methods

This study was designed as a meta-analysis study that is a synthesizing process of findings of different studies by statistical techniques (Maksimović, 2011; Fleiss, 1993). In the study 7 studies were analyzed by calculating Cohen's  $d$  effect size value and finding mean effect size value.



### ***Selection of the Publications***

For selecting the publications four databases (PubMed, Google Scholar, Proquest, National Theses Database System) were investigated by using key words “propolis, blood, glucose level, diabetes” for the time interval 2000-2015. National Theses Database System provided one thesis while Proquest provided 78 theses. Pubmed gave 34 results but Google scholar provided 6260 results. After checking abstracts and content of the publications, it was seen 7 studies reported change in blood glucose levels of diabetic organisms and they also gave 10 effect size statistics for different doses of propolis. Detailed knowledge about the publications and titles of them is seen in table 1 and table 2.

### ***Calculation of Effect Sizes and Analysis***

In this study there are two different aspects of the calculation; one for the difference between pre and post measurements and one for the difference

between experimental and control groups. In the analysis both of the differences were accepted as an effect indicator of propolis, therefore two different Cohen d formulas were applied to all differences in this study (Rosnow & Rosenthal, 1996; Cumming, 2012).

$d = (M_1 - M_2) / \sqrt{[\sigma_1^2 + \sigma_2^2 / 2]}$  for independent measures

$d = [M_1 - M_2] / SD_{\text{pooled}}$  for dependent measures,

$SD_{\text{pooled}} = \sqrt{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 / n_1 + n_2}$

All of the individual effect size per difference for each propolis dose were calculated by using two formula then mean effect size value was calculated by adding all effect sizes and dividing total into number of individual effect sizes. Therefore just one indicator of effect of propolis on blood glucose levels was gathered.

*Table 1. Descriptions of the publications in this study*

Publication Date	Name of Journal or Institution	Subject	Propolis Amount in the Application	Duration of Propolis Application
2005	Pharmacological Research	Rats	100 mg/ml	56 days
2009	Pakistan Journal of Pharmaceutical Sciences	Rats	300 mg/kg bwt	40 days
2010	Korean Journal of Veterinary Service	Rats	0.9 ml	28 days
2011	Inonu University, Health Sciences Institute	Rats	0,012 g/ml	30 days
2011	Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy	Rats	0.3 g/kg	42 days
2012	Evidence-Based Complementary and Alternative Medicine	Rats	100 mg/kg 50 mg/kg 200 mg/kg	70 days
2013	Biochemical and Biophysical Research Communications	Rats	%1 w/w %5 w/w	56 days

*Table 2. Titles of the publications*

Publication Date	Titles of the publications
2005	Effects of propolis on blood glucose, blood lipid and free radicals in rats with diabetes mellitus
2009	Experimental diabetic nephropathy can be prevented by propolis: Effect on metabolic disturbances and renal oxidative parameters
2010	Hypoglycemic effect of nano powder propolis on streptozotocin-induced diabetic rats
2011	Effect of propolis on learning and memory of streptozotocin-induced diabetic rats
2011	Glycemic control and anti-osteopathic effect of propolis in diabetic rats
2012	Effects of encapsulated propolis on blood glycemic control, lipid metabolism, and insulin resistance in type 2 diabetes mellitus rats
2013	Improvement of insulin resistance, blood pressure and interstitial pH in early developmental stage of insulin resistance in OLETF rats by intake of propolis extracts

## Results

The findings of this study showed that 6 of the effect sizes indicated positive values while 4 of them were negative. At the same time one small, two medium and 7 large effect sizes were determined. Descriptive values regarding Blood Glucose Levels, Unit of Blood Glucose Levels and Individual Effect Sizes were represented in table 3.

It seen in the table 2, the effect sizes were changed from -0.01 to +8.5. When looked at the mean effect size value, it was found that mean effect size regarding the individual effect sizes was -1.5.

## Discussion and Conclusion

The findings of the study showed that propolis is an effective agent to decrease blood glucose levels of diabetic rats. This result is in line with literature.

Chung, Cho, Ha and Kim (2010) showed that propolis decreased significantly blood glucose levels of diabetic rats. Similarly Koksall (2011) also showed that propolis decreased significantly satiety blood glucose levels of diabetic rats. The highest effect of propolis on decreasing the blood glucose levels was determined after 56 day application and by applying %5 w/w dose of propolis. Actually differences in units for propolis amount and inducing diabetic situation make evaluation of the optimum time and dose hard. But it can generally be said that propolis is an effective agent for balancing increased blood glucose levels of diabetic rats. More technically Effect size value of -.15 means that effect of propolis on blood glucose levels in diabetic rats has large effect size (Cohen, 1988). In other words the mean of the propolis applied group is at the 93 percentile of the no treatment group.

*Table 3. Descriptive Values regarding Blood Glucose Levels, Unit of Blood Glucose Levels and Individual Effect Sizes of the Studies*

Publication Date	Name of Journal or Institution	Blood Glucose Level before Propolis Application	Blood Glucose Level after Propolis Application	Unit of Blood Glucose Level	Effect Size
2005	Pharmacological Research	21.904 ± 1.99	21.954 ± 5.15	mmol/L	0,01 (+)
2009	Pakistan Journal of Pharmaceutical Sciences	61.60 ± 4.28	97.20 ± 6.57	mg/dl	6,42 (+)
2010	Korean Journal of Veterinary Service	100.4 ± 1.3	259± 51.7	mg/dl	4,33 (-)
2011	Inonu University, Health Sciences Institute	301,08±56.42 (Satiety)	253,15±100.22 (Satiety)	mg/dl	5,76 (-)
2011	Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy	143.9± 6.8	153.7 ± 4.28	mg/dL	1,72 (+)
2012	Evidence-Based Complementary and Alternative Medicine	7.30 ± 0.77	7.88 ± 0.46	mmol/L	0,92 (+)
2012	Evidence-Based Complementary and Alternative Medicine	7.20 ± 1.73	7.51 ± 0.50	mmol/L	0,24 (+)
2012	Evidence-Based Complementary and Alternative Medicine	7.17 ± 1.20	7.37 ± 0.68	mmol/L	0,21 (+)
2013	Biochemical and Biophysical Research Communications	130 ± 2	115 ± 3	mg/dl	5,90 (-)
2013	Biochemical and Biophysical Research Communications	130 ± 2	113 ± 2	mg/dl	8,5 (-)

In this study number of the publications might be seen as insufficient, but meta-analysis studies give valuable synthesis about making decisions on an effect without relying on only one study. So findings of the study has a potential for informing effect of propolis on blood glucose levels of diabetic rats. At the same time, current publications analyzed in this study represent a clear picture of current practice, so the findings of the study also inform practice using propolis for balancing diabetic blood glucose levels about dose and time of application. Moreover, this study improves power of the inferences about the effect of propolis on blood glucose levels of diabetic rats by analyzing publications more than one.

### Suggestions

In spite of advantages of the study, number of the publications should be increased in following studies to reach more comprehensive results. At the same time, other publications on organisms rather than rats should also be investigated for possible effectiveness of propolis on blood glucose levels of diabetic rats. Also time interval should also be increased for including more studies on the propolis' effect on blood glucose levels.

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# Female genital mutilation: a multi-country study

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## Abstract

**Objective:** Female genital mutilation (FGM) is a major women's health problem and human right violation. FGM has several physical and psychological consequences. The focus of the current study is the extent of FGM, the association of demographic and economic factors with FGM and women's beliefs and attitudes towards FGM in Egypt, Guinea, Mali and Sierra Leone.

**Methods:** The study used national representative, cross-sectional, household sample surveys with large sample of women of reproductive age (15 – 49 years) from each country. Multi stage cluster sampling and face-to-face interviews were used. It was cross-sectional analysis, using DHS data. Cross tabulation, multivariate analyses and bar-diagram were used.

**Results:** In Egypt 94%, in Guinea 97%, in Mali 89% and in Sierra Leone, 91%) women had genital mutilation. The majority of the respondents believe that FGM is socially acceptable and a religious obligation. Majority of the respondents believe that FGM helps to maintain virginity and that it leads to better marriage prospects and the prevention of adultery.

**Conclusions:** The majority of women in the four countries argued in favor for the continuation of FGM. There is an inverse relationship between FGM and higher education and affluence. Proper policies and awareness generation among less educated and less affluent women in rural areas are warranted in an attempt to reduce FGM.

**Key words:** Female circumcision, Genital mutilation/cutting, Egypt, Guinea, Mali, Sierra Leone.

## Introduction

Female genital mutilation (FGM) or female genital cutting (FGC) or female circumcision is a major women's health problem and a serious hu-

man right violation in respect of girls and females. It has gained increased attention in policy-making and research in the last few decades (1). More than 140 million women worldwide have experienced FGM, with a further three million more having it every year (2, 3). FGM is mainly concentrated in Africa (being practiced in 28 countries across Africa). There is also evidence of FGM in Middle Eastern, Asian, European and North American migrant-receiving countries (1, 3).

The process and typologies of FGM are described elsewhere (1). There are serious psychological and physical consequences of FGM. Physical consequences include infections (tetanus, septicemia, vulvovaginitis etc.), abscesses, hemorrhages, acute anemia, chronic pain, cheloid formation, necessity in reintervention, primary infertility, clitoral cysts and birth complications (1, 4). FGM may lead to chronic health problems such as various infections of urinary tract, gynecological and obstetric problems including difficulties with urination, problems associated with menstruation, problems during coital penetration, extreme sensitivity in clitoral remnants and sexual dysfunction (1, 4, 5, 6). Psychological consequences of FGM may include shock, anxiety, depression and post-traumatic stress disorder (PTSD) (4,5,6).

Studies have shown that of all the countries with a history of FGM, Egypt (97%), Guinea (96.6%), Mali (93.7%) and Sierra Leone (90%) had the highest FGM prevalence (7). Somalia has also very high prevalence of FGM (6). The extent, nature, determinants and consequences of FGM have been studied mostly in small areas in different individual countries (1, 7, 8, 9, 10,11 ). There has been no individual comparative study undertaken to measure the extent and nature of FGM, and the beliefs and attitudes of FGM victims by using same questionnaire and nationally representative samples, in the countries with the highest

prevalence of FGM. Efforts have been made in the present study to explore the following factors in Egypt, Guinea, Mali and Sierra Leone:

1. The prevalence of FGM.
2. The association of demographic and economic factors with FGM.
3. Women's beliefs surrounding FGM.
4. Women's attitudes towards the continuation of FGM.

## Subjects and Methods

We used latest available data from Demographic and Health Surveys (DHS) conducted between 2005 and 2008 in Egypt (2008), Guinea (2005), Mali (2006) and Sierra Leone (2008). DHS surveys were implemented by respective national institutions in collaboration with ORC Macro International Inc., and financially supported by the United States Agency for International Development (USAID). DHSs are nationally representative, cross-sectional, household sample surveys with large sample sizes.

DHS methods and data collection procedures have been described elsewhere (12, 13, 14, 15). Women of reproductive age (15-49 years) constituted the study samples. Multi-stage cluster sampling, using strata for rural and urban areas in different regions of the countries and probability proportional to sample (PPS) procedures were used to select the respondents. Standardized questionnaires were administered by the interviewers in face-to-face interviews with the participants in each country. Similar questionnaires were used in each of the countries in order to yield inter-country comparable data. The number of respondents included in the current study are Egypt, N=16523; Guinea, N=7944; Mali, N=13251 and Sierra Leone, N=7279.

### Variables of interest

Respondent has FGM or not (i.e. circumcised or not).

Types of FGM: Flesh was removed from the genital area; Genital area was nicked without removing any flesh; Genital area was sewn closed and Do not know. (Such information was lacking for Egypt).

FGM beliefs were measured by the following options: FGM has religious requirement, social acceptance, better marriage prospect; provides

better cleanliness/hygiene; provides sexual pleasure for men; preserves virginity and prevents adultery; has other benefits and has no benefits.

FGM shall be continued or not, with following options: *continued, discontinued and don't know/depends.*

### Demographic factors and economic status

Age group in seven intervals (15-19, 20-24, 25-29, 30-34, 35-39, 40-44 and 45-49 years), urban/rural residence, education (no education, primary, secondary and higher) and economic status in five quintiles (poorest, poorer, middle, richer and richest) are the main predicting variables which were utilized.

Economic status was a composite measure of the cumulative living standard of the household. It was calculated by using household level data. Elements included in the construction of the index were any economic item - specifically most household assets and utility services, including country-specific items. Principal components analysis, a generated statistical procedure, was used to estimate individual households on a continuous scale of relative wealth. The scale was standardized in a normal distribution and the standardized scores were then used to create the wealth quintiles groups as: poorest, poorer, middle, richer, and richest (16).

### Statistical issues

Prevalence estimates were calculated for each country in order to measure female genital mutilation or genital cutting or circumcision. Bar diagrams were used to represent information relating to the extent of FGM, and beliefs and attitudes surrounding FGM. Proportions and chi-square tests were used to explore the cross-relationships between circumcised women and their demographic backgrounds and economic status. Multivariate logistic regressions were used to study the potential associations between circumcised women and their demographic backgrounds and economic status. A p-value at the five percent significance level was used in the analyses. IBM SPSS version 20 was used for data analysis.

### Ethical issues

This study is based on an analysis of existing survey data with all identifier information remo-



ved. Informed consent was obtained from the participants before starting the surveys. Participants' "Right to withdraw" was emphasized throughout the survey. The surveys were approved by the Institutional Review Board of Opinion Research Corporation, Macro International Incorporated and the national agencies of the respective countries.

## Results

In Egypt 15605 (94.4%); Guinea 7691 (96.8%); Mali 11742 (88.6%) and in Sierra Leone 6619 (90.9%) women had genital mutilation. The average age of circumcision was 9 years in Egypt (mode, 10 years; standard deviation, 2.51); 6 years in Guinea (mode, infancy; standard deviation, 4.78); 32 months in Mali (mode, 1 year; standard deviation, 3.71) and 10 years in Sierra Leone (mode, 1 year; standard deviation, 5.88). Figure 1 demonstrate distribution of age of circumcision in four countries. However, a considerable number of respondents (22-31%) did not know the age of their circumcision. The following percentages of the respondents' daughters also had FGM: Egypt: 43.7%; Guinea: 87.8% Mali: 47.1%; Sierra Leone: 80.4%.

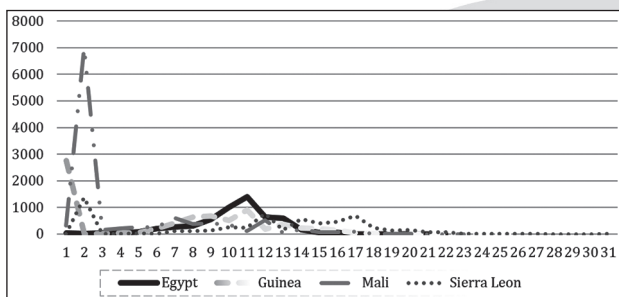


Figure 1. Distribution of age of circumcision

Table 1 indicates that in three countries, apart from Mali, the number of circumcised women increases with age. Mali had almost same proportion of circumcised women in urban and rural areas, while the other three countries had proportionally more circumcised women in rural areas. It was clear in all countries that higher educated women and economically more affluent women were less likely to be circumcised compared with the illiterate and poorest women.

Multivariate logistic regression analyses indicated that compared with teen-aged respondents (15-19 years), women aged 45 and above were five

(Egypt), eight (Sierra Leone) and 16 (Guinea) times more likely to have been circumcised. Higher educated women and women of richest economic statuses were less likely to have been circumcised, compared with the illiterate and poorest women (except in Mali).

The majority of the respondents believed that FGM has certain benefits. Religious requirements (43–63%) and social acceptability (37–67%) represent the highest proportions. Cleanliness and hygiene (13–24%); maintenance of virginity and the prevention of adultery (6–37%) are also important. Respondents also believed that FGM leads to better marriage prospects and that it is associated with enhanced sexual pleasure for men (figure 2). In Egypt 40% and in Sierra Leone 28% of respondents stated that that FGM has no benefits.

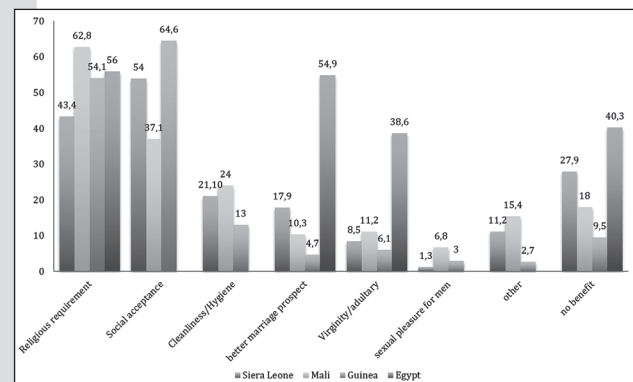


Figure 2. Beliefs of benefits of FGM

In all countries the majority of the respondents (Egypt 57%; Guinea 69%; Mali 74%; Sierra Leone 66%) strongly argued in favor of continuation of FGM. In Egypt, however, almost 30% and in Sierra Leone 27% of respondents argued for the discontinuation of FGM (figure 3).

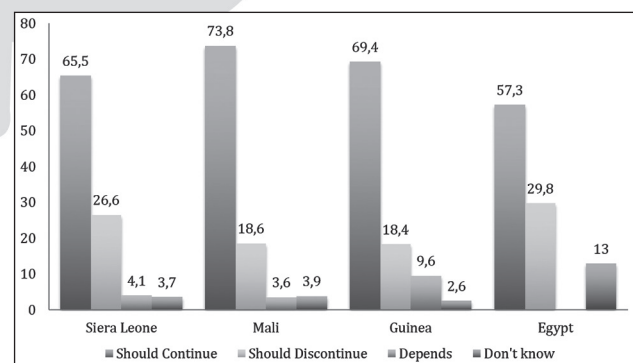


Figure 3. Attitudes towards continuation of FGM

Table 1. Demographic factors and economic status of circumcised (FGM) women in Egypt, Guinea, Mali and Sierra Leone.

	Egypt		Guinea		Mali		Sierra Leone	
	No. of respondent (N)	Circumcised n (% of N)	No. of respondent (N)	Circumcised n (% of N)	No. of respondent (N)	Circumcised n (% of N)	No. of respondent (N)	Circumcised n (% of N)
<i>Age group</i>								
15 – 19 years	636	P<0.001 582 (91.5%)	1630	P<0.001 1506 (92.4%)	2788	2473 (88.7%)	1237	P<0.001 910 (73.6%)
20 – 24 years	2621	2410 (91.9%)	1135	1085 (95.6%)	2412	2130 (88.3%)	1179	1040 (88.2%)
25 – 29 years	3318	3083 (92.9%)	1239	1203 (97.1%)	2328	2087 (89.6%)	1562	1487 (95.2%)
30 – 34 years	2702	2553 (94.5%)	1113	1094 (98.3%)	1785	1572 (88.1%)	1054	1006 (95.4%)
35 – 39 years	2553	2456 (96.2%)	1191	1182 (99.2%)	1583	1405 (88.8%)	1082	1051 (97.1%)
40 – 44 years	2439	2347 (96.2%)	825	813 (98.5%)	1261	1113 (88.3%)	655	628 (95.9%)
45 – 49 years	2254	2174 (96.5%)	811	808 (99.6%)	1094	962 (87.9%)	510	497 (97.5%)
<i>Place of residence</i>								
Urban	6674	P<0.001 6018 (90.2%)	2534	P<0.001 2251 (95.6%)	4793	4235 (88.4%)	3126	P<0.001 2641 (84.5%)
Rural	9849	9587 (97.3%)	5590	5440 (97.3%)	8458	7507 (88.8%)	4153	3978 (95.8%)
<i>Education</i>								
No education	5538	P<0.001 5341 (96.4%)	6218	P<0.001 6087 (97.9%)	10193	P<0.001 9125 (89.5%)	4553	P<0.001 4434 (97.4%)
Primary	2042	1967 (96.3%)	864	809 (93.6%)	1579	1377 (87.2%)	985	861 (87.4%)
Secondary	7134	7134 (95.4%)	816	751 (92%)	1386	1168 (84.3%)	1539	1193 (77.5%)
Higher	1809	1491 (82.4%)	46	44 (95.7%)	93	72 (77.4%)	202	131 (64.9%)
<i>Economic status</i>								
Poorest	3412	P<0.001 3284 (96.2%)	1613	P<0.001 1572 (97.5%)	2354	P<0.001 2254 (95.8%)	1305	P<0.001 1254 (96.1%)
Poorer	3368	3248 (96.4%)	1603	1578 (98.4%)	2590	2359 (91.1%)	1210	1162 (96%)
Middle	3382	3284 (97%)	1555	1496 (96.2%)	2586	2189 (84.6%)	1289	1232 (95.6%)
Richer	3210	3076 (95.8%)	1666	1606 (96.4%)	2579	2162 (83.9%)	1545	1414 (91.5%)
Richest	3151	2713 (86.1%)	1507	1439 (96.8%)	3142	2776 (88.4%)	1930	1557 (80.7%)

P-values of chi-square test.

Table 2. Multivariate regression analysis of FGM for demographic factors and economic status of Egypt, Guinea, Mali and Sierra Leone.

	Egypt				Guinea				Mali				Sierra Leone			
	Exp(B)		95% C.I. for EXP(B)		Exp(B)	95% C.I. for EXP(B)		Exp(B)	95% C.I. for EXP(B)		Exp(B)	95% C.I. for EXP(B)		Exp(B)	95% C.I. for EXP(B)	
	Lower	Upper	Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
<i>Age</i>																
15 – 19 yrs	1.00				1.00			1.00			1.00					
20 – 24 yrs	1.394	1.007	1.93†		1.511	1.069	2.136†	0.935	0.785	1.113	2.429	1.918			3.076*	
25 – 29 yrs	2.089	1.509	2.893*		2.064	1.391	3.064*	1.028	0.856	1.234	5.16	3.863			6.893*	
30 – 34 yrs	2.912	2.071	4.096*		3.659	2.218	6.036*	0.838	0.692	1.014	6.644	4.701			9.389*	
35 – 39 yrs	4.261	2.969	6.115*		8.034	4.026	16.03*	0.9	0.736	1.1	8.875	5.956			13.225*	
40 – 44 yrs	4.451	3.086	6.419*		4.266	2.319	7.846*	0.835	0.674	1.034	6.445	4.178			9.943*	
45 – 49 yrs	4.908	3.365	7.159*		16.392	5.159	52.08*	0.806	0.645	1.006	8.491	4.711			15.302*	
<i>Residency</i>																
Urban	1.00				1.00			1.00			1.00					
Rural	3.45	2.865	4.156*		0.982	0.646	1.491	0.724	0.618	0.849*	1.392	1.055			1.837†	
<i>Education</i>																
No education	1.00				1.00			1.00			1.00					
Primary	1.159	0.875	1.534		0.498	0.35	0.707*	0.778	0.658	0.921*	0.354	0.267			0.469*	
Secondary	1.409	1.131	1.756*		0.387	0.265	0.563*	0.579	0.482	0.695*	0.232	0.179			0.301*	
Higher	0.477	0.369	0.616*		0.363	0.083	1.578	0.343	0.207	0.569*	0.066	0.045			0.098*	
<i>Economic status</i>																
Poorest	1.00				1.00			1.00			1.00					
Poorer	1.15	0.886	1.493		1.791	1.079	2.972	0.449	0.352	0.572*	1.112	0.729			1.695	
Middle	1.737	1.298	2.324*		0.765	0.506	1.157	0.237	0.189	0.298*	1.284	0.849			1.941	
Richer	1.668	1.239	2.246*		1.092	0.685	1.741	0.216	0.171	0.272*	1.019	0.685			1.514	

1.0 Denotes reference category. Significance level: “\*&gt;=&gt; p&lt;0.001”; “† =&gt; p&lt;0.050”



## Discussions

The current study indicates that Egypt (94.4%); Guinea (96.8%); Mali (88.6%) and Sierra Leone (90.9%) have very high FGM prevalence rates. In Egypt (2008) and Sierra Leone (2008) there has been no mentionable change with the previous findings in 2005, since the rate only drops from 97% to 94.4% for Egypt and almost the same (90.9%) for Sierra Leone (7). In Mali there was a decrease from 93.7% to 88.6% during the same period. It is notable that during this period, Mali had taken steps towards ending FGM (8). The estimated reduction in the FGM rate in Mali might be an effect of such actions.

Higher education and better economic status have a significant impact on reduced rates of FGM. However, the majority of the women justify FGM as a religious obligation and socially acceptable, which can be described as a 'normalization of FGM' in daily life in order to continue the practice. Attempts to change such aspects of culture in daily life are usually regarded as being sensitive (1, 6, 8). Our findings suggest that women need more education in order to help lead to a reduction in FGM. The literature suggests that legislation only against FGM is not an effective way to reduce its prevalence. Legislation should also be incorporated with adequate education and awareness-raising among the general population in order to control FGM (1, 5, 6, 17, 18). It is extremely important to note that in all the countries surveyed, the majority of the respondents supported the continuation of FGM. Despite several government policies and regulation (4, 6, 8) women's attitudes are not changing. This should be very alarming to policy makers. Even though FGM rates have reduced somewhat, there is a need for general attitudinal changes, in order for more meaningful reductions to be realized. It appears, therefore, that the policies which were designed to control FGM seem to be not functioning properly or are not being sufficiently implemented. Special attention also needs to pay to the problem of FGM in rural areas (19).

The current study is based on DHS data. There are limitations with this data. For example, Somalia has a very high prevalence of FGM but we could not include Somalia in the analysis because of the unavailability of data. Even so, the statistics that were

available for use in the current study were valuable and they were suitable for examining in relation to the research questions. A cross sectional design was used in this study and as such the attribution of causality may be problematic. Thus, studies with a longitudinal design are needed to confirm potential causal links and to examine trend in FGM rates and related beliefs and attitudes.

## Key messages

Higher education and better economic status have a significant impact on reduced rates of FGM.

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# Plagiarism in scientific circles

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## Abstract

**Background:** Currently, plagiarism is one of the main concerns in the scientific community, once it affects not only the quality of the published articles, but also the reliability of the findings and the scientific progress. This is a problem that must affect not only publish editors, for reducing the number of published cases, but senior authors as well, once they are usually the supervisors of science students.

**Objective:** Here we intend to clarify the most typical cases of plagiarism, the main reasons for its occurrence and the most effective ways of restraining it.

**Results:** The most frequent cases of plagiarism are ghostwriting - when a pharmaceutical company pays a recognized author to publish favorable articles - and institutional plagiarism - when an institution takes the credit for a research developed somewhere else. In addition, there are the plagiarisms committed by graduation students, often related to inexperience in the writing process or in the English language.

**Conclusion:** The increase of plagiarism and the lack of proper punishments for those who perpetrate it causes this issue to become a major topic of discussion among the scientific community. It is essential for supervisor students to behold possible cases of plagiarism in the graduation, preventing its publication and teaching the students on how to avoid such situations.

**Key words:** academy, ghostwriting, institutional plagiarism, plagiarism.

## Discussion

In the writing scientific articles, several errors can be made by those who are not familiar with the scientific process. These research violations are not uniform: they include issues of research integrity (e.g., publishing of falsified data), where the scientific enterprise is victimized; violations of human subjects (e.g., improper or inadequate consent processes), where individual (and potentially vulnerable) human beings are victimized; and animal care violations, where nonhuman animals are victimized<sup>1</sup>. One of the standout mistakes committed by the writers is the plagiarism, which occurs when an author takes credit for writing or ideas that have come from someone else<sup>2</sup>.

Unfortunately, this has often occurred in the academic world, undermining the transparency of various articles. While in universities students may be caught for plagiarizing a whole paper or a group of sentences, reports of plagiarism in scientific publication have centered around the stealing of data (duplication of the research itself), but rarely, if ever, concerns the copying of a few sentences (duplication of segments of text)<sup>3</sup>. A recently published meta-analysis of survey data on this topic estimates that almost 2% of scientists have admitted fabricating, falsifying, or modifying data at least once and, when asked about colleagues' behavior, over 14% of scientists surveyed reported knowing of data falsification<sup>4,1</sup>.

One type of plagiarism that has grown is ghostwriting. A growing literature<sup>5,2</sup> is documenting the practice of medical ghostwriting, whereby pharmaceutical companies draft favorable scientific articles, send them to academic physicians or researchers who sign on as author, and publish the articles



in medical journals<sup>2</sup>. In this aspect, is important for academics to educate undergraduate and postgraduate students about plagiarism and its avoidance<sup>6</sup>.

Often the pharmaceutical company contracts the work out to a medical education and communications company, which executes key steps in the process such as drafting the article, inviting academics to be author, paying these honorary authors typically \$1,000 to \$2,000 per article<sup>7,2</sup>, and a range of other 'publication planning' services<sup>8,2</sup>.

Another type of plagiarism is often observed when the regulations for submissions to these Research Assessment Exercises (RAEs) gave full credit for any published work to the institution employing an author at the end of the assessment period, regardless of the fact that all the published work may have been carried out elsewhere<sup>6</sup>. At the same time, plagiarism by business students in higher education institutions appears to be on the rise (Park, 2003)<sup>9</sup>. The rules of metrics-based systems can encourage universities, if not to pass work off as their own, at least to gain credit from research in which they played no part<sup>6</sup>. For some Chinese scientists text-based plagiarism, which typically involves the replication or patch writing of sentences from a source or sources, should be discussed in a framework of ethical research conduct<sup>3</sup>. However, the scientific community should question the desirability of systems that deny credit to those institutions in which work has been carried out (providing the research environment and support), only to reward recruiting institutions with credit for work in which they played no role. Alternative assessment systems could be devised to eliminate or reduce the effects described above<sup>6</sup>.

The reasons for plagiarism are too many, and the main problems in the conduct of research arise in part due to self-serving biases, faulty mental models for research, stress, and the failure to forecast long-term consequences of actions, including especially consequences to others<sup>10-19,1</sup>. Usually, the copying was not "ill-intentioned" but it was above all, due to the students' "lack of scientific perspicacity" (from their novice status in conducting scientific research) and their "difficulty in English"<sup>3</sup>.

To solve this frequent problem in the scientific environment, university policies incorporate many and varied penalties and strategies to address the problem of plagiarism<sup>20,9</sup>. Such resolutions are

essential for the academic community, once plagiarism is undetected, it distorts the scientific record, substituting marketing and persuasion for the balanced exchange of views and the search for sound answers that characterize the contents of medical journals at their best<sup>21,2</sup>. There is another way to circumvent the plagiarism. Journal editors have suggested "senior authors"<sup>22,3</sup> or "mentors of young researchers"<sup>23,3</sup>, should take responsibility in disseminating the ethics of research and publication, including in the prevention of "linguistic plagiarism"<sup>3</sup>.

In the moment that students can understand the gravity of this problem, the indices of plagiarism may fall. An understanding of the factors underpinning student plagiarism will better inform curriculum design and policy development aimed at reducing the incidence of plagiarism<sup>9</sup>. Universities should have vigorous recruitment policies to attract research active members of staff who publish regularly in highly-rated journals to enhance departmental research capabilities (although as investors are warned, past success cannot be taken as an indicator of future performance)<sup>6</sup>.

Furthermore, honorary authorship should be frowned upon by the research community in the same way that other types of plagiarism are frowned upon<sup>2</sup>. Clearly it is important for academics to educate undergraduate and postgraduate students about plagiarism and its avoidance. However, we should be aware of the wider context that is set by universities and governments as we assess the ethical framework that surrounds this issue and the message that is sent to students and the wider community<sup>6</sup>.

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## Instructions for the authors

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# Preparing Article for HealthMED Journal

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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 mm<sup>2</sup>) 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)

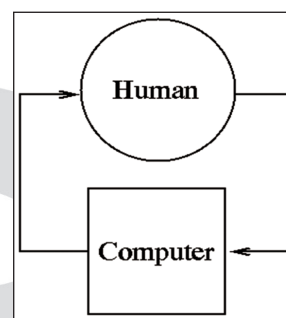


Figure 1. Text here

### 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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