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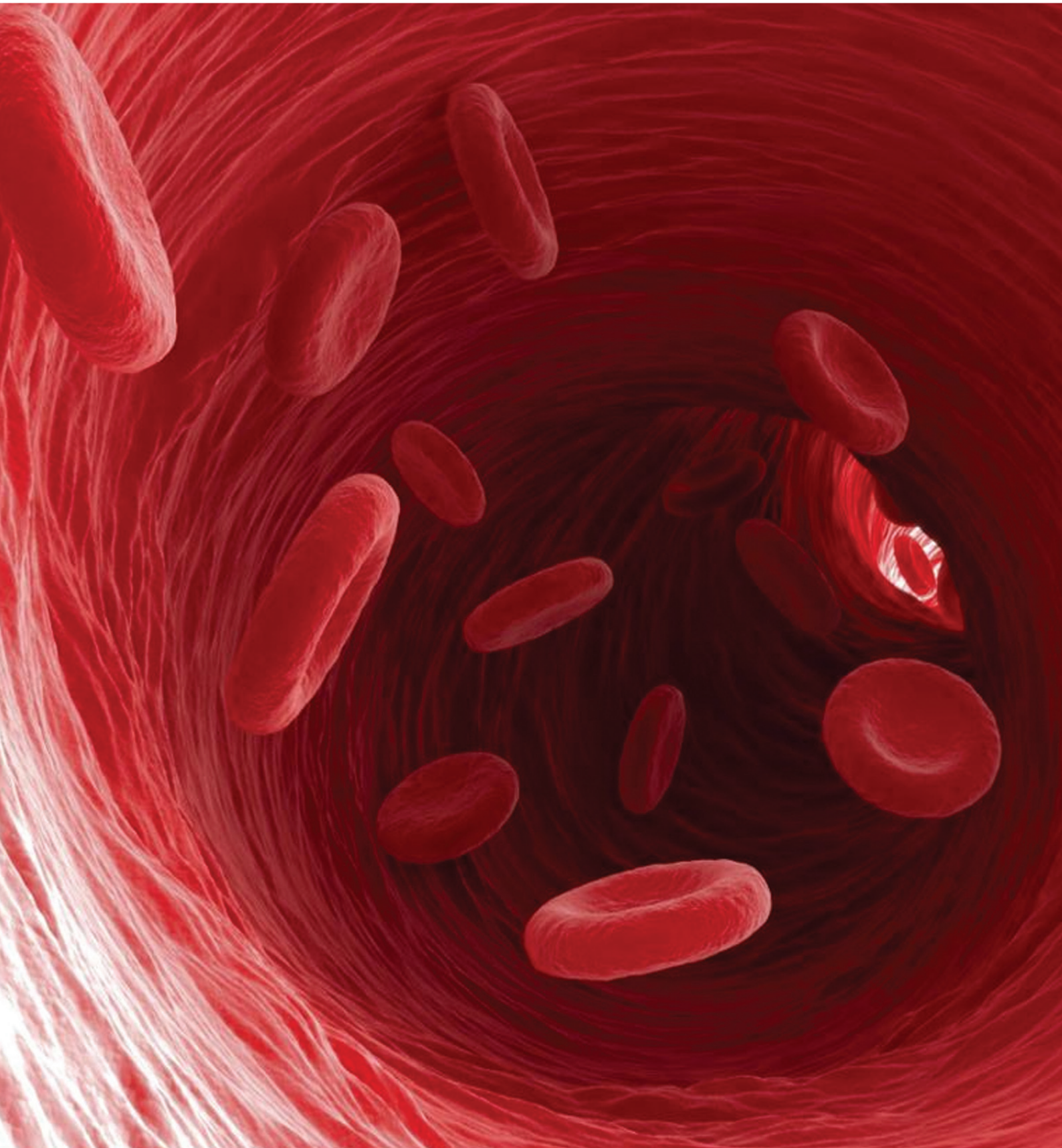

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Multyvariate analysis of Cranioscopic and Craniometric parameters in gender determination of skulls

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Abstract

Objective: Current trend in forensic medicine and anthropology when it comes to gender determination based on human skulls is to apply discriminant function analysis of data obtained from measurement of linear dimensions. Aims of this study were to determine the prediction (effectiveness) of gender assessment based on quantitative and qualitative analysis of the skulls. The next step was to compare the craniometric and cranioscopic methods used for gender assessment based on human skulls and investigate correlation relationships between linear dimensions and cranioscopic characteristics in observed skulls.

Methods and material: The material used for this study were samples of 211 macereted and degreased human skulls, 139 male and 72 female, with average age of male skulls was 52.6±19.84, while average age of female skulls was 52.0±20.66. The operating method was the craniometric and cranioscopic. We analyses seven linear dimensions and ten cranioscopic characterisitcs on each skulls.

Results: Using multivariant binary logistic regression some of linear dimensions stend out with predictiv power that allows creating the model for gender prediction. In our study that are D2 (porion-porion), D4 (zygion-zygion) and D5 (nasion-prosthion). Combining the mentioned D2, D4 and D5 dimensions with cranioscopic characteristics we derive the "D1" probability pattern for gender determination using linear dimensions and cranioscopic characteristics that showed statistically significant effect on gender dimorphism in skulls in our investigated sample. Accuracy of gender assessment of our investigated sample using "D1" probability patternwas 91% for male skulls, while the accuracy of gender assessment for female skulls was lower and is 67.7%.

Conclusion: We would like to propose the application of D1 probability pattern for gender determination of skulls. It is exactly the combination of cranioscopic and craniometric parameters with population proliferation and application of discriminant function analysis that gives the best results in gender determination based on human skulls.

Key words: skull, discriminant function analysis, binary logistic regression, craniometry, cranioscopy, gender dimorphism.

Introduction

Gender assessment based on human skeleton has its significance and aplication in forensic medecine, anatomy, physical anthropology, archaeology (1). Morphological differences between male and female skeletons start developing even before birth and continue to increase during childhood and adolescence, which increases the precision of sex determination of the skeleton (2).It is well-known that skeletal features vary between populations, therefore each regional variation should have its own specific standard for sex determination. The gender will be assessed with the highest accuracy if it is possible to make analyze of the whole intact human skeleton. Numerous qualifications distinguish pelvis, as marker part of skeleton for profiling the gender, due to numerous details that are gender dependent and caused by functional morphological differences (birth channel in females). Besides the pelvis, the most discriminate ability for gender identification has skull, moreover if mandible is present. (1) Krogman ranks the precision of sex determination as follows: based on pelvis 95% of accuracy, skull with 92% accuracy, onlymandible90% accuracy, and pelvis and skull 98% accuracy, other bones 80% accuracy (3).

Methods of sex determination based on human skull have developed over time, therefore, nowadays, in addition to the classical visual methods, we also use methods based on univariate and multivariate statistical analysis, with special focus on discriminant function analysis of obtained data. Gender assessment based on osteoscopic analyze can be very subjective and depends on the experience of the person determining sex. Morphometric methods based on measurements are accurate and can be used for sex determination with higher reliability (4). The list of researchers that widen our knowladge related to qualitative and quantitative gender assessment by human skull is numerous (Bubić Ilduza 1973; Zavando MDA et al 2009; Maina et al; Altayeb Ahmed et al; Nidugala et al; Vidya et al; Marinescu et al; Deshmukh AG). (5, 6, 7, 8, 9, 10, 11, 12).

There is no doubt that gender predilection based on human skull is still one of the most interesting craniology issues. (1) The next step was to compare the craniometric and cranioscopic methods used for gender assessment based on human skulls and investigate correlation relationships between linear dimensions and cranioscopic characteristicsin observed skulls.

Methods and material

The material used for this study were samples of 211 macereted and degreased human skulls, 139 male and 72 female, with average age of male skulls was 52.6±19.84, while average age of female skulls was 52.0±20.66. The operating method was the craniometric and cranioscopic. We analyses seven linear dimensions and ten cranioscopic characterisitcs on each skulls. All skulls are from Osteologic collection of the Department of Anatomy, Medical Faculty University of Sarajevo.

Qualitative (cranioscopic) analysis was performed through observation: expression of parietal tuber; expression of frontal tuber; expression of superciliary archand glabella; appearance of supraorbital margin; appearance of orbit; width of nose root; size of mastoid process; expression ofmuscle insertions; size of teeth; face shape.

Characteristic male sings of skulls we observed lack of un-expressed frontal and parietal tuber, expressed superciliary archand glabella, thicker supraorbital margin, square orbits, narrow nose root, massive mastoid process, prominent muscle insertions on bones, big teeth and oblong face shape. Characteristic female sings of skulls are opposite of male.

Craniometric analysis included measurement seven craniometric dimensions wich represent in table 1. All measured dimensions are expressed in centimeters (cm).

Table 1. Review of linear dimensions

Craniometric dimensions	Measuring device
D1 glabella-opisthion (max. cranial length)	Kephalometer
D2 porion-porion (max. cranial width)	Kephalometer
D3 vertex-porion (max. cranial height)	Slide divider
D4 zygion-zygion (max. viscerocranium width)	Kephalometer
D5 nasion-prosthion (upper facial height)	Slide divider
D6 nasion-basion	Kephalometer
D7 basion-prosthion	Slide divider

Prior measurement itself, skull is put in position of "Frankfurt horizontal plane" (Figure 1)

Statistic methods

For statistical data analysis, we used SPSS for Windows (version 19.0, SPSS Inc., Chicago, Illinois, USA). By univariate binary logistic regression, we examined the influence of the size of the individual dimensions and cranioscopic characteristics of the skull (expressed/not expressed) on the prediction of the binary output-gender (male/female). Variables that showed a statistically significant influence were examined in the multivariate binary logistic regression and we defined pattern for gender determination using their standardized coefficients for calculating the constants of the equation:

$X = Constant_{(pattern)} + B1Y1 + B1Y2 + BnYn$ where B1-n is the standardized coefficients for each non-invariant variable, and Y1-n is the independence of the variable with a statistically significant prediction on the envelope of the gender variable.

The pattern is reprezented by formula: Pattern "D1" = exp (X) / (1+ exp (X)).

The pattern was derived from the log or natural logarithm of the chance: log it (p) = ln (p/1-p). By

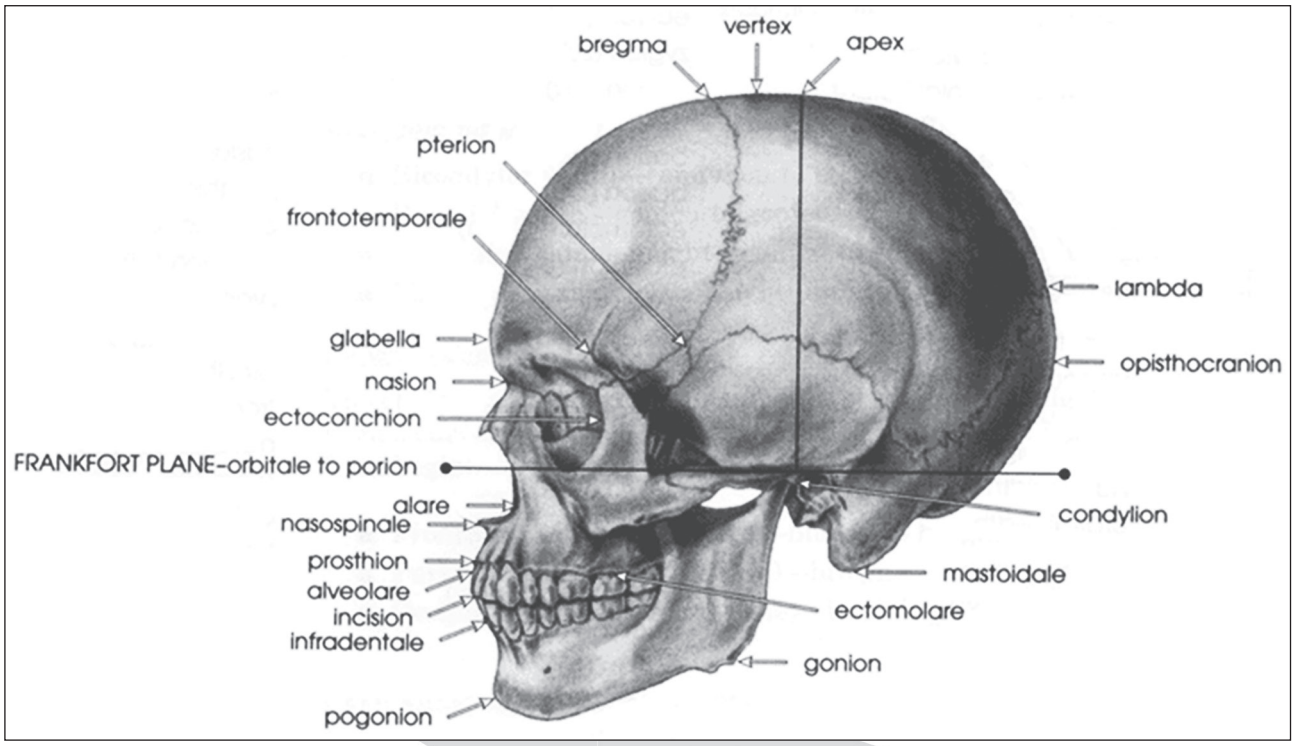

Figure 1. Frankfurt horizontal plane and anthropometric points – side view (23)

exploring the logarithm, ie by using EXP (logit) we get the chance of e $^{logit(p)=}$ (p) = EXP (ln (p / 1-p)). For the limit of statistical significance we took the value α = 0.05

Results

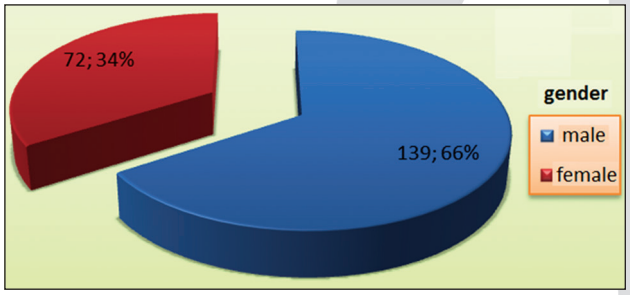

Chart 1. Age structure of the sample

Using multivariate binary logistic regression, five-step BackvardWald method, we assaying the influence of autonomous variables (craniometric measurements-linear dimensions) on gender dimorphism in the skull.

Statistically significant multivariate influence on gender dimorphism have shown three linear dimensions D2 (porion-porion), D4 (zygion-zygion) and D5 (nasion-prosthion) p<0.05. Linear dimensions D1 (glabella-opisthion), D3 (vertex-porion), D6 (nasion-basion) and D7 (basion-prosthion) have not shown any statistically significant multivariate influence on gender determination in skulls (p>0.05).

Table 3 and table 4 shows the number of craniоscopic characteristics of male skulls on a sample N (male) =139, except for the width of the nose root, where the number of the observed sample was N¹=100 due to a certain number of skulls with transitory form of this characteristic; and except for the teeth size, where the number of the ob-

Table 2. Multivariate influence of linear dimensions on gender dimorphism in skulls

		B	S.E.	Wald	Df	P	Exp(B)	95,0% C.I.for EXP(B)	
								Lower	Upper
Step 5(a)	D2	-,464	,163	8,134	1	**,004**	,629	,457	,865
	D4	-1,562	,359	18,899	1	**,000**	,210	,104	,424
	D5	-1,144	,338	11,468	1	**,001**	,318	,164	,618
	Constant	33,366	5,147	42,019	1	,0005			

Table 3. Frequency of cranioscopic characteristics in male and female skulls

Gender/ number of skulls	Parietal tuber		Frontal tuber		Superciliary arch and glabella		Supraorbital margin		Orbit	
	Not prominent♂	Prominent ♀	Not prominent ♂	Prominent ♀	Not prominent ♀	Prominent ♂	Blunt ♂	Thickened♀	Round ♀	Quadrangular ♂
M/139	60	79	79	60	21	118	102	37	102	37
F/72	17	55	21	51	46	26	33	39	33	39
Σ	77	134	100	111	67	144	135	76	135	76

Table 4. Frequency of cranioscopic characteristics in male and female skulls

Gender/ number of skulls	[1]Root of nose (100M; 61F)		Mastoid process		Muscle insertions		[2]Teeth (67M;39F)		Face shape	
	Wide ♀	Narrower ♂	Not prominent ♀	Prominent♂	Not prominent ♀	Prominent ♂	Small ♀	Big ♂	Elongated ♂	Round ♀
M/139	48	52	48	91	14	125	25	42	114	25
F/72	38	13	49	23	28	44	31	8	47	25
Σ	86	65	97	114	42	169	56	50	161	50

Table 5. Multivariate influence of cranioscopic characteristics on gender dimorphism

		B	S.E.	Wald	df	p	Exp(B)	95,0% C.I.for EXP(B)	
								Lower	Upper
Step 4(a)	Frontal tuber	,646	,376	2,954	1	,086	1,908	,913	3,985
	Superciliary arch and glabella	-1,852	,383	23,426	1	**,000**	,157	,074	,332
	Mastoid process	-,733	,368	3,967	1	**,046**	,481	,234	,988
	Muscle insertions	-,875	,450	3,770	1	,052	,417	,172	1,008
	Face shape	,786	,422	3,475	1	,062	2,196	,960	5,019
	Constant	,992	,544	3,316	1	,069	2,695		

served sample was N²=67 due to lack of teeth in other observed skulls. Fields highlighted in blue mark the number of characteristics in male skull.

The same table shows the number of cranioscopic characteristics of female skulls on a sample N (female) =72, except for the width of the nose root, where the number of the observed sample was N¹=61 due to a certain number of skulls with transitory form of this characteristic; and except for the teeth size, where the number of observed sample was N²=39 due to lack of teeth in other observed skulls. Fields highlighted in yellow show the number of characteristics in female skull.

Using the multivariate binary logistic regression, method BackWard:Wald in four steps, we estimated the influence of autonomous cranioscopic characteristics on gender dimorphism in skulls (table 5).

Cranioscopic characteristic expression of superciliary arch and glabella and cranioscopic characteristic size of mastoid process have shown statistically significant influence on gender dimorphism in skulls p<0.05. Other cranioscopic characteristics have not shown any statistically significant multivariate influence p>0.05.

Using the multivariate binary logistic regression, we estimated the impact of autonomous craniometric and cranioscopic variables on genderdimorphism in skulls (table 6).

Expression of superciliaryarch and glabella, D4, D5 and D2 have shown significant statistical influence p<0.05 on prediction of gender dimorphism in skulls. The influence of size of mastoid process is indicative p=0.067.

Probability pattern "D1" for gender identification in skulls

Based on the results presented in Table 6 we decided to constructed the "D1" pattern for prediction of gender in skulls.

The pattern is presented by equation:

Pattern „D1"= exp(X) / (1+ exp(X)) * 100

*D1=26,67 – (1.022 * D4) – (0.944 * D5) - (0.482 * D2) – (1.641 * superciliary arch and glabella")*

1-"arch prominent; 0-"arch not prominent

Table 7 indicates that the "D1"pattern indicated 121 skull as male, out of which 110 were actually male skulls. Therefore, prediction of male gender using the "D1"pattern is 110/121=0.91 or **91%** accuracy.

The "D1"pattern indicated 90 tested skulls as female, out of which 61 tested skulls were actually female. Therefore, prediction of female gender using the "D1"pattern is 61/90=0.677 or **67.7%** accuracy.

Discussion

Craniometrical method is consistent of measuring skull single linear dimension using kephalometer or slide divider, according to precise defined

Table 6. Multivariate influence of autonomous craniometric and cranioscopic characteristics on gender dimorphism in skulls

Y		B	S.E.	Wald	df	p	Exp(B)	95,0% C.I.for EXP(B)	
								Lower	Upper
Model „D1"	Superciliary arch and glabella	-1,641	,408	16,153	1	**,0005**	,194	,087	,431
	Mastoid process	-,718	,392	3,364	1	,0670	,488	,226	1,051
	D4	-1,022	,396	6,663	1	**,0100**	,360	,166	,782
	D5	-,944	,369	6,529	1	**,0110**	,389	,189	,803
	D2	-,482	,173	7,772	1	**,0050**	,618	,440	,867
	Constant	26,677	5,313	25,209	1	,0005			

Table 7. Gender prediction using the "D1"pattern

		Pattern "D1"		Total
		<= 40,62	40,63+	
Gender	Male	**110**	29	139
	Female	11	**61**	72
Total		*121*	*90*	*211*

methodology of measuring skull linear dimensions among two specific anthropometric points. (1). In our study we measured:

D1 (glabella-opisthion);
D2 (porion-porion);
D3 (vertex-porion);
D4 (zygion-zygion);
D5 (nasion-prosthion);
D6 (nasion-basion);
D7 (basion-prosthion).

Certainly, each of these linear dimensions has an impact on the determination of gender of the skull in its domain that can be proven by binary logistic regression. However, only by application multivariate binary logistic regression, it is proven multivariate influence of certain linear dimensions on the determination of gender of the skulls.

Using method BackWard:Wald in four steps, in our research stand out, in terms of statistical significance, three linear dimensions D2, D4 and D5 ($p<0.05$), while D1, D3, D6 and D7 have not shown any multivariately statistically signifikant influence ($p>0.05$).

Especially, we distinguish effect of linear dimension D4 (zygion-zygion) which has shown the greatest effect in the group (Wald=18.9 $p<0.0005$, OR=0.21, and 95% CI (0.10-0.42)). Each increase of D4 by 1 cm reduces the chances of the skull being female by 79%, or in the interval from 90 to 58%. When it comes to the greatest multivariate effect of D4 (zygion-zygion) dimension on gender determination, the answer to it could probably be found in the very involvement of the temporal bone in the structure of the middle and posterior cranial fossa where the process of cranial base kyphosis takes place. These processes result in pyramid torsion and rotation of the whole temporal bone. Pyramid torsion affects the labyrinth which is fitted in it and is, at the same time, of great significance for the formation of cranial asymmetry. It is exactly the cranial base flexion (cranial base angulation and kyphosis) that enables an optimal position of sensory organs. Eyes are placed so that in dominant position adopted by humans in upright posture they can encompass distant horizon. Distance between the nose and ground increased and the entire facial region of the skull is reduced. No special explanation is required for mutual connection between the temporal and zygomatic bone, which then consequentially is affected by changes occurring at the level of the temporal bone. Therefore, this determines it as primarily significant in this matter i.e. gender determination.

D5 (nasion-prosthion) has the second greatest effect in the group (Wald=11.5 $p=0.001$, OR =0.32, and 95% CI (0.16-0.62)). Each increase of D5 by 1 cm reduces the chances of the skull being female by 68%, or in the interval from 84 to 38%.

D2 (porion-porion) has the third greatest effect in the group (Wald=8.1 $p=0.004$, OR=0.63, a 95% CI (0.46-0.87)). Each increase of D2 by 1 cm reduces the chances of the skull being female by 37%, or in the interval from 54 to 13%.

Using multivariate binary logistic regression, we estimated the impact of observed cranioscopic characteristics on gender assessment, where only the cranioscopic characteristic of expression of superciliary arch and glabella; as well as the size of mastoid process demonstrated statistically significant influence on gender assessment. Multivariate influence of expression of muscle insertions on gender assessment is borderline ($p=0.052$).

We thought that further progress in gender prediction based on human skull could be achieved by testing the influence of certain autonomous craniometric and cranioscopic variables on gender assessment in skull. Statistically significant influence of linear dimensions was shown D4, D5 and D2 linear dimensions, while, out of the observed cranioscopic characteristics, only the expression of superciliary arch and glabella indicated statistically significant influence ($p<0.05$).

We find that the expression of superciliaryarch and glabella stands out as an independent cranioscopic characteristic which has a predictive effect with craniometric dimensions on gender prediction in human skull due to the fact that it is a most frequent cranioscopic characteristic in both male and female skulls.

Based on the obtained results, we derived a probability pattern "D1" for gender predictionusing linear dimensions and cranioscopic characteristics which showed statistically significant influence on gender dimorphism of skulls in our tested sample.

Accuracy of gender prediction of our investigated sample using "D1" probability pattern is 91% for male skulls, while the accuracy of gender prediction for female skulls is much lower and is 67.7%.

Formulas for discriminate functional analyze for skull gender dimorphism predilection between Afro-Americans and Americans that have European origins are less precise when they are used on other populations compared to population that they are formed for. (1) Therefore, we compared our pattern D1 with data from recent literature.

In 2009, Daniela Alejandra Zavando Matamala et. al conducted a research on 225 macerated skulls Brasilian individuals of known sex. They tested the possibility os sex determination based on linear skull diameters. They measured the following diameters: eurion-eurion, glabella-opisthion, basion-bregma, nasion-prosthion, zygion-zygion, nasion-spina nasalis anterior, and maximum width of piriform aperture. Multivariately, two linear diameters was shown statistically significant effect on gender determination zygion-zygion and nasion-anterior nasal spine which agrees with our results. (6)

We found similar results in the research conducted by Nigerian scientists on 300 macerated skulls (150 male and 150 female) belonging to individuals from three ethnic groups in North-East Nigeria. They measured skull length, width and height, as well as circumference of male and female skulls from all three ethnic groups (Fulani, Tangale and Tera). They concluded that all diameters of male skulls were larger than the respective diameters of female skulls within the same ethnic group, except the skull width which was larger in female skulls belonging to individuals from the Fulani ethnic group than in the male skulls belonging to the same group. (7)

In research, Altayeb A. Ahmed et. al conducted a research on 110 CT scans of skulls belonging to individuals form Northern Sudan where they tried to prove the existence of sexual dimorphism. They observed seven linear diameters and determined that all linear diameters were larger on CT scans of male skull compared to CT scans of female skulls. Accuracy of sex determination based on all observed linear diameters in this research was 83.6%. The greatest effect on sex determination have three linear diameters glabella-inion, basion-nasion, basion-bregma. (8)

In research conducted in 2013 on 80 macerated and degreased skulls (40 male, 40 female) belonging to members of South Indian population, Nidugala et. al tried to determine gender based on linear skull diameters. They measured bizygomatic width, total facial length, upper facial height, orbital height, orbital width, biorbital widht, interobital width, nasal height, nasal width and length of nose bone. Using univariate logistic regression, they tested all diameters in terms of gender determination. All diameters were longer in male skulls compared to the same diameters in female skulls, except upper facial height, orbital height and length of nasal bone. Multivariate binary logistic regression indicated statistical significance of all measured diameters for gender determination based on human skull with accuracy of 68.8% in this tested group (9), as opposed to our research where three diameters stand out: D2 (porion-porion), D4 (zygion-zygion), D5 (nasion-prosthion).

In the research conducted in 2012 on 80 macerated skulls belonging to individuals of South Indian origin, Vidya C. S., Prashantha B., Gangadhar M. R. measured linear diameters and calculated skull, upper facial and nasal index for the purpose of determining sexual dimorphism of skulls. They measured maximum length of skull, maximum width of skull, facial height bizygomatic diameter, nasal height, nasal width and length of left and right mastoid process. Univariately, nasal length, nasal width and length of right mastoid process proved to be significant; while multivariately, only nasal height and length of left mastoid process showed statistical significance for sex deterimination of the tested sample of South Indian population. (10)

In 2014, Marinescu M. et. al conducted a study on 200 macerated skulls of known gender (100 male, 100 female) belonging to members of Rumunian population, where they measured eleven linear diameters and, using discriminatory functional analysis observed their effect on sex determination. Discriminatory functional analysis of all measured diameters showed that length of skull (glabella-opisthion), facial width (zygion-zygion), height of skull (basion-bregma), nasal height (nasion-anterior nasal spine) had statistically significant effect on sex determination. Therefore, using these four linear diameters, the authors created their own probability model F1 for sex determination with 88% accuracy.

Discriminatory functional analysis of the linear diameters of the calvaria (skullcap) indicated statistical significance of skull length, skull height

and bimastoid width; therefore, using these three linear diameters of the skullcap, the authors proposed a probability model F2 for sex determination with 81% accuracy in this research.

Discriminatory functional analysis of linear diameter of face bones indicated statistical significance of facial width, orbit width, orbit height and nasal height. Using these four linear diameters, the authors proposed a probability model F3 whose accuracy in sex determination in this research was 83.5%. (11)

In 2006, Deshmukh A.G. and Devershi D.B. conducted a research on 74 macerated skulls of known gender (40 male, 34 female). Their objective was to compare sex determination using univariate and multivariate analysis of linear skull diameters. They measured 16 linear diameters, out of which, univariately, ten showed statistically significant effect on sex determination with 32.41% accuracy. Based on a multivariate analysis of all 16 linear diameters, sex of 90% of male skulls and 85.29% of female skulls from the tested group was correctly determined. Total percent of correctly determined gender based on multivariate analysis in this study was 87.84%. (12)

In 2012, Osvaldo et. al conducted a research on a sample from Brasilian population using 100 macerated skulls (50 male, 50 female) trying to prove the existence of sexual dimorphism using craniometric analysis. Their objective was to define the parameters that would be most reliable for sex determination and linear diameter zygion-zygion proved to be the best for sex determination, which is in accordance with our results. (13).

Based on measurement performed on 200 macerated skulls (100 male, 100 female), L. Francesquini Junior et. al analyzed sexual dimorphism by measuring linear skull diameters. The authors defined a formula which can be used for sex determination in the tested group with 79.9% accuracy. (14)

In the research conducted on 100 macerated skulls, Narasimha Murthy S. et. al used only the linear diameter nasion-bregma for sex determination where the accuracy of male skull identification was 6%, and female skulls 14% in the tested group. (15)

In the research conducted on 100 macerated skulls of Nigerian individuals of known sex (78 male, 22 female), Orish C. N. et. al used the surface of the triangle between antropometric points inion, opisthion and asterion. Their results indicate that this method of sex determination is more reliable for determination of male sex, and the accuracy of determination of male sex using the triangle surface on the right side was 47.19% and 46.42% using the triangle on the left side; while the accuracy of determination of female sex was slightly lower - 38.87% for the right triangle and 37.40% for the left triangle. (16)

In his paper from 2010, Bidmos MA. et. al gave an overview of researches that used quantitative, qualitative, geometrical and morphological or molecular method for sex determination of skeletal remains in South Africa. They concluded that the accuracy of sex determination of skeletal remains was lower when one method was used, while application of two or more methods increased the accuracy of sex determination. (17) In our research is also confirmed that utilization of parallel and combined qualitative and quantitative analyzes is grant for the most effective anthropological provement and as such may be suggested to forensic expertise based on human skulls.

In 2005, Franklin D. et. al conducted a research on skulls of Bantu South Africans. The objective of this research was to test sexual dimorphism of the skulls using discriminant functional analysis of linear diameters transformed by a special three dimensional software. Univariately, bizygomatic diameter proved to be the best for sex determination, which is in accordance with our results. Discriminant functional analysis of eight linear parameters indicated statistical significance of eight linear parameters with 77 to 80% accuracy in sex determination. Sex determination accuracy using either the skullcap or facial bones was 75-76% on this research sample. (18)

In his research, Lopez M. C. observed sexual dimorphism based on pear-shaped opening (aperture piriformis). The research was conducted on 90 skulls of Brasilian individuals of know sex, age and race. The results indicated that all measured diameters (height of aperture piriformis, lower and upper width of aperture piriformis) were larger in male skulls compared to female skulls belonging to the same race. (19)

In the research conducted on 112 skulls of Indian individuals of known sex, Saini V. et. al used discriminant functional analysis to observe the ef-

fect of ten standard linear skull diameters on sex determination and bizygomatic width proved to be most efficient in the tested group with 85.5% accuracy (20), which is in accordance with our results.

Numerous researches have indicated skull differences between population suggesting the need for the establishment of a population-specific standard that would be used for sex determination of skulls.

In her research conducted on macerated skulls of African-Americans and European-Americans, Amanda Grace Kittoe concluded that sexual dimorphism varies among different populations. The research showed that same-sex skulls, both male and female, with different racial background have different values of measured diameters. Furthermore, the author states in her paper the reasons that might have contributed to these variations, which are of genetic and nutritional nature. (21)

Finally, we compared the probability model "D1" and our model "L" (13) and concluded that the "D1" model, which, in addition to linear dimensions (D5, D4, D2), also uses the expression of superciliary arch and glabella, significantly contributes to determination of male gender compared to model "L", which uses only three linear diameters (L5, L4, L2) and increases the accuracy of sex determination by 6% (from 85% to 91%), while in determination of female sex it showed approximate accuracy like model "L" (L=68%, D1=67.7%, the difference being 0.3% in favor of the "L" model). (22)

Based on the obtained results, we can say that there is a large number of male skulls which, based on both their cranioscopic characteristics and linear dimensions, incline to female skulls and vice versa. Given that most of the skulls are of transitional type, identification of gender based on quantitative or qualitative analyses of skulls is not an easy task, therefore, we derived model "D1" which integrates both quantitative and qualitative analysis for the purpose of gender determination. We would like to propose the application of D1 probability model for gender determination of skulls. It is exactly the combination of cranioscopic and craniometric parameters with population proliferation and application of discriminant function analysis that gives the best results in gender determination based on human skulls.

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The relationship between emotional intelligence and social media attitudes of university students

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Abstract

Introduction: High emotional intelligence is related with academic success, and good skills in interpersonal communication. In our study, emotional intelligence (EI) levels of university students, the factors affecting EI and the relationship of emotional intelligence with social media attitudes were evaluated.

Materials: EI was evaluated with Bar-On Emotional Quotient inventory (Bar-On EQ-i). To evaluate social media attitudes of the university students Social Media Attitudes Scale (SMAS) was used.

Results: This study included 208 university students from Trakya University. SMAS scores and Bar-On EQ-i scores were significantly higher in participants who were using social media for a longer period of time than new users. SMAS scores were significantly higher in participants who used social media more frequently. Also, Bar-On EQ-i scores tended to be higher with increasing frequency of social media use.

Discussion: SMAS and Bar-On EQ-i scores were significantly higher in those who started to use social media earlier in our study. Also, participants who used social media for a longer duration each time had higher mean scores in both scales. With increasing social media use Bar-On EQ-i also increased. Education programs should be established to improve younger generations' EI.

Key words: Social media, nursing student, university student, EI

Introduction

Emotional intelligence has recently attracted an increasing interest in the whole world and scientists add dimensions such as adaptation to and relationship with the environment to the definition of intelligence. Previous research has shown that although intelligence quotient (IQ) gives a quantitative measurement for cognitive intelligence, emotional quotient determines success in life and maintains quality of life [1, 2]. High emotional intelligence is related with academic success, and good skills in sociocultural, interpersonal communication and stress management areas; low emotional intelligence is related with unusual behaviors, alcohol and substance dependence and insufficient interpersonal relations [3]. Emotional intelligence is the sum of all non-cognitive skills and abilities that affect coping of the individual with external influences. Studies have shown associations between emotional intelligence and achievements such as creativity and communication skills [4, 5].

Using empathy and interview skills developed since the education period in professional life may help to reduce the anxiety encountered and increase the harmony in the working environment. Studies that evaluated the relationship between emotional intelligence and sociodemographic features of individuals demonstrated that the lessons they have learned during their education life and their parents' attitudes may support the development of emotional intelligence. In the studies, there were also positive correlations between personal skills, interpersonal skills, compatibility, coping with stress, and general mood subscales of emotional intelligence, understanding people and establishing relationships [6, 7].

Research has shown that use of internet and especially social media is getting more and more important place in lives of young people. In recent years 95% of young people aged 18-33 reported active use of social media. Social sharing sites help to share experiences and social relations and the use of social media programs among young people seems to be widespread. The use of social media is rapidly becoming a central part of young people's lives, and more than 90% of them now use social media almost every hour of the day [8, 9].

It is important to determine the sociodemographic characteristics and lifestyle features which

affect emotional intelligence in order to determine the needs of young people and to develop education programs for them. As the communication skills are very important nowadays, it is important to develop curriculums in order to promote emotional intelligence of young people while they are attending to school and before they enter to professional life.

In our study, emotional intelligence levels of university students, the factors affecting emotional intelligence and the relationship of emotional intelligence with social media attitudes were evaluated.

Material and method

Emotional Intelligence was evaluated with Bar-On Emotional Quotient inventory (Bar-On EQ-i) which was validated in Turkish (13-15). Bar-On EQ-i is a Likert type measure consisting of 87 items. During rating the items 5 means fully agree, 4: agree, 3: undecided, 2: disagree, and 1: strongly disagree**.** This scale measures 5 subscales of emotional intelligence (personal skills, interpersonal skills, compatibility, stress management, and general mood). Construct validity and criterion validity studies of Bar-On EQ-i were conducted during Turkish validity and reliability study. Construct validity studies included comparison of extreme groups and item-total score correlations and demonstrated that both discriminative features and correlations with relevant dimensions were statistically significant; criterion validity studies demonstrated that emotional intelligence was a theoretical construct apart from intelligence quotient but related with personality features. According to these results it was concluded that Turkish version of Bar-On EQ-i can be used in scientific studies [10-12].

Personal information form includes questions about age, gender, school, socioeconomic status, parental education, and the longest place of residence of the volunteers.

To evaluate social media attitudes of the university students Social Media Attitudes Scale (SMAS) which was developed by Otrar and Argın was used. This scale includes a total of 23 items (6 of which are positive and 17 of which are negative) and 4 factors (need to share, social competence, social isolation, and relationship with authorities) [13].

This study evaluated the levels of emotional IQ and social media use in university students and the relationship between them. The data were summarized using appropriate descriptive statistics. Mean and standard deviation were used for numerical variables and frequency and percentage were used for categorical variables.

Approval was obtained from Trakya University Scientific Research Ethics Committee and informed consents were obtained from all participants. All statistical analyses were performed with SPSS 20.0 Package Program. The data were summarized with appropriate descriptive statistics. Mean and standard deviation were measured for numerical variables and frequency and percentage were measured for categorical variables. Normal distribution of data was controlled with Shapiro-Wilk test. Student t test was used for comparison of two groups. One-way ANOVA was used for comparison of more than two groups. Multiple comparisons after one-way analysis of variance were evaluated with Bonferroni test. Chi-square test was used for the relationships between categorical variables. Significance level for all statistical analyses was defined as 5%.

Results

Our study included 208 university students between 18-24 years of age. This study included voluntary students from Trakya University Health Vocational School and Applied Sciences High School. Distribution of the students to classes showed that 58 were at grade 1, 91 were at grade 2, 34 were at grade 3 and 25 were at grade 4 (Table 1).

Table 1. Distribution of participants according to grades

Grade	N
1	58
2	91
3	34
4	25

Among the participants 135 were females and 73 were males. The mean SMAS score of the female students was 64.3 and the male students was 68.6. The mean score of the male students was statistically significantly higher. Although no significant difference could be found in emotional intelligence the mean Bar-On scale score of the females was higher than the males.

Health Vocational High School Students formed 38% of all students and Applied Sciences High School students formed 62%. The mean SMAS and Bar-On scale scores of Applied Sciences High School Students were higher than Health Vocational High School students. Also, Bar-On emotional quotient increased with increasing SMAS score (Table 2).

No difference was found in SMAS and Bar-On EQ-i scores of the participants according to education level of the parents of the participants. However, scores from both scales tended to increase as the level of mother's education increased. With increasing father's education only SMAS scores tended to increase.

Although there wasn't a statistically significant difference students with a better socioeconomic status tended to have a better score in Bar-On emotional intelligence scale. However, there was not a trend for increasing SMAS scores. Also, familial attitudes were asked and the mean scores from both scales did not change according to familial attitudes.

Table 2. Sociodemographic features and the mean SMAS and Bar-On EQ-i scores of the study participants

	N	%	SMAS	Standard Deviation	P	Bar-On EQ-i	Standard Deviation	P
Gender								
Male	73	35.1	68.68	14.6	.032*	295.9	33.6	.361
Female	135	64.9	64.33	13.4		299.7	25.5	
School								
Health Vocational High School	79	38	62.73	12.6	.012*	291.9	22.1	.035*
Applied Sciences High School	129	62	67.77	14.5		300.5	31.6	
Mother's Education								
Primary school or lower	146	70.2	64.8	14.1	.254	296.9	27.3	.834
Secondary School	59	28.4	68.0	13.8		297.5	32.3	
University or higher	3	1.4	72.0	8.0		307.0	10.3	
Father's Education								
Primary school or lower	92	44.2	64.36	14.4	.365	298.02	27.1	.944
Secondary school	99	47.6	66.84	12.7		296.61	28.9	
University or higher	17	8.2	68.24	18.1		297.18	35.9	
Socioeconomic status								
Moderate	165	79.3	66.1	13.7	.628	297.05	27.0	.821
Good	43	20.7	64.9	15.0		298.16	34.6	
Family attitudes								
Protective	119	57.2	65.92	13.617	.991	297.66	27.8	.668
Authoritarian	28	13.5	66.0	15.171		300.68	32.0	
Democratic	61	29.3	65.66	14.490		294.97	28.9	
Father's Occupation								
Public Servant	23	11.1	65.9	17.2	.880	298.4	28.9	.973
Worker	56	26.9	66.0	12.8		295.7	25.1	
Self Employed	72	34.6	64.8	12.8		297.8	32.8	
Other	57	27.4	66.8	15.3		297.5	26.7	
Mother's job								
Public Servant	2	1.0	65.5	2.1	.653	296.0	7.0	.178
Worker	23	11.1	69.9	15.2		300.3	40.3	
Self Employed	8	3.8	69.5	6.1		310.2	43.0	
Housewife	165	79.3	64.6	14.1		295.9	26.3	
Other	10	4.8	73.1	12.3		301.6	24.2	

**indicates statistically significant difference.*

There was not a difference in scores from the scales according to mothers' occupations however, children of housewives tended to get lower scores from both scales. Also there was not a statistically significant difference according to fathers' occupations (Table 2).

Young people were connecting to social media sites more commonly by mobile devices. They were using social media not only during their leisure times at home but also during when they are outdoors (Table 3).

Table 4, shows social media use and scores from both scales of the participants. SMAS scores and Bar-On EQ-i scores were significantly higher in participants who were using social media for a longer period of time than new users. No significant difference could be found according to the time spent at each entry to social media. However, there was a tendency for higher SMAS and Bar-On EQ-i scores in participants who stayed longer at each entry. SMAS scores were significantly higher in participants who used social media more frequently. Also, Bar-On EQ-i scores tended to be higher with increasing frequency of social media use but the difference was not statistically significant.

Discussion

In our study the mean Bar-On EQ-i scores of the participants were at moderate level. Previous studies also reported moderate level emotional intelligence scores in students attending health related schools [14, 15].

Although there was not a statistically significant difference in emotional intelligence according to gender, the mean score of females from Bar-On EQ-i was higher than males. Some studies detected that emotional intelligence differ according to gender with a 90% confidence interval and females have higher emotional intelligence than males [16]. The mean SMAS score was higher in males.

Table 3. Preferences for social media connection device and place of the participants

	N	%
The most common place to connect to social media		
Home	60	28.8
Outside	148	71.2
The most common device to connect to social media		
Mobile device	204	98.1
Computer	4	1.9

Table 4. Social media use, SMAS and Bar-On Scale scores of the participants.

	N	%	SMAS	Standard Deviation	P	Bar-On Scale	Standard Deviation	P
Duration of social media use								
Less than 1 year	5	2.4	44.4	8.5	.002*	271.4	15.6	.041*
1-2 years	8	3.8	61.3	10.9		285.5	17.5	
2-3 years	31	14.9	63.6	11.4		291.0	28.3	
More than 4 years	164	78.8	67.1	14.1		299.8	28.8	
Time spent at each entry to social media								
5-10 min	29	13.9	62.3	12.5	.391	295.2	31.5	.543
11-30 min	54	26	64.9	13.9		297.3	21.7	
31-60 min	53	25.5	65.8	13.5		292.8	28.9	
61-120 min	31	14.9	66.7	16.1		299.1	27.5	
More than 121 min	41	19.7	68.9	13.8		302.8	34.6	
Frequency of social media use								
More than once every day	184	88.4	67.1	13.4	.006*	299.2	29.2	.086
Once or less every day	24	11.6	56.4	12.3		283.3	19.9	

**indicates statistically significant difference.*

SMAS and Bar-On EQ-i scores were significantly higher in those who started to use social media earlier in our study. Also, participants who used social media for a longer duration each time had higher mean scores in both scales although the difference was not statistically significant. Those who used social media more frequently in 24 hours had higher SMAS scores than others. With increasing social media use Bar-On EQ-i also increased but the difference was not statistically significant. Other studies showed that young people who used social media a few times a month had lower social media attitude scores than those who use everyday or at least once a week. This shows that increasing frequency of social media use also increases attitudes towards social media [17].

Studies on use of internet and social media show that most young people spend at least half an hour every day. With the rapid progress in technology, people tend to meet the internet and social media at an earlier age and tend to use it more often and for a longer period of time than older adults. Due to their age, it is normal for young people to be in an effort to establish close relationships with their surroundings. The use of social media is a tool that enables young people to communicate with each other and introduce themselves to other people [9, 18]. In our study 88.4% of the participants used social media more frequently and their SMAS scores were higher than those who used social media less frequently. Although it was not statistically significant Bar-On EQ-i scores were higher as frequency of social media use increased.

The nature of mankind is not suitable for living alone and this leads him to communicate in various forms. The increasing use of social media also provides new communication environments for people. Thus, individuals who do not want to be separated from the society they belong use the possibilities of technology in this area in order to keep communication alive [19, 20].

Studies that evaluated mother's working status, its effect on emotional intelligence scores, and academic achievement found a positive relationship between emotional intelligence scores and mother's working status [21]. In our study, no difference could be found both in SMAS and in emotional intelligence scales according to mother's occupation but children of mothers who were not working had higher scores in both scales than children of working mothers.

In our study no significant difference could be found in scores from the scales according to father's occupation. Previous studies also couldn't prove a relationship between father's occupation and emotional intelligence. Under normal conditions emotional connection between the mother and the child begins as soon as the baby has first formed in the mother's womb and continues as the child grows, and develops, which is always more prominent than the connection with the father. Although the relationship with the father increases in some stages of life it is not easy to replace the relationship with the mother. Father's occupation may affect the quality and duration of time he spends with his child; but no evidence could be detected to prove that father's occupation has a strong influence to affect emotional intelligence score of his child.

Studies that investigated the correlation between emotional intelligence and education level of mother found a positive correlation. Mother's love to her child starts when she first feels it and this love enables to provide the most emotional support possible throughout her life as long as it is not adversely affected by some external factors [22]. In our study no statistically significant difference could be found in SMAS or Bar-On EQ-i scores according to mother's education. Regardless of their education level mothers try to support the development of their children with their endless love and affection. However, mothers with higher education find better opportunities which facilitate their efforts. Our study also showed a trend of increase in both scales as mother's education level increases.

Previous studies couldn't find a relation between fathers' education level and emotional intelligence of the participants [22]. In our study only SMAS scores tended to increase with increasing paternal education. Increased education level decreases financial concerns of the father. This increases the time he spends with his child and facilitates sharing mother's responsibilities. But with increasing education level father's expectations on academic and social grounds increase, leading to a pressure on the child and affecting emotional development adversely. Considering all these factors together, low level of education does

not always mean low emotional support from the father. Development of emotional intelligence will be better when father enjoys a good time with his child, tries to form strong bonds and shows timely support. Increased paternal education level also decreases socioeconomic concerns and increases access to social media.

There wasn't a statistically significant difference according to socioeconomic status of the patients however, Bar-On EQ-i scores tended to be better in participants with a better income status. Some previous studies have found a positive relationship between emotional intelligence level and socioeconomic status of the family [23]. As families' financial concerns decrease they respond more easily to needs of their children. This increases sociocultural development of individuals but can't support emotional development by itself. Emotional intelligence strengthens with family bonds and their affection, respect and support towards each other.

General attitudes of families as protective, authoritarian, or democratic were not associated with scores on SMAS or Bar-On scales. There are other studies which investigated the association between family attitudes and emotional intelligence. Some studies have found significant relations between emotional intelligence and family attitudes of individuals [24]. We didn't question familial problems of the participants. Considering that they won't want to share their familial problems they were only asked to choose from the options as authoritarian, protective, or democratic. Inability to find an association between social media use, emotional intelligence and attitudes of families may be due to the wide spectrum of family attitudes.

Emotional intelligence was first defined by scientists as "the ability of individuals to evaluate their own and others' feelings, to distinguish between these feelings, and to use them in shaping their thoughts and behaviors". Research has shown that emotional intelligence is indispensable to be successful in social, cultural and academic grounds [25, 26].

Some studies have indicated that cognitive intelligence measured by tests is not an important indicator of success in life; instead those who recognize and control feelings of themselves and others are more successful in academic and sociocultural grounds. Emotional intelligence may be developed with personal effort. Research has shown that cognitive intelligence alone can give young people the profession they desire but it can't provide pleasure from the life [26-28].

Appropriate use of social media by young people has positive effects in terms of communication with the environment, socio-cultural development, sharing feelings and thoughts in a clear and understandable way, communication and mental relaxation; its inappropriate use may cause losing too much time, decreasing efficiency and performance at work, distracting attention, and adverse effects on socialization. Social media may be helpful to support learning, but caution should be exercised to avoid its negative consequences.

Education programs should be established to improve younger generations' emotional intelligence in order to improve their abilities to cope with social problems and catching up with their times. Sociodemographic features that affect emotional intelligence should be identified, its relationship with social media use to which young people spend a great deal of time should be determined, and both should be included in education programs to contribute to young people's personal development.

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Treatment success evaluation between two surgical treatments of pelvic organ prolapse: colporrhaphy and mesh implant

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Abstract

Aim: Our goal was to compare two surgical techniques in the treatment of pelvic organ prolapse, and to determine which of these methods have better outcomes and offer better quality of life of patients after surgery.

Methods: It is a prospective study, which included 70 patients who were surgically treated after diagnosed pelvic organ prolapse. The subjects were divided into two groups, experimental group which included 35 patients having pelvic organ prolapse corrected with a mesh implant (Prolift system), while the control group with 35 patients had their pelvic organ prolapse corrected with the classical vaginal surgical method, colporrhaphy. Preoperative preparation included a detailed anamnesis and physical examination, taking urine culture and bacteriological vaginal smears, and classification of the prolapse with the POP-Q system. Performed questionnaires and forms were evaluated, diagnostic ultrasound and gynaecological and clinical examinations which were performed prior to surgery and after surgery (examinations in the first, third and sixth month after surgery) were also evaluated in the aim of investigation of outcomes after treatment. We also did a quality of life analysis before surgery, and in the first, third and sixth month after surgery.

Results: A significant difference between the two groups has been found in the treatment outcome, postoperative quality of life and in additional parameters related to the length of rehabilitation.

Conclusion: The outcome of the treatment of pelvic organ prolapse in women is better after the application of the mash implant.

Key words: Pelvic organ prolapse, Prolift system, colporrhaphy

Introduction

Pelvic organ prolapse progressively involves an increasing number of women with age advancement. Although mortality is negligible, morbidity caused by prolapse is significant. These conditions are not only frequent, but also a challenge for surgeons because surgical care has so far shown unsatisfactory results- about 30% of the used procedures (Olsen et al., 1997).

Performed surgery that is only manipulating the tissue of the patient can only restore about 50% of the preoperative firmness. Because of this, recurrences occur in about 30% of patients, with the recurrence occurring time progressively decreasing if recurrences occur several times (Fatton et al., 2007).

Using a technique similar to the transopturatory sling, Debodinance and associates in 2004 used a polypropylene mesh by twisting the ends of the mash through the obturathory opening for cystocele correction, and in the posterior segment of the small pelvis, they used pararectal space and slit the ends of the mash through both sacrospinal ligaments (Debodinance et al., 2004).

Numerous publications have been published in the last few years with a large number of patients using synthetic meshes in vaginal surgery with a high percentage of satisfactory results (from 75 to 100%) with longer average follow-up (from 1 to 36 months) (Gauruder-Burmester et al, 2007).

The use of artificial materials significantly improved the mechanical strength of the restored prolapse. The development of soft prolene mashes enabled better tolerance and lower morbidity of operations (Fatton et al., 2007).

Material and methods

The study was prospective and involved 70 patients who were surgically treated after diagnosed pelvic organ prolapse. A multicentre study was carried out at the Gynaecological Department of the Regional Hospital in Bihac, as well as at the Gynaecological Department of the Clinical Centre in Ljubljana. The duration of the study was unlimited.

The inclusion criteria was a clinically diagnosed and surgically treated pelvic organ prolapse without prior operative or corrective treatment, and the sample was determined consecutive. Exclusion factors were patients with arteriosclerosis, diabetes, and severe heart, lung, and kidney diseases.

The experimental group consisted of 35 patients, which were treated with a polypropylene mash-Prolift system and the control group of 35 patients where the prolapse was corrected with the classical vaginal surgical method. Patients of the experimental group included a front, back or total Prolift kit, while the control group included colporrhaphy or colporrhaphy with vaginal hysterectomy.

Among other parameters, postoperative objectification, gynaecological ultrasound diagnostics, gynaecological and clinical examinations were performed prior to surgery and on controls in the first, third and sixth month after the operation and the results were evaluated for examining outcomes after treatment. In addition, a quality of life analysis was performed prior to the surgical procedure and after the surgery (1, 3 and 6 months).

The following questionnaires were used: POP-Q reference guide (Bump et al., 1996), intraoperative details and treatment results questionnaire, Urinary incontinence objectification test, Quality of Life Questionnaire (P-QOL) (Barber et al., 2001).

Statistical analysis

Detailed statistical analysis of the data was carried out during this study. Descriptive statistics was used for the data analysis. To test the statistical significance of differences between samples parametric and non-parametric tests were used. P<0, 05 was considered statistically significant. Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences) software.

Results

The type of surgical treatment performed in both groups is presented in Table 1.

Table 1. Type of surgical treatment

Type of surgical treatment	Number of patients	%
Prolift anterior	16	23,0
Prolift posterior	15	21,0
Total set	4	6,0
Colporrhaphyy	30	43,0
Colporrhaphy+hysterectomy	5	7,0
Total	**70**	**100**
x ± SD	14,00±10,512	

As shown in the previous table, 16 patients had an implantation of a prolift anterior set, 15 patients got a prolift posterior implant, and the total set was used on four patients. The anterior and posterior vaginoplasty was performed on 30 patients, while hysterectomy with vaginoplasty was performed on five patients.

General comparisons between the two groups, such as mean operative time, hospitalization length, and intraoperative, early and late postoperative complications are shown in Table 2.

According to the results, it is clear that all the examined parameters indicate the advantage of the Prolift method. The average operating time at the Prolift group was 55.68 minutes, ranging from 35 to 90 minutes, while performing vaginoplasty the average operating time was 58.02 minutes, ranging from 44 to 90 minutes. The average length of hospitalization was 5.14 days for the Prolift group, while the average hospitalization rate for patients treated with vaginoplasty was 8.28 days. Looking at the complications in the Prolift group, fewer operative and postoperative complications were reported compared to the group of patients treated with vaginoplasty. The results of treatment after 1/3/6 months in patients treated with Prolift system are shown in Figure 1.

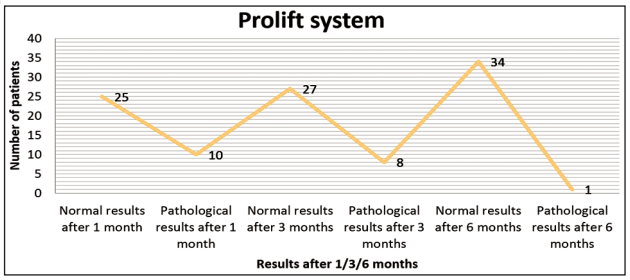

Figure 1. Treatment results after 1/3/6 months (Prolift method)

Table 2. General comparisons between Prolift and vaginoplasty

General comparisons	Prolift system	Colporrhaphy	T	χ^2	dF	p
	Nr. of patients	Nr. of patients				
Mean operative time	55,68	58,02		0,597		
Length of hospitalisation	5,14	8,28		0,270		
Intraoperative complications	0	3		2,491		
Early postoperative complications	2	5		0,796		
Late postoperative complications	8	11		0,081		
Total			**-0,147**	**4,236**	**4**	**0,375**
x ± SD	14,16±23,4	17,06±23,1				

From the previous chart it can be seen that the level of recovery and regular findings through controls was significantly increasing, so from the initial 71% of patients with normal findings in the first month postoperatively, after three months the percentage of normal findings was 77%, while the percentage of normal findings on the last check went up to 97%.

The treatment results after 1/3/6 months of patients treated with the classical method are shown in figure 2.

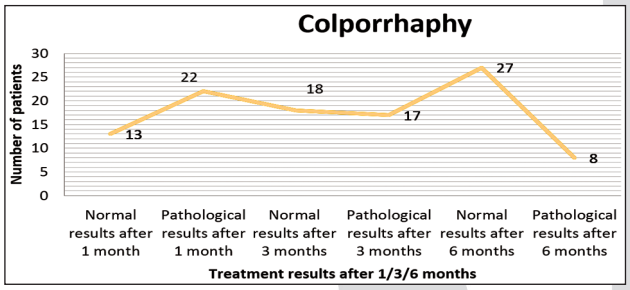

Figure 2. Treatment results after 1/3/6 months (classical method)

The previous chart shows that the recovery rate and the regular findings were lower in the control group, so from the initial 37% of patients with normal findings in the first month postoperatively, after three months the level of normal findings was 51%, while the after 6 months the percentage of normal findings went up to 77%.

A questionnaire was following the patient's quality of life after the operation. Results for both groups are presented in Table 3.

By analyzing the quality of life of patient after the performed surgery, the following results were obtained. In the Prolift group 77% thought that their quality of life was significantly improved, 20% of patients had some improvement, while one patient was without improvement. In the classically treated patients, 51% had a significant improvement, 26% had some improvement and 23% had no improvement.

In order to assess the success of prolapse treatment the time of postoperative rehabilitation in both groups was monitored and the results are presented in Table 4.

During the postoperative rehabilitation period in the Prolift group, all patients were ready for physical work and business life up to 30 days after surgery, with rehabilitation lasting up to 10 days in five patients, 18 patients needed between 11 and 20 days to rehabilitate, while 12 patients needed 21 to 30 days. On average, recovery time in the Prolift group was 17,543 ± 6,586 days.

In the control group, 6 patients were ready for physical and business activities after 10 to 20 days, 22 patients were rehabilitated after 21 to 30 days, and seven patients needed more than 30 days of

Table 3. Quality of patient life after operative surgery

Outcome	Prolift system		Colporrhaphy		T	χ^2	dF	p
	Nr. of patients	%	Nr. of patients	%				
Significantly improved	27	77,0	18	51,0		1,800*		
Improved	7	20,0	9	26,0		0,250		
Not improved	1	3,0	8	23,0		5,444*		
Total	35	100	35	100	0,0	7,494*	2	0,024
x ± SD	11,67±13,61		11,67±5,51					

Table 4. Duration of postoperative rehabilitation in both groups

Postop. rehabilitation (days)	Prolift system		Colporrhaphy		T	χ^2	dF	p
	Nr. of patients	%	Nr. of patients	%				
Up to 10 days	5	14,0	0	0,0		5,000*		
11 – 20	18	34,0	6	20,0		6,000***		
21 - 30	12	52,0	22	63,0		2,941**		
> 30 days	0	0,0	7	17,0		7,000**		
Total	35	100	35	100		20,94*	3	0,0001
x ± SD	17,54±6,586		24,77±6,039		-4,786*	72,49*	34	0,0001

rehabilitation. The average rehabilitation time for this group was 24.77 ± 6.039 days, which was approximately three days longer than the patients treated with the Prolift method.

Discussion

A significant aspect of pelvic organ prolapse is the impact on the quality of life. Due to the large number of women with pelvic floor defects, the success of treatment depends on good diagnosis and well-chosen treatment. By using modern diagnostic and therapeutic procedures, the optimal treatment effect is achieved and ensures a patient's quality of life. The risk of surgical intervention due to pelvic organ prolapse in the general female population is 11%, and 29% of women in surgery are undergoing reoperation due to the failure of the first operation in the period of five years after surgery (Olsen et al., 1997).

The analysis of the outcome of the two surgical treatments showed that the level of recovery and normal findings in Prolift group was significantly increased through controls, so that from the initial 71% of patients with a regular finding in the first month postoperatively, after three months the level of regular findings was 77%, while the degree regular findings on the last control rose to 97%.

Analysing data in a group of patients treated with the classical method showed that the level of recovery and normal findings through controls had a smaller increase, so that from the initial 37% of patients with a regular finding in the first month postoperatively, after three months the level of regular findings was 51% while the level of regular findings at the last control rose to 77%.

By analysing the implemented objective methods and the subjective statements of patients about the outcome of the operation, the following results were obtained: in the Prolift group 77%, were considered cured, 20% of patients had an improvement after surgery, while one patient had no improvement. In patients treated with the classic method 51% of them were cured, 26% had an improvement after the operation and 23% were not cured.

In a randomized study of Weber and associates, only 30% of patients had satisfactory results with standard coloporrhaphy, while the combination of the front colporraphy with polyglycine mesh gave satisfactory results in 42% of patients (Weber et al., 2001).

Dwyer and associates came in their study to the following conclusions: the repair of anterior defect in 47 patients who were followed for 12 months yielded the following results: 2% recurrence, 6% erosion (Dwyer et al., 2004).

Sand and associates analysed the degree of recurrence of cystocele in patients who had already performed one of the methods of repairing pelvic defects after 12 months (regression analysis), and they came up with the following results: patients with a sling implant had a relapse in 19% of cases, while in patients undergoing surgery without sling, the rate of recurrence was 42% (Sand, 2011).

Migliari and associates also tested the success of the methods and the occurrence of recurrence. Under failure or recurrence, they considered prolapse in stage III or IV in any segment of the pelvic floor, whether symptomatic or asymptomatic. The percentage of relapse was 6.25%. Three patients had asymptomatic prolapse in the front segment, one after placing total prolift, and two cases of asymptomatic cystocele after the posterior Prolift. In addition, three patients had a relapse in the last segment, which was manifested three months after the anterior Prolift, which was symptomatic only

in one patient and required subsequent correction with the insertion of the posterior Prolift (Migliari et al., 2000).

Cervigni and associates in their 2001 study had a success percentage of 98% by using the mash implant (Cervigni et al., 2000).

De Tayroc and associates published their 48-cystocele operation experience using polypropylene mashes with a follow-up of 18 months. The results showed that there were 8.3% erosion and 97.9% success rate (De Tayroc et al., 2002).

In other studies using synthetic meshes in vaginal surgery the outcome was a large percentage of satisfactory results (75 to 100%) with longer median follow-up (1 to 36 months) (Gauruder-Burmester et al., 2007).Abdel-Fattah and Ramsay in a series of 289 patients found a recurrence of 5% (Abdel-Fattah et al., 2008).

In general, the success of back colporrhaphy is 76-90%. A possible complication is the "de novo" emergence of dyspareunia that occurs in up to 26% of patients with back colporrhaphy. As a result of the change in the mechanical properties of the vagina wall, the efficacy of these procedures decreases with time, so the success in the five-year period is about 52%, with a significant number of complications (Milani et al., 2005).

Until the Prolift method occured vaginoplasty was the method of choice, although the current position of the American Urological Association is that the front plastic is the least successful operation for a longer period compared to other methods (Leach et al., 1997).

By analysing the quality of life of patients after the surgery, the following results were obtained: in the Prolift group 77%, considered that their quality of life was significantly improved, 20% of patients had an improvement in quality of life after surgery, while one patient was without improvement. In patients treated with the classic method, 51% had significant improvement, 26% had improved quality of life after surgery and 23% had no improvement.

The analysis of postoperative rehabilitation in the Prolift group showed that all patients were ready for personal and business life up to 30 days after surgery, in five patients the rehabilitation lasted up to ten days, in 18 patients the rehabilitation lasted between 11 and 20 days , and 12 patients needed 21 to 30 days rehabilitation. In patients treated with the classic method, six were ready for physical and business activities after 10 to 20 days, 22 patients were rehabilitated after 21 to 30 days, and seven patients needed more than 30 days for their rehabilitation.

Conclusions

Based on the obtained results, we concluded the following:

The Prolift method is more effective than the classic method. In the control examination, six months after the surgery and after clinical trials, there were more regular findings in the Prolift group than in the vaginoplasty group, which led to a better outcome of treatment in the Prolift group, which proved to be statistically significant.

The quality of life of a patient undergoing Prolift treatment was statistically significantly better in comparison to patients treated with the classic method.

Postoperative rehabilitation after application of the Prolift system is statistically significantly shorter compared to the classical method.

The conducted research confirmed the benefit of using the mash implants in comparison to the classical vaginal surgical method. It also showed shorter duration of surgery and shorter hospitalization, fewer early and late postoperative complications, which would allow patients greater comfort and easier resolution of prolapse problems. A better quality of life for patients after the use of the Prolift system was also demonstrated.

The conducted research concluded that the problem of resolving pelvic organ prolapse consists in selecting a method that will be best for the patient, as well as the most economical for a health institution. Some of the criteria are that surgical intervention should be minimally invasive, postoperatively achieve minimal morbidity, which implies minimal tissue damage, short hospitalization and absence from the workplace, which significantly affects economic cost-effectiveness and, above all, the highest possible rate of treatment success with a minimum number of recurrences, and the complete restoration of the quality of life that the patient had before the illness. The fulfilment of these criteria in our research was demonstrated by using the Prolift method.

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Assistive technology for people with visual impairment in the prevention of Sexually Transmitted Infections: clinic validation

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Abstract

Objective: This study aimed to validate the assistive technology *STI - in order to prevent, it is necessary to learn* among men and women with visual impairment.

Method: It was a methodological study conducted with 180 subjects from five associations for blind people. An assessment tool proper to the evaluation was used and access happened through audio recorded in mp3 format. Answers with agreement of 95% of the subject or more than this percentage were considered satisfactory. Inferential statistical analyzes were considered significant when $p < 0.05$. The project was approved under protocol 1,018,682.

Results: Eleven items were evaluated as adequate according to more than 95% of the subjects. Only the items "It allows you to search for information without difficulty"; "It makes the resources available to use"; "The information content is adequate for your needs"; "It has attractive presentation strategy"; "It awakens your interest to use it"; and the item "it stimulates a change of behavior in you" got rating below 95%.

Conclusion: As the established methodological framework, assistive technology is validated. Despite suggestions for its improvement, statistical analyzes indicate it is an appropriate resource use of health promotion and education for the proposed clientele.

Key words: Sexually Transmitted Diseases, Self-help devices, Visually impaired persons; Health promotion.

Introduction

Historically, people with visual impairments are excluded as for the media and information, and despite the search for accessibility, there are difficulties in their full inclusion in everyday environments and activities(1). In this scenario, there is the support for sexual and reproductive health.

Sex is presented as an important component of a healthy sexual experience. Sexually Transmitted Infections (STIs), inserted in this context, correspond to conditions arising from the lack of prevention during sex. Factors such misinformation may contribute to the occurrence of the STIs.

Therefore, people with visual impairment, when deprived of the development of a healthy sexual experience may have increased exposure to diseases in the context of sexual and reproductive health. For example, they may get some STIs.

The promotion of the health of people who have disabilities includes the use of Assistive Technology (AT), understood as an aid to enlarge a poor functional ability or to support the achievement of the desired function which is prevented by disability or aging(2). Using such technologies makes it possible to overcome limitations previously considered impassable. With the aid of creative projects, the involvement of people with visual impairment in health learning process is possible.

The nurse must have the resources to make information accessible and consolidate effective interventions. In this study we chose to use a rhyming text to teach individuals with visual impairments. The rhyme may be interpreted as an educational strategy. Its effect comes from the

musicality and power of synthesis inherent in the verse when composing texts that join, in addition to the literary language, a scientific knowledge[3]. It is believed that a differentiated language is able to arouse greater interest for reading and learning.

Given the above, in seeking to promote the health of people with visual impairment, and believing in the unquestionable importance of prevention, and to tailor health education strategies to the needs of this clientele, there is the AT *STI - in order to prevent, it is necessary to learn*, presented in mp3 in rhyming, approaching the main STIs (aids, syphilis, condyloma, herpes, hepatitis B, chlamydia, gonorrhea and trichomoniasis), and focus on condom use as a way to prevent these diseases, arranged in 52 verses lasting about 15 minutes, available on the site www.labcomsaude.ufc.br.

The objective, therefore, was to perform the clinical validation of the AT *STI - in order to prevent, it is necessary to learn* among men and women with visual impairment.

Method

This is a methodological study whose purpose is to evaluate and optimize materials and methodological strategies[4]. In this research, we adapted the method to the theory and the model of building psychological instruments of Pasquali (2010)[5] to validate the AT.

The study was conducted in five associations for blind people, after obtaining permission from the institutions, two from Fortaleza and three from Recife. We chose these cities because these where the place of residence of the researchers, facilitating data collection, which took place between the months of December 2014 to September 2015 and was carried out in person by two researchers and three trained fellows.

Participants were women and men with impaired vision selected upon availability of the subjects who were, in turn, found through previous contact in the associations. The inclusion criterion was to have age of 18 years or older and the exclusion criterion was the presence of intellectual disability and associated hearing disability.

The sample size calculation was based on the number of visually impaired people with these characteristics and application of the calculation of "n" in the formula for infinite populations, deriving a total of 180 subjects.

Access to AT occurred through recorded audio in mp3 format in mobile phones, portable CD stereos, provided by researchers, or in computers of the associations. An instrument was individually answered after the researcher conducted the reading of items and recorded the answers.

When the pilot test was completed, rewriting of the AT was performed. The empirical/experimental polo was then executed, with assessment of the AT by a statistically significant number of people with visual impairment.

Finally, we held the statistical analysis of the results, analytical pole. The number of subjects in the pilot test composed the final sample for this analysis that was performed by clinical validation through binomial test, comparing the suitability of each item according to the responses of subjects when assessing the AT. Items with agreement equal or above 95% of subjects (mean equal or above 1.95) were considered satisfactory. Inferential statistical analyzes were considered significant when $p < 0.05$.

With the statistical analysis, it was possible to see if any aspect of the AT was disapprove by the target population. Thus, adjustments were necessary to thereby validate the AT. When necessary, the resulting suggestions for improving the AT were conducted through literature review to support the changes.

Respect to the legal precepts and all subjects signed an informed consent form (ICF). The project was approved by the Ethics Committee of the Federal University of Ceará and approved under protocol 1,018,682.

Results

The study enrolled 180 subjects, 94 men and 86 women, 106 with blindness and low vision 74, 66 subjects aged between 18 and 30, 74 aged between 31 and 50 years and 40 aged between 51 and 80 years. The analyzes are presented as the six attributes for the assessment tool: objectives, access, clarity, structure and presentation, relevance and effectiveness, and interactivity.

Table 1 shows the results according to each attribute.

Table 1. Assessment of items corresponding to the topics objectives, access, clarity, structure and presentation, relevance and effectiveness, and interactivity

Item	Part.Adeq./ Inadequate		Adequate		p	Average
	N	%	n	%		
Objectives						
1 It relates the content covered in your day to day	5	2.8	175	97.2	<0.0001	1.96
2It clarifies doubts about the content addressed	1	0.6	179	99.4	<0.0001	1.99
3 It stimulates learning about the content addressed	5	2.8	175	97.2	<0.0001	1.97
4 It stimulates learning new concepts or facts	2	1.1	178	98.9	<0.0001	1.96
Access						
5 It allows you to search for information without difficulty	12	6.7	168	93.3	<0.0001	1.93
6 It offers the appropriate and necessary resources to its use	32	17.8	148	82.2	<0.0001	1.81
Clarity						
7 It presents information needed to better understand the content	7	3.9	173	96.1	<0.0001	1.96
8 The information content is suitable for your needs	29	16.1	151	83.9	<0.0001	1.84
9 I presents the information in a simple manner	-	-	180	100.0	<0.0001	2.00
Structure and presentation						
10 It displays the content in a organized manner	-	-	180	100.0	<0.0001	2.00
11 It has an attractive presentation strategy	10	5.6	170	94.4	<0.0001	1.93
Relevance and effectiveness						
12 It allows you to reflect on the content presented	-	-	180	100.0	<0.0001	2.00
13 It awakens your interest to use it	15	8.3	165	91.7	<0.0001	1.90
14 It encourages a change in behavior on you	22	12-2	158	87.8	<0.0001	1.83
15 It reproduces the content addressed in different contexts	5	2.8	175	97.2	<0.0001	1.97
Interactivity						
16 It offers interaction, active involvement in the educational process	-	-	180	100.0	<0.0001	2.00
18 It provides autonomy to the user in relation to its operation	5	2.8	175	97.2	<0.0001	1.97

Scale: 0 = Inadequate; 1 = Partially Adequate; 2 = Adequate; Binomial test.

According to Table 1, it appears that 11 items were evaluated as adequate by more than 95% of subjects. All inferential statistical analysis obtained p <0.05, thus considered significant.

Only six items obtained rating below 95% and average below 1.95, namely: "It allows you to search for information without difficulty" (93.3% - 1.93); "It offers the appropriate and necessary resources to its use" (82.2% - 1.81); "The information content is suitable for your needs" (83.9% - 1.84); "It has an attractive presentation strategy" (94.4% - 1.93); "It awakens your interest to use it" (91.7% - 1.90); and the item "It encourages a change in behavior on you" (87.8% - 1.83), which correspond respectively to the domains access, clarity, structure and presentation, and relevance and effectiveness.

In the item "It allows you to search for information without difficulty", 6.7% of subjects reported difficulty with the handling of the audio to search each STI quickly. Thus, the audio division into groups to enable this handling was suggested and implemented.

In relation to the resources available for the use of AT (item 6), 17.8% considered this item inadequate or partially adequate due to the difficulty with the use of audio in MP3 format, also suggesting to make it available in Braille.

Regarding the adequacy of the information to the needs of the subjects (item 8), 16.1% of subjects did not consider this as adequate. Further deepening suggested in each disease, and they scored the addition of content: taboos involving the context of the person with HIV (Human Immunodeficiency Vi-

rus), vertical transmission, and the citation of both types of condoms, as well as their gratuity. The specific suggestions were added, considering that the deepening on the content for each STI was not feasible. Concerning vertical transmission, this theme and the importance of prenatal care are already presented in the AT in relation to syphilis. However, as suggested, information on HIV was also added.

The AT highlights the use of condoms and focuses on prevention generally implying that when referring to condoms, the two existing types are considered. However, because the need for disclosure and specification as to the existence of the female condom was recognized, this item was strengthened on the back.

Table 1 shows the back before and after the changes performed in the AT as suggested by subjects.

The evaluation of the absence of the sense of vision in the way the characteristics of some diseases were described was questioned. According to some subjects, the *Human Papilloma Virus* (HPV) wart, exemplified as cauliflower, could only be understood by those who have had physical contact with the structure. When considering the possibility that many subjects have the tactile understanding of cauliflower, this characteristic was kept.

In turn, another aspect, it would not be possible for the visually impaired person to identify colorations of urine and feces, which are changed in cases of hepatitis B and of the discharge caused by gonorrhea and trichomoniasis. Thus, the typical colorations of gonorrhea and trichomoniasis were removed and specific colorations and features of the skin and physiological eliminations present in the case of hepatitis B were kept, making it clear that they could be identified with the help of a person that can see.

For the evaluation of the audio with the rhyme, in the item "It has an attractive presentation strategy", 5.6% of subjects considered it inadequate or partially adequate. Problems were detected in the recording, suggesting that the reading of the contents should be slower and the background music softer in relation to the voice.

About item "It awakens your interest to use it", 8.3% of subjects did not considered this items adequate, as they find the text too extense. However, the length of the AT was not changed because there are too many diseases, it is not possible to further reduce the length of the audio because it is already prepared with a view to clarifying key information relevant to the theme, portraying key aspects of this content.

Finally, in relation to stimulate change in behavior, 12.2% of subjects rated as inadequate or partially adequate because they considered that people that have a steady partner does not use a condom to prevent STIs and also is not aware of the occurrence of these diseases .

Discussion

It is necessary to include people with disabilities in the development of technologies since its construction in order to ensure accessibility and usability for all users[6]. The visually impaired people, just like any other people, have their preferences, interpret in their own manner the environment, know how to communicate and express their needs. Their perception is based on life experiences and visual impairment only makes these manifestations to be expressed differently, where the feature of vision does not exist or is very limited.

A study reinforces that sex education aimed at this population may be an effective strategy provided as far as it is effectively accessible, with resources tailored to their reality, with the use of information in Braille, anatomical drawings in high relief, audios, movies and videos with audio description[7].

The use of sound as a resource, when properly presented to what it is proposed, therefore allows, in addition to transmission of information, the idealization of structures by persons with visual disabilities.

The difficulty reported by the subjects in handling the audio to seek STIs individually led to the division of the audio into tracks, separated to each disease. As the AT developed here is intended to serve as a consultation vehicle with general information about the various STIs, accessible to people with visual disabilities, releasing it this way would facilitate the identification of the disease of interest at that moment.

Similarly, the spoken journal adopts strategy of division into tracks. According to the researchers, as well as in printed, people can instantly flip through the pages that they do not have interest. Once the listener is not interested for the track he is listening, he can go to the next[8].

The resources available for the use of AT, some people reported difficulty in handling the audio in mp3 format and, above all, on the computer, suggesting the need to make it available in Braille. It is believed that the inferred difficulty is due to the lack of opportunity and training in the use of technological resources.

A research on the use of AT for learning among visually impaired people reveals the use of resources using, among others, the Braille at the expense of the computer and reinforces the issue of the difficulties of acquisition, access and use of computer equipment at home and even in educational institutions, which still do not have these resources[9].

As for the still existing prejudice in the context of HIV-positive persons, the literature states that the fear of contagion and lack of information are factors that cause social stigma. Authors cite reports of withdrawal of other people related to fear of any physical contact like handshake and kiss on the cheek, for example[10].

On the types of condoms, despite the positive characteristics of the feminine condom, access is still insufficient, both for their lack in health facilities, the little incentive of their use that professionals provide[11]. Strengthening such a strategy would, therefore, preserve the right of autonomy and empowerment of women, highlighting those with visual impairment.

The characterization of the structures presented in AT, points to ways in which corrections were requested by subjects. It depends on the perception of each individual to understand that there is an image that can infer the idealization of the same structure being understood in different ways in a given group[12]. In relation to the senses, besides speech, blind people also perceive the world using the touch and hearing, and learning to memorize how a given structure is constituted[13].

On the corrections made on the recording, about the attractive presentation strategy, a study sought to evaluate the preference of the means of access to content used by blind people. It was found that, in relation to the audio, the voice of the narrator can have an influence on the use of audio resource. For subjects, a nasty voice, as well as excessive breaks or too fast narration can cause disinterest[14]. Thus, specific care is necessary. The final version of the AT was re-recorded by reading more slowly, with soft background music in relation to the voice.

In the assessment of the AT on the changeof behavior in face of the scenario of STIs by adopting condom use, it was reported a lack of interest in STIs prevention and this is justified by the existence of a steady relationship and not by the use of this device.

Thus, the testimonials of men and women with visual impairment during workshops in which they discussed sexuality, STIs and use of male and female condom, reinforce sexual ability and insinuate the occurrence of betrayal among couples[15].

Thus, as it happens in the general population, the existence of fixed partners does not reduce the risk of STIs, and therefore, should be a theme to be worked with these clients. The fact that the subjects themselves requested details of each disease reinforces the importance of this topic for them.

Conclusion

According to the established methodological framework, the validation of assistive technology "*STI - in order to prevent, it is necessary to learn*" led to the conclusion that the methodological approach is validated. All recommended steps were followed.

As for the evaluation of the AT, statistical analyses showed that despite the suggestions for its improvement, all items of the assessment tool were adequate in more than 80% and the majority in more than 95%, suggesting that it is an adequate resource for the promotion and health education for the proposed clientele.

The main changes made were: a recording with paced reading and background music at lower volume in relation to the voice; division into audio tracks separated by each disease; mention of the two types of condoms and their gratuity; taboos involving the context of the person with HIV and its vertical transmission. The expressions that could not be included in the scenario of lack of vision, such as colorings, were eliminated or made explicit as for the person who can see.

Limitations of this study primarily correspond to the difficulty of access to subjects for data collection, especially because it is a theme still surrounded by social stigmas, especially in the context of this clientele.

As a future perspective, in an attempt to reduce the difficulty of access to information for people with visual impairments, particularly in the context of prevention of STIs, it is intended to make the technology available for all sites where assistance to this population is provided, as well as for users.

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Peak injection pressure is a better monitor of intraneural injection than the rate of pressure rise

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Abstract

Introduction: Abnormal resistance to injection (>11 psi) may indicate intraneural needle placement and increase the risk of neurologic injury. Because during peripheral nerve block (PNB), the peak pressure can not be determined until it has passed, we sought to determine whether the rate of rise of injection pressure (IP) is superior to peak IP in predicting the development of neurologic injury. Our hypothesis is that real-time evaluation of momentary rate of rise of IP provides a window of opportunity during PNB to detect intraneural injection and abort the injection.

Materials: Fifty adult Wistar rats (300 g) both sexes were studied. After induction of general anesthesia, the sciatic nerve was exposed bilaterally (n=100). Under direct microscopic guidance, a 25 G needle was placed either perineuraly (n=50) or intraneurally (n=50) and 4 mL of preservative-free lidocaine 2% was injected using an automated infusion pump (4mL/min). IP data were acquired using an in-line manometer coupled to a computer via an analog-digital conversion board. After nerve block injection, the animals were awakened and subjected to serial neurological examinations. The earliest injection time at which the peri- and intraneural groups diverged was assesed graphically with means and confidence limits. Discriminant analysis was used to determine whether the rate of rise of IP was superior to peak IP to segregate injections into peri- and intraneural injections. The co-linearity among the neurological measures was assessed with correlation analysis to determite if there was redundancy.

Results: All perineural injections resulted in preassures <5 psi, and 8 of the intraneural injections resulted in high pressures (11.6±3.0 psi) at the beginning of the PNB. Motor function returned within 3 h with all perineural injections. Neurologic deficits persisted throught the study period with all 8 intraneural injections resulting in high IP (>11 psi). A discriminant analysis to determine which aspect of the IP recording provided the best segregation of peri- and intraneural injections showed peak pressure and slope-to-peak both provided 100% sensitivity and specificity. Examination of means and 95% confidence limits of the peri- and intraneural group exceeded the highest pressure of the perineural group was 72 sec.

Conclusion: Since high IP (>11 psi) during PNB predicts the development of neurologic injury, we attempted to identify whether real-time evaluation of the build-up of nerve block injection pressure could provide a basis for action to prevent intrafascicular injury. In discriminant analyses both peak pressure and average slope-to-peak well discriminated the peri- and intraneural preparations. However, since the average time-to-peak in the intraneural group was 80 sec, rate of pressure did not offer a meaningful informational advantage over simple monitoring of absolute pressure.

Key words: Intraneural injection, High injection pressure, Local anesthetic, Neural injury

Introduction

Peripheral nerve blockade (PNB) with local anesthetics is common practice in providing pain control for wide range of surgical procedures and pain syndroms (1). Unintentional intraneural injection of local anesthetics may cause mechanical injury and pressure ischemia of the nerve fascicles. Peri-operative nerve lesions can mark a successful surgical procedure, handicap a patient with severe functional disability and leave the medical team facing possible protracted and unpleasant litigation. The true incidence of peri-operative nerve

damage remains unclear and, as a complication, it is probably under-reported (2). Neurologic injury associated with peripheral nerve block is a rare but serious potential consequence of PNBs. Unfotunately, the mechanism by which unintented intraneural injection causes nerve injury is not well understood (3). Possible mechanisms of neurologic injury may be related to mechanical needle injury, injection force, vascular injury or neurotoxicity of local anesthetics and their additives (4).

In current clinical practice, there is no consensus on techniques or methods to reduce the risk of intraneural injection. Much on the debate on how to prevent intraneural injection and nerve injury associated with PNB has focused on methods of nerve localization (e.g., paresthesia versus nerve stimulation). However, there is still no evidence that one method is safer than the other, and neurologic injury can occur even with experienced practitioners (1).

One study performed im small animals suggests that intraneural injection may be associated with high initial injection pressure (5). Intrafascicular needle placement associated with high opening injection pressure can result in neurologic injury in animal models (3, 6).

Our hypothesis is that real-time evaluation of momentary rate of rise of IP provides a window of opportunity during PNB to detect intraneural injection and abort the injection.

Material and methods

Animals

The study was conducted in accordance with the Principles of Laboratory Animal Care (7) and was aproved by the Laboratory Animal Care and Use Comittee of the Medical and Veterinary Schools of the University of Sarajevo. Fitfy Wistar rats, both sexes (300-350 g, 3 months old) were used in this study. Animals were housed in central animal care facility. Food and water were provided ad libitum. Animals were monitored for appropriate postsurgical recovery.

Experimental design

On the day of experiment, the rats were anesthetized with pentobarbital sodium (50 mg per kilogram of body weight) administrated intraperitoneally. Thereafter, by using an aseptic technique for survival surgey, the sciatic nerves (n=100) were exposed bilaterally through a gluteal muscle-splitting incision. Under idrect vision by using light microscope, a 25-gauged insulated nerve block needle (ProBlock, LifeTech, Stafford, TX) was placed either intraneurally (n=50) or perineurally (n=50). For perineural injections needle was placed within the epineural tissue but outside the perineurium, while for intraneural injections the needle was placed intraneurally inside the perineurium. The needle was mechanically stabilized without suturing, and 4 mL of preservative-free lidocaine 2% was injected under direct vision using an automated infusion pump (PHD2000; Harvard Apparatus, Holliston, MA) with speed of 4 mL/min.

In this study the following methods have been used:

1. Measuring and analysing of intraneural and perineural injection pressure
2. Evaluation of neurological status of the animals
   - Injection pressure data were acquired using an in-line manometer (PG5000; PSI-Tronics Technologies Inc, Tulare, CA) coupled via an analog-to-digital conversion board (DAQ card 6023; National Instruments, Austin, TX). The manometer was placed proximal to the needle and in line with nondistensible high durometer polyvinyl chloride injection tubing (84“ arterial pressure tubing; Abbot Critical Care Systems, Abbot Laboratories, North Chicago, IL). The data on pressures have been analysed using the special software package BioBench 1.2 (National Instruments, Austin, TX) assigned for registration and processing of data obtained in various medical researches as well as for educational purposes. In this study BioBench program has been used in the term of registration and processing of pressure values during intraneural and perineural application, while also at the same time registering the time interval needed for application.
   - After executed injection application and awakening of animals from general anesthesia the methodic neurological

examination was performed. A veterinarian who was unaware of the specific injection performed monitored neurologic function (immediately after awakening, each two hours during the 12 hours of first day, and one time during next 7 days). Neurological examination has been conducted by modified Thalhammer's neurological examination (7), and included assessment for the presence and severity of paresis and ataxia by following criteria:
Paresis : 0 – absent, 1 – slight paresis; 2 – moderate paresis; 3 – severe paresis; 4 – flaccid extremity.
Ataxia : 0 – no ataxia; 1 – slight ataxia; 2 – moderate ataxia; 3 – severe ataxia.

On day 7, the rats were killed by using an overdose of sodium pentobarbital and potassium chloride.

Statistical analysis

One hundred sciatic nerves (50 rats) were required for the power of 0.80 to detect a significant difference in proportions of nerve injury between intraneural and perineural injections at α=0.05 (8). Statistical analyses were performed by using SPSS program, version 11.5. Maximum pressure values during intraneural and perineural njections were compared by using paired t-test. Rates of neurologic injuries were compared between intraneural and perineural injections by using McNemar's test for paired proportions. Fisher's exact test was used to compare injury rates during the intraneural injection between groups of rats, based on injection pressure threshold values. A P value < 0.05 was considered to be significant.

Results

Pressure Data

The average peak pressure for the intraneural injection group was 11.6 + 3.0 psi versus 3.1 + 0.8 psi for the perineural injection group ($p < 0.0001$). The time to attainment of peak pressure was comparable in the two injection groups (381 + 71 versus 327 + 133 seconds) ($p < 0.23$). The pressure gradient slope from the beginning of the procedure to the attainment of the peak pressure was also different in the two groups (0.032 + 0.009 versus 0.015 + 0.008) ($p < 0.0001$). This data indicates that the mechanics of the approach to the administration of the injections was carried out at a pace that was similar in the two preparations, although a higher pressure was involved in the intraneural injection. The lowest pressure attained in the period following the peak pressure was significantly higher in the intraneural group (4.4 + 1.6 versus 1.4 + 0.4) ($p < 0.0001$) showing, as expected, that the pressure required to infuse is higher within the intraneural capsule than in the adjacent periphery. The number of seconds required to hit a nadir pressure following the attainment of peak pressure was somewhat longer in the intraneural group (825 + 216 versus 647 + 191 perineural group), but this difference barely achieved statistical significance ($p<0.05$). Finally the total injection pressure subjected to the injection site (area under the curve for pressure for 2400 seconds), was significantly greater by 3.5-fold in the intraneural versus the perineural group (7157 + 2556 vs 2026 + 541) ($p<0.0001$), (Table 1.).

Table 1. Pressure values in intraneural and perineural groups

	Intraneural Preparations		Perineural Preparations	
	Mean	SD	Mean	SD
Pressure Peak (psi)	11.6	3.0	3.1	0.8
Seconds to Peak	381	71	327	133
Slope to Peak	0.032	0.009	0.015	0.008
Pressure Nadir (psi)	4.4	1.6	1.4	0.4
Seconds to Nadir	825	216	647	191
AUC (2400sec)	7157	2556	2026	541

A discriminant analysis was used to identify which of the derived parameters from the pressure tracinings were superior for correctly classifying intraneural versus perineural injections. Knowledge of the peak pressure attained during procedure correctly classified all perineural injections and 85% of intraneural injections. Two intraneural preparations achieved peak pressures under 5-psi, only slightly higher than the maximum peak pressure reported in the perineural group, which accounts for the misclassification rate of 15% for the intraneural group. Neither the number of seconds to pressure peak nor the slope of the pressure gradient to peak showed a better capacity to classify intraneural injections (60% and 73%, respectively).

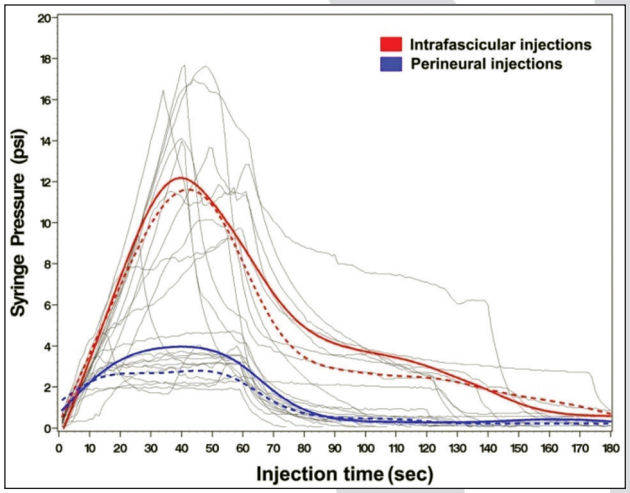

Figure1. Injection pressures during intrafascicular and perineural injections

Neurologic Data

On awakening from general anesthesia, all rats exhibited signs of sciatic nerve blockade during neurologic examination. Motor function fully recovered within 3 hours after all perineural and low-pressure injections, where none of the extremities exhibited residual paresis or ataxia. In contrast, after 8 intraneural injection with high injection pressure (>11 psi), severe and persistent motor deficits were recorded in all 4 extremities throughout the study period.

After executed neurological exam, it has been established that all injections joined with high application pressure resulted with failing, which lasted more than 24 hours, and neurological deficits were evident yet at the end of experiment, after 7 days, which clearly shows that intraneural injection caused the nerve injury.

On contrary, all injection combined with low injection pressure, whether they were intraneural or perineural did not result with neurological sequels at the end of experiment.

Discussion

It is known from previous studies that all the local anesthetics are potentially neurotoxic if used in excessively high concentration or acting on the nerve through a prolonged period of time (9, 10, 11). However, previous experience has shown that perineuraly placed local anesthetic are significantly deprived of a neurotoxic potential and carries a small risk of nerve injury. The reason for this is probably that in normal circumstances injection bolus of local anesthetic reaches the equilibrium pressure with the surrounding tissue. At that moment happens diffusion into the surrounding tissue, interstitial fluid quickly dilute local anesthetic and its concentration decreases with further systemic absorption (12). The results of our study have shown that all of perineural injection of local anesthetic did not result in significant damage to the nerve fibers.

In contrast to perineural injections, intraneural (intrafascicular) injection of local anesthetic can result in nerve injury. In a several previous studies, in which is used a different injection agents, it is showed that only intrafascicular injection can cause a nerve damage (3, 13, 14). The relatively low incidence of complications associated with peripheral nerve blockade, together with the lack of objective monitoring and precise monitoring procedures during nerve blocks, makes a retrospective analysis of cases with nerve injury very speculative with respect to the current mechanism of nerve injury in clinical practice. Mechanism of injury after intrafascicular injection involves direct mechanical injury, neurotoxic effects of local anesthetics, changes in the permeability of blood-nerve barrier, edema, ischemia due to pressure, increased pressure of a endoneuronal fluid. All that contributes to the formation of nerve injury (12). Our results are consistent with findings from previous studies showing that intraneural injection increases the risk of nerve injury. In our study most of the intraneural placed

injection, combined with high injection pressure (11.6±3.0 psi), resulted in persistent neurologic deficits throughout study period, which shows that the mechanical factor, caused by needle, crucial in the formation of nerve injury.

The neurological consequences of an intraneural needle insertion and injection of local anesthetic depend on many factors such as the design of the needle tip, injection pressure and the chemical structure and concentration of injectate. However, the anatomic specifies of a given nerve and how its organization can protect it against extrenal injury may be the most significant factor that determines the likehood of injury (15). In other words, our results indicate that the site of application is a crucial factor in determining the degree of neuronal injury. In order to achieve neurological injuries, injection should be injected intrafasciculary while extrafasciculary injection of the same injection chemicals, usually do not cause nerve injury. The extent of damage of the nerve after intrafascicular injections can vary from minimal to severe axonal and myelin degeneration (16, 17).

In current clinical practice, there is still no consensus on the technique or method that reduces the risk of intraneural injection, which has imposed need for the development of the objective and reliable monitoring for preventing intraneural injection and consecutive neurological injury. There is still much debate about how to prevent intraneural injection and nerve injury associated with peripheral nerve block and all are focused on methods of nerve localization (paresthesia versus nerve stimulator). However, there is still no evidence that one method is more secure than others, because of neurological sequelae follow both methods (18, 19, 20, 21, 22).

The oldest method in the detection of neural structures during peripheral nerve blockade is a method of paresthesia. Today there are many data showing that a sharp, lancinating pain may suggest intraneural placement of injection of local anesthetic and increase the possibility of nerve injury. However, in clinical practice it is difficult to distinguish when the pain of injection of pareshesis is „normal“ and when it is an ominous sign of intraneural injection. Paresthesia can be compromised with sedated or anesthetized patients. This method is also unacceptable in pediatric patients, because child is not able to precisely report paresthesia or to distinguish it from other discomforts during the block execution (13, 20).

In today's clinical practice in the detection of neural structures commonly used are nerve stimulators. However, it should be noted that nerve stimulators used for peripheral nerve blockade vary in their characteristics, frequencies of stimulation, the maximum production power, the duration of the stimulus and their accuracy. The main problem with peripheral nerve stimulator is that the current is flowing in all directions, and not necessarily only to the nerve. The occurrence of nerve injuries despite the use of a peripheral nerve stimulator in the localization of nerves, indicates that nerve stimulator can provide only a rough and approximate information about the relationship between the needle-nerve (23, 24, 25).

Latest method in attempts to identify the neural structure is ultrasonic method. However, the use of ultrasound has significant disadvantages: high price, which makes it less accessible and size, which makes it less portable. Also, presently available ultrasound technology does not differentiate between peripheral nerves and tendon fibers, which with sometimes poor picture resolution presents additional disadvantage of this method (26).

During nerve blockade procedures clinicians often rely on the sense of resistance to injection, or how much power is needed to produce adequate flow. Anesthesiologists often rely on subjective assessment of abnormal resistance to injection when performing peripheral blocks, knowing that intraneural injection results in greater resistance to the injection and subsequent nerve damage. Hadžić and associates showed that the perception of the appropriate resistance varies considerably among anesthesiologists, and that this method is inconsistent and that it can affect different design of the needle (3). Resistance to injection is greater for needles with smaller diameter, and such needles are used for peripheral blocks. The achived pressure during injection significantly varies among the needles of same lenght and diameter but to different manufacturers.

Pressure monitoring is another possible modality to help determine needle tip placement, and is recommended by some authors (27), though clinical data is very limited, and animal studies have

yielded variable results (3, 6, 28, 29). A recent clinical study of interscalene block with pressure monitoring suggests that high opening injection pressure may consistently detect contact of the needle tip with the nerve root, which may allow prevention of injection when the tip is inadvertently advanced into the nerve (30). In 1978. Salander with associates performed a study in rabbits. The authors indirectly concluded, without any futher analysis, that generally a greater pressure (i.e. ≥11 psi) is required to inject solutions intraneurally than perineurally (5). Until 2004., when a group of authors performed series of studies on dogs, pigs, rats and rabbits, and demonstrated that intraneural, intrafascicular injections are accompanied by high injection pressure (3). Indeed, in our study 8 of the intraneural injections were associated with injection pressures greater than 11 psi, whereas none of the perineural injections resulteted in a pressure greater than 5 psi. Intraneural injection involves significantly higher injection pressure prior to penetration of the internal bundle and continued high injection pressure probably due to the restricted diffusion space within the bundle relative to that observed for perineural preparations. More importantly, intraneural high-pressure injections in our study were also associated with persistent neurologic deficits. Our results do not offer an explanation as to why some, but not all, of the intraneural injections performed in our study resulted in high injection and nerve injury. It is possible that in the low-pressure intraneural injections that did not result in neurologic consequences, the needles were not lodged intraneurally but between the fascicle instead of intrafascicular. This is because it is difficult to ensure intrafascicular placement of the needle even under direct vision. In that case local anesthetic is deposited out of fascicle and such blockade lats for hours after injection, but there in no histological evidence of nerve fibers damage. In our study fascicular injury and neurological deficit were developed only after intraneural injection joined with high injection pressure. High injection pressure may rupture the perineum, interrupt capillary blood flow and cause neural ischemia. It is obvious that intraneural injection do not always lead to nerve injury. However, high injection pressure during intraneural injection may be indicative of intrafascicular injection and predicts development of neurologic injury. The pressure information displayed by manometer reliably indicate the pressure rate the tip of the needle, regardless to the size of the needle or the rate of injection applied. Simple monitoring of absolute pressure is suficient to determine where the tip of the needle is and to predict nerve injury as well. Avoiding high injection pressure prevents from lodging the needle into intraneural space followed by consequential complications.

Results from our study shows some limitations. First, injection pressure limits that predicted nerve injury in our study may be specific for the needle type and injection configuration in an open animal model. Second, we used a slower rate of injection (4mL/min) than what is commonly used clinically (9-27 mL/min) (31). Third, there may be species differences between dogs and humans. Fourth, the sciatic nerve in our study was surgically exposed without the surrounding connective tissue and needles were inserted under direct vision. It is possible that such a dissection may have increased the potential for nerve injury because in clinical practice nerves may be more moveable in the connective tissues and less likely to be penetrated by an advancing needle.

Conclusion

Based on our research it is obvious that measuring of pressure during the nerve blockade is very important in order to decrease the risk of neurological complications. In our dog model of relatively slow intraneural injection into sciatic nerve, the combination of intraneural needle placement and high injection pressures resulted in severe neurologic injury. Knowledge of the peak pressure attained during procedure correctly classified all perineural injection and 85% of intraneural injection. With careful analysis of the value of pressure in all three scenarios, we concluded that peak injection pressure is a better monitor of intraneural injection than the rate of pressure rise. If these results are applicable to clinical practice, avoidnig excessive injection pressure during nerve block administration may reduce the risk of neurological injury.

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The value of Rv0220 as a stimulus of interferon-gamma release assay in detecting latent infection of tuberculosis

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Abstract

We prospectively evaluated Rv0220 protein as a stimulus of interferon-gamma release assay (IGRA) using 30 cases TB-positive patients' peripheral blood tested in Xinjiang Kashi Tuberculosis Control Center (lung nuclear hospital), IFN-γ responses were detected in 66. 67% (20/30) of Rv0220 antigen protein, and there was no significant difference observed between the positivity of commercially available control group (ESAT-6/TB7. 7/CFP-10 mixed antigen protein). Rv0220 is a potential antigen protein, and combined with other antigen proteins, can be advantageous for the detection of latent tuberculosis infection via IGRA.

Key words: IFN-γ, LTBI, IGRA, Rv0220 protein

Background

Latent tuberculosis infection (LTBI) is a special clinical manifestation consisting of imaging changes or bacteriological evidence of nonactive tuberculosis after being infected with *Mycobacterium tuberculosis* (MTB) [1,2]. If not treated and controlled in time, 5%–10% of cases will develop into active tuberculosis (TB), and a higher proportion will develop among people with immunosuppressive disorders [3].

There are many shortcomings in existing diagnostic methods. Clinical symptoms and imaging features are helpful for discovery in suspected patients, but further laboratory diagnosis is needed. At present, the existing early diagnosis methods mainly rely on smear microscopy, molecular biology detection, tubercle bacillus culture, and TB antibody detection [4]. In recent years, interferon-gamma (IFN-γ) release assays (IGRA), based on the region of differences (RD) area antigen of MTB, have been used to diagnose MTB infection, and have been proven to have good specificity and sensitivity [5,7], so they are widely used at present. To confirm efficient diagnostic antigens for M. tuberculosis, the serodiagnosis value it is in dire need of screening the specific antigen protein of MTB with high detection rate in IGRA. In recent years, more and more emerging evidence indicates that bacterial cell walls or surface-localized enzymes can be used as antigenic proteins to determine an immune reaction or the invasion of host cells [8,9].

In this study, we used a special antigen protein, Rv0220, and explored its diagnostic value with IGRA. Rv0220 is a member of the Lip family based on the presence of the consensus motif GX-SXG, which is characteristic of esterases, and is a cell surface protein that is present in both the cell wall and the capsule of MTB. RV0220 is a new cell surface associated MTB esterase with high highly Immunogenicity and induction of antibodies and cytokines/chemokines, which could cause a strong humoral immune response in HIV negative (HIV) and HIV positive (HIV+) TB patients [10]. In the present study, we used Rv0220 for the first time as the stimulus in IGRA.

Materials and methods

Bacterial strains, plasmids, and serum

Bacterial strains and plasmids used in this study are listed in Supplementary Table 1. Unless otherwise stated, all of the strains were grown in LB medium. An aliquot (20 μL) of overnight culture was inoculated into 20 mL medium, and the bacterial cultures were incubated at 37°C.

Expression and purification of recombinant protein

We used Primer5. 0 software to design upstream and downstream primers with reference to the nu-

Supplementary Table 1. Bacterial strains, plasmids, and serum

Strain, plasmid and serum	Source
Strain	
E. coli DE3	Zoonosis Laboratory of Shihezi University.
E. coli DH5α	
plasmids	
PMD18-T	Zoonosis Laboratory of Shihezi University.
pET-28a	
serum	
Negative serum	Affiliated Hospital of Shihezi University
Positive serum	Xinjiang Kashi tuberculosis control center (lung nuclear hospital)

cleotide sequence of the Rv0220 (1220 bp) gene combined with mycobacteria published by NCBI, and the primers were synthetized by Sangon Biotech. PCR amplification was then performed using *M. tuberculosis* genomic DNA as the template. The amplified products were observed by 1% agarose gel electrophoresis. A DNA recovery kit was used to recover the Rv0220 PCR product, which was ligated into a PMD18-T cloning vector. The recombinant vector was used to transform *E. coli* DH5α cells. The Positive PMD18-T vectors were extracted and digested by EcoR I and HindⅢ enzymes, and the target sequence was ligated into a pET-28a expression vector, which was then used to transform *E. coli* DH5α. After culturing in LB solid medium overnight, single clones were chosen and verified for the presence of the Rv0220 gene by PCR. According to the results of agarose gel electrophoresis after PCR, the recombinant pET-28a expression vectors were extracted.

Recombinant plasmids expressing Rv0220 protein were transformed into *E. coli* DE3. Cells were induced by 1mmol L^{-1} isopropyl thiogalactoside (IPTG) for 6 h at 37°C, then collected. Cells were disrupted by sonication then applied to a His Trap FF Ni ion affinity chromatography column with binding buffer, and then eluted with elution buffer. The collected products were transferred to nitric acid fiber after SDS-PAGE electrophoresis, then blocked by 5% skim milk for 2 h. Western blotting was conducted with a 1: 50 dilution of the positive tuberculosis serum as the first antibody and sheep anti-human IgG-HRP (1: 8000 dilution) as the second antibody. After coloring with DAB (1: 1), deionized water was used to terminate the reaction.

Indirect ELISA detection

First, 50 cases of negative serum were used to test the negative and positive cut-off values of IgM and IgG, respectively. According to the indirect ELISA matrix titration, a concentration of Rv0220 protein of 0. 25 μg ml^{-1} was used to coat a 96-well ELISA plate at 100 μL per well. Then 5% skim milk (200 μL/well) was added, and the plate was incubated at 37°C for 2 h. After washing by PBST, 100 μL serum (1: 50 dilution) as the first antibody was added to each well at 37°C for 30 min, and after washing, 100 μL sheep anti-human IgM-HRP or IgG-HRP (1: 20,000 dilution) as second antibodies were added to each well at 37°C for 30 min. After washing, 50 μL of the coloring agent TMB was added to each well and incubated for 15 min at 37°C, then the reaction was terminated by 50 μL H_2SO_4. The absorbance at 450 nm was measured within 10 min by an ELISA instrument. Primer17. 0 software was used for analysis of the data. The criteria were established based on the measured average OD450 nm value (X) and standard deviation (SD). X+3*SD was used as the positive cut-off value and the X+2*SD as the negative cut-off value, and it was doubtful between the two situations.

According to the established ELISA procedure, the specificity and sensitivity of Rv0220 antigen protein were analyzed in 336 healthy human TB-negative serum samples and 86 TB-positive serum samples.

Interferon-gamma release assay (IGRA)

Using 24-well ELISA plates, each test sample was divided into two experimental groups. First, RPMI-1640 cell culture medium was added to each well. To the negative control group (N) and positive

control group (P) respectively were added PBS and ConA, both at a final concentration of 10 μg mL^{-1}. The two experimental groups (T) were the ESAT-6, TB7. 7, and CFP-10 protein mixed solution, and the Rv0220 protein solution, and both were added at a final concentration of 10 μg mL^{-1}. Then, 400 μL lymphocytes (2. 5×106/mL, separated from peripheral blood samples in heparin sodium vacuum blood collecting anticoagulant tube) were added to each reaction well such that the number of cells and volume of each well were equal. Using sterile centrifuge tubes (endotoxin-free, RNAse-free), samples were collected and centrifuged at 3000 rpm for 15 min, and the supernatants were stored at -20°C until use. The content of IFN-γ in serum (pg mL^{-1}) was detected by ELISA.

To standard sample dilution wells were added 100 μL of freshly prepared concentration gradient (400, 200, 100, 50, 25 and 12. 5 pg mL^{-1}); the blank well contained no liquid. Plates were incubated at 37°C for 1 h after the IFN-γ antibody of 50 μL was added. After washing, 50 μL of coloring agents A and B were added to each well and incubated for 15 min at 37°C. The absorbance at 450 nm was measured within 10 min by an ELISA instrument with termination solution. The standard curves of the antigen content and the corresponding absorbance were applied, and the correlation coefficient was > 0. 99, otherwise the test was not effective. According to the standard curve, the IFN-γ content of T, N, and P groups were calculated. IFN-γ release results provided by an IGRA-TB kit are shown in Supplementary Table 2.

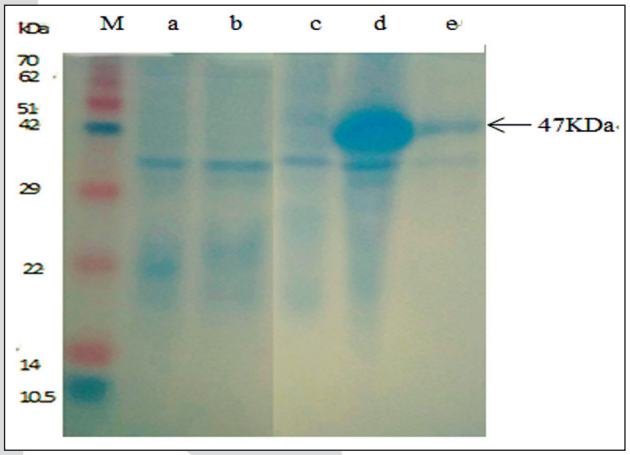
Statistical analysis

Data were entered into Microsoft Excel and analyzed using SPSS 17. 0; $p<0.05$ was regarded as significant.

Results

Purification of recombinant antigen protein

The pET-28a-Rv0220-DE3 expression vector was induced by IPTG and purified by Ni-ion affinity chromatography. The purified proteins were analyzed by SDS-PAGE. The molecular weight of the protein was about 47 kDa, which is consistent with the expected value, and the purified protein appeared as only one band (Supplementary Figure 1).



Supplementary Figure 1. Rv0220 protein SDS-PAGE electrophoresis.

Supplementary data #1 (Figure 1): M: protein marker; a: Negative check E. coli DE3; b: PET-28a vectors; c: Supernatant after ultrasound; d: Inclusion bodies after ultrasound; e: Purified Rv0220 protein

Identification by Western blot

Identification by Western blotting using a 1: 50 dilution of TB-positive serum as the first antibody and sheep anti-human IgG-HRP (1: 8000 dilution) as the second antibody showed that Rv0220 had good immunoreactivity (Supplementary Figure 2).

Supplementary Table 2. IGRA test results for determine the standard

N	P—N	T—N	Result
≤400	Any value	≥14且≥N/4	Positive
	≥20	<14	Negative
	≥20	≥14但≤N/4	Negative
	<20	<14	Indeterminate
	<20	≥14且<N/4	Indeterminate
>400	Any value	Any value	Indeterminate

Supplementary Figure 2. Western blot analysis of recombinant Rv0220.

ELISA specificity and sensitivity

Fifty cases of TB-negative serum were used to test the negative and positive cut-off values of IgM and IgG, respectively (Supplementary Table 3). Healthy human TB-negative serum (336 cases) and TB-positive serum (86 cases) were used to test the specificity and sensitivity. For the IgM antibody, 301 samples of negative serum were detected and 39 samples of positive serum were detected, which showed that the specificity and sensitivity for IgM were 89. 58% and 43. 35%, respectively. For the IgG antibody, 277 samples of negative serum were detected and 56 samples of positive serum were detected, which showed that the specificity and sensitivity of IgG were 82. 44% and 65. 12%, respectively. The above results are shown in Supplementary Tables 4 and 5. Moreover, the positive predictive value (PPV) and negative predictive value (NPV) of IgM were 52. 70% and 86. 49%, as well as the PPV and NPV of IgG were 48. 70% and 90. 23%, respectively. Overall, Rv0220 protein had better specificity and sensitivity than the mixed proteins.

Comparison between interferon-gamma release assays between experimental groups

The IFN-γ release was assayed from the 30 cases of TB-positive peripheral blood of (The sputum smear experiment showed positive). 20 cases had a positive IGRA of Rv0220 antigen protein, and 22 cases had a positive IGRA for ESAT-6/TB7. 7/CFP-10 mixed antigen protein. In comparing the two groups, the IFN-γ positive response rate between ESAT-6/TB7. 7/CFP-10 mixed antigen

Supplementary Table 3. Sheep anti-human IgM, IgG cut-off values

	Average OD value X	Standard deviation SD	Negative cut-off X+2*SD	Positive cut-off X+3*SD
Group	IgM	IgM	IgM	IgM
	IgG	IgG	IgG	IgG
Rv0220	0. 55034	0. 186634	0. 923608	1. 110242
	0. 42868	0. 1111	0. 65088	0. 76198

Supplementary Table 4. 336 negative serum specificity detection results of Rv0220

Group	Negative	Positive	Specificity
IgM	301	35	89. 58%
IgG	277	59	82. 44%

Supplementary Table 5. 86 positive serum sensitivity detection results of Rv0220

Group	Negative	Positive	Sensitivity
IgM	47	39	45. 35%
IgG	30	56	65. 12%

Supplementary Table 6. Comparison of IGRA of ESAT-6/TB7. 7/CFP-10 mixed antigen protein results in 30 positive serum.

Group	IFN-γ content				Positive rate	χ^2	p
	Mean	Maximum	Minimum	Standard deviation			
ESAT-6/TB7. 7/CFP-10	103. 95	382. 34	20. 82	72. 07	73. 33%(22/30)	0. 317	0. 573
Rv0220	173. 20	402. 74	41. 00	81. 72	66. 67%(20/30)		

protein and Rv0220 antigen protein was not statistically different (χ^2=0. 317, p=0. 573). Results are shown in Supplementary Table 6. Rv0220 antigen protein can be used as stimulator in IGRA for LTBI.

Discussion

According to the World Health Organization (WHO), it has been estimated that one-third of the world's population is latently infected with MTB and approximately 10% of individuals with LTBI will develop active disease during lifetime [11]. However, many developing countries have problems with low detection rates. Based on cytokine/chemokine secretion (IL-4, IFN-γ, tumor necrosis factor-α, IL-10, IL-12, and G-CSF), TST can measure skin induration response as an *in vivo* test [12]. This method is simple and cheap, but a reduction in the specific inoculation of Bacille Calmette-Guerin (BCG) will affect the result.

T-SPOT uses tuberculosis-specific activated T lymphocytes to determine *M. tuberculosis* infection in the peripheral blood, but because its commercialized kits are relatively expensive, they are not suitable for poor areas. T-SPOT is also an immunological method that cannot effectively distinguish between latent infection and active tuberculosis. Progress has been made with T cell-based detection *in vitro* via the stimulation of T cells to release IFN-γ *M. tuberculosis* antigen [13,16].

In recent years, the gamma interferon release test has been widely used in clinical diagnosis of tuberculosis. Compared with other tests, IGRAs can effectively divide the BCG vaccinated population and other non pathogenic Mycobacterium infection, provide guidance for clinical treatment, the whole test time is short (within 48 hours), and the sensitivity and specificity are higher. The principle is based on the immune response of T cells. T cells are exposed to specific antigens of *Mycobacterium tuberculosis* and reexposed T cells to these specific antigens (such as ESAT-6, CFP-10, etc.). They will differentiate into effector T cells and release a variety of cell factors, including high level IFN- gamma, and through the changes in IFN- gamma content or release the number of IFN- gamma T lymphocytes to determine whether the organism is infected with Mycobacterium tuberculosis. Meta analysis showed that the sensitivity of IGRAs in clinical detection of tuberculosis was between 78%~100% and specificity between 87. 5%~100% [17,18]. The results of the study found that the results of low sensitivity and specificity were mainly in the high burden countries of tuberculosis. Park HJ and Basirudeen and so on, found that has the same diagnostic effect as TST in the economically advanced areas where TB is a high burden [19,20]. The main factors of the difference in sensitivity and specificity in different countries and regions may be caused by the high polymorphism of human leukocyte antigen (HLA) in different populations and the different levels of cellular immunity in different populations, resulting in the different immune intensity of T cells caused by antigen. Therefore, it is of great significance to screen out IGRAs stimulated antigens suitable for the Chinese population on the basis of stimulating antigens.

In this study, we used Rv0220, which is located in both the cell wall and capsule of *M. tuberculosis*, as a stimulus of IGRA to explore its diagnostic value in LTBI. As demonstrated by ELISA，Rv0220 showed highly specificity and sensitivity. Based on the national corresponding standard of IFN-γ release, IFN-γ responses from 30 cases of TB-positive serum were 66. 67% (20/30) for Rv0220 antigen protein. In comparison with the control group of ESAT-6/TB7. 7/CFP-10 mixed antigen protein, the IFN-γ positive response rate between two groups was not statistically different (χ^2=0. 317, p=0. 573). Only using Rv0220 an antigen protein as a stimulus in IGRA, IFN-γ responses results almost closed to commercially available group (ESAT-6/TB7. 7/CFP-10 mixed antigen protein) Therefore, it is believed that Rv0220 antigen have great potential as IGRAs stimulus antigen, and a combination with other antigen proteins or fusion with other antigen protein genes will be become a new combination of stimulus with great potential, which can greatly improve the detection rate of LTBI and provide effective measures for the prevention and control of tuberculosis.

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The role and importance of diagnosisation of asimptomatic bacteriuria at pregnancy

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Abstract

Introduction: Data in contemporary literature show that significant asymptomatic bacteriuria occurs with fetal maternal complications in pregnancy.

Purpose of the paper: The aim of the paper is to analyze the role and significance of asymptomatic bacteriuria in the appearance of fetal maternal complications in pregnancy and to determine the incidence of asymptomatic bacterium in pregnancy by type of bacteria in our population.

Material and methods: The study included 110 pregnancies, which were selected according to the random method. Pregnant women were examined in clinical, laboratory and ultrasound form, including pregnancies from the 13th week of gestation until week 40. These pregnancies were urinary culture, and urinary tract results were grouped in the group of patients with sterile urinary tract with significant asymptomatic bacteriuria and non-significant asymptomatic bacteriuria.

Results: Through the obtained results we have ascertained that; premature birth, spontaneous abortion, uterine death, increased intrauterine growth restriction (IUGR), preeclampsia and anemia have been reported with higher incidence in patients with significant asymptomatic bacteria compared to sterile urinary patients. Pyelonephritis, Glomerulonephritis and Cystitis are presented only in cases with significant asymptomatic bacteriuria.

Conclusion: Fetal-maternal complications; premature birth, spontaneous abortion, death of uterine fruit, IUGR, preeclampsia and anemia have been reported with higher incidence of asymptomatic bacteria significant compared to sterile urinary patients. Pyelonephritis, Glomerulonephritis and Cystitis are presented only in cases with significant asymptomatic bacteriuria.

Key words: Significant asymptomatic bacteriuria, urinary culture, pregnancy, fetal-maternal complications.

Introduction

Asymptomatic bacteriuria is the presence of bacteria in the urine but without the presence of infection symptoms. Asymptomatic bacteriuria important for diagnosis and recovery is bacteriuria which; in 1 ml of urine accounts for more than 100,000 (105) microbes. It has been verified that the presence of significant bacterial infections in pregnancy is associated with pregnancy complications as well as fetal complications. In addition to (significant) bacteriuria there is also insignificant asymptomatic bacteriuria (not significant) if the presence of microbes is in small numbers. The frequency of occurrence of asymptomatic bacteriuria is 5 to 12%. It is diagnosed by the urine analysis in urine (urinary tract) during pregnancy. Most females carrying asymptomatic bacteriuria during pregnancy had asymptomatic bacteriuria even before pregnancy. Asymptomatic bacteria during pregnancy most commonly occur to women who suffer from diabetes, hypertension, kidney disease, and urinary tract abnormalities, primary and secondary immunodeficiency, autoimmune diseases, and the like. The authors (5, 10) found that in the period after puberty the frequency of asymptomatic bacteriuria is from 2 to 10%. While (1) in 1989 has verified that if asymptomatic bacteriuria does not cure, in 40% of cases, acute symptoms of urinary tract infection will develop. It should be noted that in these 40% of pregnant women with these acute symptoms 2% of them will have "sterile urinoculture". This is explained by the fact that a number of pregnancies at the beginning of pregnancy have some symptoms that are: disori-

entation, pain in the urinary bladder region, polarization, etc. In these cases it is a so-called cystalgium of endocrine origin. This is explained in this way that the rectal epithelium of the urinary vesicle of origin is receptive to estrogens. In some women with a low threshold of excitement of this epithelium by estrogens, the effects of estrogens in this epithelium are reported, such as hyperemia, pathology, edema and vasodilatation, whereby the mentioned symptoms are manifested. In order to eliminate this phenomenon, in all doubtful cases it should be a more careful diagnosing: repeat the analysis. For analysis should be taken the middle of the urine flow, the catheter analysis, the analysis after the disinfection of the periurethral region and the ostium of the vagina.

It should be considered that the small amount of bacteria in the urine does not speak for real bacteriuria. The authors (10, 13, 16) found that 70 to 90% of Escherichia colitis is caused by urinary infections, while the remaining 10 to 30% are caused by the proteus, staphylococcus, pseudomonas, klebsiellosis, and in some cases the flora Bacterial is mixed with two or more bacteria.

It is known that asymptomatic bacteriuria is not manifested with clinical symptoms and if it does not recover, complications appear on the urinary system, which are associated with clinical signs. Urinary tract infections may be local in the urinary vesicle, pyelone, kidney, and the infection may involve the entire urinary system. If asymptomatic bacteriuria does not cure in 20 to 40% of cases, acute pyelonephritis occurs. From this we observe how important the adequate healing of symptomatic bacteriuria is.

It has been verified that asymptomatic bacteriuria, acute pyelonephritis, chronic pyelonephritis, acute glomerulonephritis, chronic glomerulonephritis belong to the group of endangered pregnancies (15). In this group of endangered pregnancies, more often, spontaneous abortion, premature births, increased stagnation (IUGR), anemia, hypertension, acute pyelonephritis, and other fetal complications (5, 14, 15, 16, 17) are commonly reported.

Purpose of the paper

The aim of the paper is to analyze the role and significance of asymptomatic bacteriuria in the appearance of fetal maternal complications in pregnancy and to determine the incidence of asymptomatic bacteriuria in pregnancy by type of bacteria in our population.

Material and methods

In our study are included 110 pregnancies that are randomly selected. The study included pregnant women from 2015 to the beginning of 2017. All pregnancies were examined and monitored continually, during pregnancy and at birth. Pregnancies were examined in clinical, laboratory, and ultra-sonographic form including pregnancies from week 13 gestation to week 40. The gestation age was determined based on the last menstruation as well as the Hadlock formula. These pregnancies have become urinary cultures, and urinary tract results are grouped in the group of patients with sterile urinary tract, with significant asymptomatic bacteriuria and non-significant asymptomatic bacteriuria.

The material for microbiological analysis is prepared sterile. For microbiological analysis, the median flow of urine flow has been accepted, following the patient's previous preparation with the disinfection of the ostium of the vagina and the urethra.

Reading uroculture is done in the department of microbiology, while other hematological and biochemical analyses have been done at the "General Hospital" in the biochemical laboratory. All the pregnant women were hospitalized at the Regional Hospital in the Gynecology and Obstetrics Department, whereby their complete examination was done. All the time, follow up and record of fetal maternal complications presented during pregnancy. The pregnancies were also monitored during the act of birth, and the assessment of the mother's and neonates' health condition was performed.

Results

From the tabulation we notice that significant bacteriuria is present in the percentage of 25.45% in the examined pregnant population.

Table 1. Table of application of bacteriuria in the pregnant population

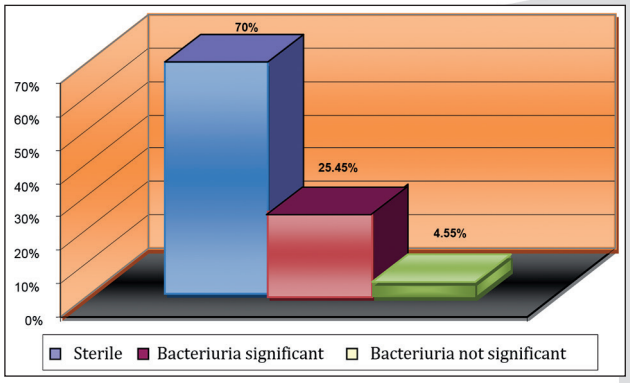
Urinoculture	Number	Percentage %
Sterile	77	70 %
Asymptomatic bacteriuria significant	28	25,45 %
Asymptomatic bacteriuria Not significant	5	4.55 %
Total:	110	100 %

Table 2. Chart presentation of bacteriouria by type of microbes

Uroculture	Type of microbes							
	Escheriae coli		Proteus mirabilis		Staphylococcus aureus		Klebsiella	
	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Asymptomatic bacteriuria significant	15	45.45	1	3.03	4	12.12	2	6.06
Asymptomatic bacteriuria Not significant	0	0	1	3.03	1	3.03	1	3.03
Total:	**15**	**45.45**	**2**	**6.06**	**5**	**15.15**	**3**	**9.09**

Table 3. Chart presentation of bacteriouria by type of microbes

Uroculture	Type of microbes							
	Staphylococcus epidermidis		Enterococcus		Acinetobacter calcoaceticus		Citrobacter	
	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Asymptomatic bacteriuria significant	0	0	3	9.09	2	6.06	1	3.03
Asymptomatic bacteriuria Not significant	2	6.06	0	0	0	0	0	0
Total:	**2**	**6.06**	**3**	**9.09**	**2**	**6.06**	**1**	**3.03**



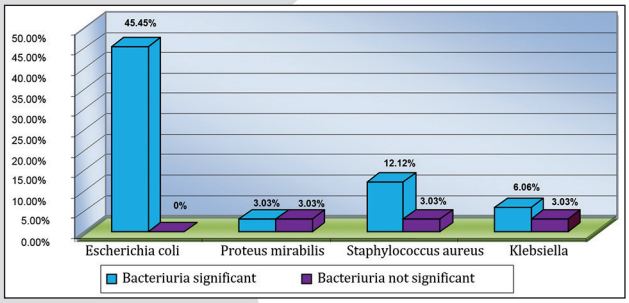
Graphic 1. Graphic presentation of bacteriuria in the pregnant population

From the graphic 1 we notice that significant bacteriuria is present in the percentage of 25.45% in the examined pregnant population.

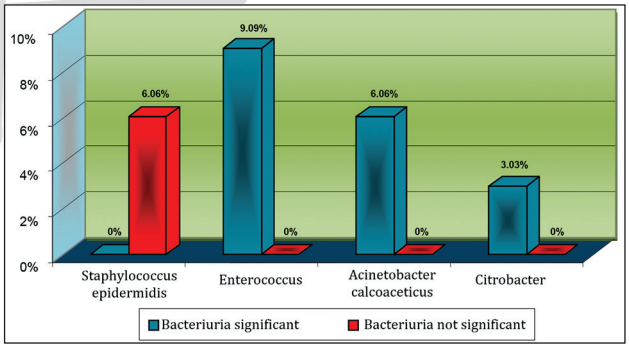
Based on the table 2 we observe that Escherichia-coli present the highest percentage (45.45%).

From the graphic 2 we notice that Eschichia coli is presented with percentage (45.45%).

From the table 3 we observe that the citobacter presents in the lowest percentage (3.03%).



Graphic 2. Chart presentation of bacteriouria by type of microbes



Graphic 3. Chart presentation of bacteriouria by type of microbes

Table 4. Chart of presentation of faetomaternal complications during pregnancy in significant asimptomatic bacteriouri

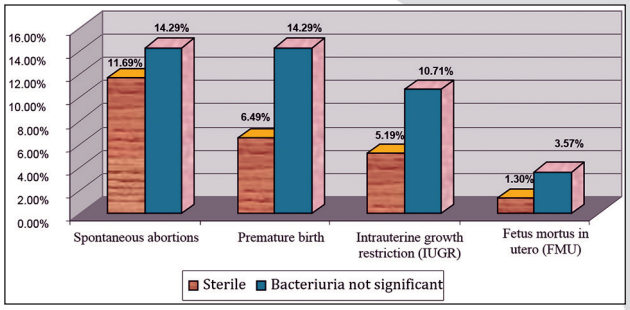
Urinoculture		Fetomaternal complications							
		Spontaneous abortions		Premature birth		Foetal growth stagnation (IUGR)		Foetal intrauterine death (FMU)	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%
Sterile	77	9	11.69	5	6.49	4	5.19	1	1.30
Asymptomatic bacteriuria significant	28	4	14.29	4	14.29	3	10.71	1	3.57
Asymptomatic bacteriuria Not significant	5	0	0	0	0	0	0	0	0
Total:	**110**	**13**	**11.82**	**9**	**8.18**	**7**	**6.36**	**2**	**1.82**

Table 5. Chart of presentation of faetomaternal complications during pregnancy in significant asimptomatic bacteriouri

Uroculture		Fetal-maternal Complications									
		Pyelonephritis		Cystitis		Glomerulonephritis		Preeclampsia		Anemia	
		Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Sterile	77	0	0	0	0	0	0	6	7.79	15	19.48
Asymptomatic bacteriuria significant	28	6	21.43	5	17.86	1	3.57	3	10.71	8	28.57
Asymptomatic bacteriuria Not significant	5	0	0	0	0	0	0	0	0	1	20.00
Total:	**110**	**6**	**5.45**	**5**	**4.55**	**1**	**0.91**	**9**	**8.18**	**24**	**21.82**

From the table 4 we observe that the citobacter presents in the lowest percentage (3.03%).

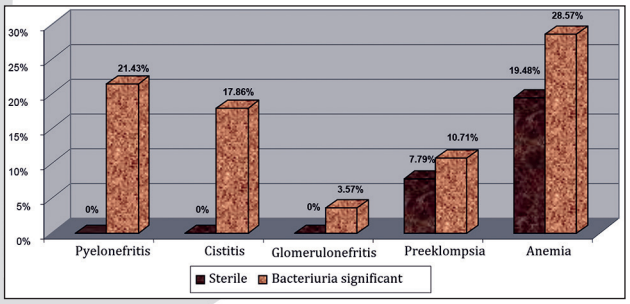
From the chart we notice that spontaneous abortions appear more frequently in asymptomatic significant bacteriuria. Premature birth, fetal growth stagnation and intrauterine death of the fetus appear asymptomatic bacteriuria significant.



Graphic 4. Graphic presentation of foetomaternal complications during pregnancy in significant asimptomatic bacteriouri

From the graph 5 we notice that spontaneous abortions appear more frequently in asymptomatic significant bacteriuria. Early birth, fetal growth stagnation and fetal intrauterine death occur as often as significant asymptomatic bacteriuria.

From the chart we observe that pyelonephritis, cystitis and glomerulonephritis appear only in cases with significant asymptomatic bacteriuria. Pregnancy anaemia is the most common complication.



Graphic 5. Chart of presentation of faetomaternal complications during pregnancy in significant asimptomatic bacteriouri

From the graphic we observe that pyelonephritis, cystitis and glomerulonephritis appear only in cases with significant asymptomatic bacteriuria. Pregnancy anaemia is the most common complication.

Discussion

In the study, we analyzed the frequency of asymptomatic bacterium disease in pregnancy and estimated that the frequency of asymptomatic bacteriuria in the investigated pregnancies was higher (25.45%) compared to the authors' data (5.10) who in their study have presented this frequency of asymptomatic bacteria (5 to 10%). This higher percentage of asymptomatic bacterium in pregnancy has been explained by the fact that in pregnancy the hormonal status changes which plays an important role in changing metabolic and immunological processes in pregnancy. Progesterone is known to progressively increase progesterone and estrogen concentrations. Progesterone with its own effect on smooth muscle makes peristalsis inhibitory, and makes urethra dilation, allowing the urinary tract and transmitting infections upwardly. The same effect also causes the urinary bladder by reducing tonus and making it difficult to empty the urinary bladder and thus contributes even more to the increase in urinary tract infections.

It has been verified that estrogen showed a sufficient effect on the immune system. It has been argued in experimental form that the administration of estrogen reduces the tuberculin-like reaction. It has also been verified that estrogen delivery inhibits the laying of corneal homograft. Therefore, the growth of estrogen in pregnancy through the immune system can also affect the incidence of infections in pregnancy.

It should be noted also the role of corticosteroids which also increase in pregnancy. It is known that corticosteroids in large doses reduce the effect of phagocytosis and the production of antibodies. Based on these facts we can conclude that the increase in asymptomatic bacterial frequency in pregnancy is higher. In addition to the hormonal factor important role should also be on the nutritional factor. It is clear that poor nutrition is a cause of secondary immunodeficiency. Protein-related deficiency causes cellular immunity suppression by inhibiting the phagocytosis process and inhibition of complement activation. In addition to protein, it has been proven that even lipids are used in small amounts during pregnancy and increase sensitivity to infection. Sensitivity to infection increases due to the suppression of humoral immunity. Lack of lipids causes destabilization of the lymphocyte lysozyme membrane. Lipid deficiency also hampers the preparation of antigens that must be phagocytized by the macrophage.

Some other substances which are: folic acid, pantothenic acid, and pyridoxine are very essential substances for cell metabolism. If these substances are used in small quantities will cause metabolic disorders, and at the same time will bring about immune and humoral immunity. Based on these data we can conclude that the nutritional factor is very important for the normal development of pregnancy and the prevention of infections. It is known that poor nutrition is also manifested in other complications of pregnancy and fetus.

In our study we have observed that Escherichia coli presents with higher frequency (45%) of cases with asymptomatic bacteriuria. The authors (10, 13, 15, 16) have found the percentage of (70 to 90%) of Escherichia coli. The lowest percentage of Escherichia coli in our study is explained by the best maintenance of hygiene in the perineum region as well as maintaining hygiene in the vaginal and urethral ostium region. It is known that the source of Escherichia coli infection is fetal and urinary.

Another important factor for explaining the lower frequency of Escherichia coli in our work is that most patients requiring treatment of iron-containing preparations are reported late and are not treated adequately. It is known that long treatment of iron-containing preparations increases the frequency of urinary infections and especially the infections caused by Escherichia coli. Iron is an important element for the metabolism of this microbe. Since our pregnancies are not subject to this regime with iron preparations, there is a possibility to avoid iron exploitation by this microbe.

The highest presence of this microbe compared to other microbes is explained by the fact that the Escherichia coli with its fimbria on the surface contain an affinity for the receptors of carbon hydrate in cell membranes of the urinary tract epithelium. Urine flow can be very difficult to carry and avoid these microbes by urine.

According to some authors of asymptomatic bacteriuria, the vaginal immunoglobulin responsible for the "O" antigen of Escherichia coli is absent, and therefore there are no leukocyte protective reactions against this microbe. This fact can

also serve as an important element for a higher frequency of Escherichia coli in asymptomatic bacteriuria. The bacteria that is presented with lower frequency in our material has been the Citrate bacterium with percentage (3.03%). The lowest citrate bacteria frequency is explained by the fact that personal hygiene is adequately maintained.

The presence of Acinetobacter calcoaceticus microbes in our work has been in the percentage of (6.06%). Such a percentage is not met in the literature of other authors. This high percentage of this microbe in our material explains that in our pregnancies we have to do with a diminished immunity (due to the nutritional factor) that is characteristic for infections caused by this bacterium.

Regarding fetal maternal complications, in our study, we have verified that premature births occur with higher frequency of asymptomatic bacteriuria compared to pregnant women with sterile urine culture (without significant presence of bacteria in the urine). The authors (5) have verified that premature births are more common in asymptomatic significant bacteriuria. This is explained by the fact that pregnancies with asymptomatic bacterium are significant, complications are caused by the release of certain so-called "pyrogene" substances which can initiate uterine contractions and premature birth. Spontaneous abortions in our material are also presented with higher frequency of significant symptomatic bacteriuria (13, 14, 16, 17). The mechanism of spontaneous abortion can be explained through the action of these pyrogenic substances (interleukins) which initiate uterine contractions, directly acting on myofibrils. It is possible that these substances (interleukins) act directly on the hypothalamus and release the oxytocin which then initiates contractions in the uterus. Another mechanism for explaining these phenomena is based on the action of endotoxins of gram negative bacteria in initiating uterine contractions. Endotoxins activate the phospholipase that cleaves the cell membrane phospholipids and enables the formation of prostaglandins. Prostaglandins act on myofibrils and initiate co-incubations (14).

The intrauterine death of the fetus is presented with higher frequency of asymptomatic bacteriuria, compared to the group of pregnant women who had sterile urinary culture. Authors (5) have found the same frequency. This phenomenon can be explained by the action of endotoxins in the fetus. In severe cases of acute pyelonephritis, endotoxins released from the mother are transmitted to the fetus and cause widespread fetal death. The other mechanism is based on the fact that in severe cases with bacteria, the possibility of transfusion of bacteria is not excluded, a severe fetal seizure and fetal sepsis that causes widespread fetal deaths (13, 14, 15, 16, 17).

Growth Failure (IUGR) manifests more in cases of significant bacteriuria compared to pregnant women who have had sterile urinoculture (17). It is known that severe cases of pyelonephritis acute and hypertension associated with significant bacteriuria also present stagnation in fetal growth. This phenomenon is explained by the fact that prolonged hypertension can cause damage to endothelial blood vessels in the uteroplacental circulation, and may lead to fetal hypoxia.

The other mechanism is that increased stasis may be caused by the action of small-dose endotoxins for a longer period of time, which can cause degenerative changes in the fetus's body (14, 17).

Preeclampsia is presented at higher frequency of asymptomatic bacteriuria. It is known that severe cases of asymptomatic bacteriuria pass into acute pyelonephritis associated with preeclampsia.

The author (5, 8) has verified that severe cases of Pylonefrit may also be associated with respiratory distress syndrome. Regarding the appearance of pyelonephritis, glomerulonephritis and cystitis, these are presented only in cases where we had significant asymptomatic bacteriuria. Pyelonephritis is presented with higher frequency (21.45%), while glomerulonephritis with much lower frequency (3.57%). The appearance of pyelonephritis in the general population of pregnant women with a frequency of (5.45%), was something higher than the percentage of pathologist meeting in the literature with frequency of (1.5 to 3%). The highest percentage of pylonefrit in our population is explained by the fact that the diagnosis of asymptomatic bacteriuria is delayed (15, 16).

In our glomerulonephritis material is presented with frequency (3.57%). The author (7, 4, 13) has verified that pregnancy worsens the condition of chronic glomerulonephritis, which may be associated with hypertension. The author (5) has verified that kidney function is weakened in pregnant women who have had prolonged hypertension.

In our study, anemia was the most frequent complication with a percentage of (21.82%) of the total number of pregnant women.

The frequency of anemia was higher in pregnant women with significant bacteriuria, compared to pregnancies in which urinary culture has been sterile. This is explained by the fact that severe and unhealthy cases of significant bacteriuria can be associated with severe forms of pyelonephritis associated with anemia.

Conclusion

Fetal-maternal complications; premature birth, spontaneous abortion, death of uterine fruit, IUGR, preeclampsia and anemia have been reported with higher incidence of asymptomatic bacteria significant compared to sterile urinary patients. Pyelonephritis, Glomerulonephritis and Cystitis are presented only in cases with significant asymptomatic bacteriuria.

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The effect of the size of strabismus angle, visual acuity, age and gender on the functionality of binocular vision

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Abstract

The purpose of this research is to examine the effect of the size of strabismus angle, visual acuity, age and gender on the functionality of binocular vision. The research covers 58 examinees with esotropia. Data used herein were collected on the basis of history data of examinees treated at Department for orthoptic and pleoptic of the Clinic for eye diseases, University Clinical Center Tuzla. Their age is from 3 to 14. The examinees were divided into two age groups: 3-6 and 7-14. SPSS 17.0 for Windows program package was used for data processing applying analysis of a variant (ANOVA), i.e. p-value and regression analysis. Upon completion of data processing, taking into consideration results obtained, it may be concluded the functionality of binocular vision is influenced by the size of the strabismus angle, the age of the subjects, while gender and visual acuity have not shown statistical significance.

Key words: strabismus angle size, visual acuity, age, gender, binocular eyesight

Introduction

The sight is the most important sense in humans and upper vertebrae for perceiving the environment, space and behavior in it (Čelić i Dorn 2004). Good visual acuity is crucial for the proper physical and mental development of children. Receiving equal information visually on both eyes is essential for the development of a visual center in the brain (Lee et al., 2001). Poor vision affects the ability to perform daily activities (Gallaway et al, 2002). In order to better understand the causes of visual disturbance, it is necessary to know about the normal vision development. Newer knowledge of embryology, histology and anatomy of the eye, and new types of search and possibilities of examining vision and its acuity since the first days of life are essential in the attempt to detect an oculomotor impairment, an impairment of the object fixation and changes on the eye bottom, which is easier to detect if the visual function of the child is normal (Čupak, Čupak-Zergollern, 1997). For the purpose of any visual impairment it should be acted preventively in the prenatal, natal, childhood and the adult age or in pre-school and school institutions and in the working environment (Čupak, 1990). Binocular single vision means the coordinated use of both eyes which produces a unique visual observation in the brain. Binocular vision means viewing with two eyes which, in the normal case, results in a single, unified visual observation. Binocular vision is enabled by the proper functioning of the ocular muscles and is conditioned by the sensory correspondence of both retinas (Stanković, 1995). Strabismus occurs when it is impossible to point both lines of vision, at the same time, toward the point being fixed. Strabismus is an anomaly of the eyes position and the disturbance of the binocular visual function, that is, the motor and sensory anomaly. As a result of these complications, amblyopia develops at a squint eye. Visual functioning and visual capacity not only relate to the precision of observing the form, details and colors of static objects or objects on the move, but also the success of processing and interpreting received visual information (Gligorović i Vučinić, 2010). Compulsory screening assessment of visual functions of children under four years of age, at pre-school, younger school and younger adolescent ages (12 to 13 years of age), is a prerequisite for effective therapeutic approach to visual distur-

bances and disorders (Ohlsson, 2003). Amblyopia is a common occurrence, which can affect up to 5% of the total population. In the UK, there are currently screening programs for identifying children who have or who are at risk of developing amblyopia (Carlton and Kaltenthaler, 2011). Amblyopia is a sensory disorder that arose due to the inability to develop visual acuity on one or both eyes (Litričin et al., 2004). The best period for correcting amblyopia is during early childhood. The treatment aims to achieve the best possible visual acuity in the amblyopic eye (Litričin et al., 2004). Working with visually impaired people is considered very complex. It demands extreme patience and the most frequent engagement of a team of experts from different fields (Li, Levi, 2007). Early detection and monitoring of eye disease is essential to prevent further loss of vision and other visual problems that might follow (Stamenković i Stankov, 2010). Treatment of vision disorders, such as strabismus and diplopia, are key factors for binocular vision (Vukićević et al., 2013). Prevalence of strabismus shows great variations in different ethnic groups and populations. The highest frequency is in the European and English population and ranges from 3-5% of children of school age (Zhang, 2007). It is important to do a precise clinical examination in order to make a decision on proper treatment (Miller, 2005). Visual rehabilitation includes a series of activities aimed at strengthening visual capacity and improving the quality of life and independence of people with visual impairment resulting from illness or injury (Scheiman et al, 2005).

Aim of research

The effect of the size of strabismus angle, visual acuity, age and gender on the functionality of binocular vision

Method of operation

Sample of examinees

The research includes 58 examinees, 30 females and 28 males. They are 3 to 14 years old.

Examinees were divided into two age groups: 3-6 year olds and 7-14 year olds.

Amblyopia (poor eyesight), as accompanying phenomenon, was present at 36 examinees, while 22 of them were without amblyopia.

Sample of variables

The research includes examination of multiple perception of characteristics. The examined independent variables are: age, eyesight acuity on the right and left eye, strabismus angle size, and amblyopia, while dependent variable is a binocular vision.

Mode of research conduction and instruments of measurement

Data used for this research were gathered on the basis of history of patients processed and treated at Department for orthoptic and pleoptic, Clinic for eye diseases, UCC Tuzla.

Criteria for data processing were examinees with esotropia. The following methods were used within the research: analysis of medical documentation, examination of eyesight acuity, refraction, examination of eye motility and occulomotor balance of eye muscle, cover-uncover test, measurement of objective deviation, i.e. of objective angle of strabismus (on synaptophore with obliged altering occlusion before measuring the angle), examination of binocular vision (on synaptophore).

Data processing methods

SS 17,0 for Windows program package SP was used for data processing.

For observed variables at each examination, for every group of examinees established according to their age, measures of central tendency, measures of dispersion and quartiles were calculated. To test presence difference of middle values among the groups established according to the age of patients (independent samples), and according to other criteria, analysis of variance (ANOVA), i.e. p-value, was applied. To see whether there is a statistically significant connection (interdependence) among some variables, was used regression analysis.

Results and discussion

Visual impairment has a significant impact on the socioeconomic development of individuals and society. Its consequences are an important health problem in developed countries, where

Table 1. Respondents by age and sex

Age groups	Sex					
	Male		Female		In total	
	f	%	f	%	f	%
3-6 year	18	46,20	21	53,80	39	100,00
7-14 year	10	52,60	9	47,40	19	100,00
In total	28	48,30	30	51,70	58	100,00

Table 2. Parameters of distribution of respondents by age

Distribution parameters	Age groups		
	3-6 year	7-14 year	Age
Sample size	39	19	58,00
Arithmetic middle	4,51	10,42	6,45
Median	5,00	10,00	5,50
Modus	3,00	8,00	3,00
Standard deviation	1,14	2,41	3,25
Variation range	3,00	7,00	11,00
Minimum	3,00	7,00	3,00
Maximum	6,00	14,00	14,00
Q1 (first quartile)	3,00	8,00	4,00
Me (median)	5,00	10,00	5,50
Q3 (third quarter)	6,00	13,00	8,25

damage occurs in 80% of cases (Congdon et al., 2003). So the differences in the visual acuity of the right and left eye are one of the factors limiting binocular co-operation in visually impaired (Mitchell, 2008). According to the results in Table 1, in the total number of subjects, 28 are male and 30 female. By grouping the subjects by age, 39 subjects were between the ages of 3 and 6, while 19 of them were between the ages of 7 and 14.

Observing the age of the above mentioned groups of subjects, the results obtained during the research are presented in Table 2. The average age in the group of subjects from 3-6 years old is 4.51 years with an average deviation from the group average of 1.14 years. The average age in the group of subjects from 7-14 years old is 10.42 years with an average deviation from the group average of 2.41 years. The average age for all subjects is 6.45 years with an average deviation from the average of all subjects is 3.25 years. The medial age in the first group of subjects is 5 years, while the most common age in the same group is 3 years. The youngest subject in the first group was 3 years old, the oldest 6 years in the same group, with the age in the variation range of 3 years. The medial age in the second group of subjects is 10 years, while the most common age in the same group is 8 years. The youngest subject in this group was 7 years old, the oldest 14 years old in the same group, with the age range variation of 7 years.

Below, the study included examining the visual acuity of the left and right eye in the two age groups of subjects. Multiple observation of features (visible acuity and strabismus angle) were examined. Table 3 shows the parameters of the observed features. On the basis of the obtained data, the average VOD value in the group of subjects of 3-6 years is 0.61 with an average deviation of 0.33, while the average VOD value in the group of subjects of 7-14 years old is 0.81 with an average deviation from the average of 0.20. The average VOD value for all subjects is 0.68 with an average deviation from the average of 0.30. Furthermore, the average VOS value in the group of subjects of 3-6 years is 0.59 with an average deviation of 0.33, and the average VOS value in the group of subjects of 7-14 years old is 0.82 with an average deviation of 0.24 from the average. The average VOS value for all subjects is 0.66 with an average deviation of 0.32 from the average. Finally,

Table 3. Parameters for viewed features during review

Distribution parameters	3-6 year			7-14 year			In total		
	VOD	VOS	Angle strabismus	VOD	VOS	Angle strabismus	VOD	VOS	Angle strabismus
Sample size	39	39	39	19	19	19	58	58	58
Arithmetic middle	0,61	0,59	26,28	0,81	0,82	25,21	0,68	0,66	25,93
Median	0,70	0,60	27,00	0,90	0,90	23,00	0,80	0,75	25,00
Modus	0,90	0,90	30,00	0,90	0,90	20,00	0,90	0,90	30,00
Standard deviation	0,33	0,33	5,59	0,20	0,24	7,44	0,30	0,32	6,21
Variation range	0,96	0,96	23,00	0,60	0,96	26,00	0,96	0,96	26,00
Minimum	0,04	0,04	17,00	0,40	0,04	17,00	0,04	0,04	17,00
Maximum	1,00	1,00	40,00	1,00	1,00	43,00	1,00	1,00	43,00
Q1 (first quartile)	0,40	0,40	20,00	0,70	0,70	20,00	0,40	0,40	20,00
Me (median)	0,70	0,60	27,00	0,90	0,90	23,00	0,80	0,75	25,00
Q3 (third quarter)	0,90	0,90	30,00	0,90	1,00	25,00	0,90	0,90	30,00

the average value of the strabismus angle in the group of subjects from 3-6 years is 26.28 with an average deviation of 5,59, and the average value of the strabismus angle in the group of subjects from 7-14 years is 25,21 with an average deviation of 7, 44 from the average. The average value of the strabismus angle for all subjects is 25.93 with an average deviation of 6.21 from the average.

Regression analysis was used to examine the effect of the size of the strabismus angle, visual acuity, gender, and age, determined during the examination for achieving binocular vision. The method of encoding the variables is shown in Table 4, where it is a dichotomous variable encoded in two values.

Table 4. Encoding of dependent variables

The name of the outcome	Code
Not	0
That	1

The model proved to be statistically significant because χ^2= 14.955 and p<0.0106 and explains the possibility between 22.7% -30.9% of variance for the presence or achieving binocular vision.

The overall correspondence between the observed and estimated presence of binocular vision is 72.41%. Therefore, 72.41% is the percentage of correct estimations of the model. The percentage of accurate predictions for the outcome NO is 86.11%, and for the outcome YES 50.0%. It is not accordant in 5 cases of NO, which according to the predictors are classified in cases of YES. Also, 11 cases of YES that are classified in NO are not accordant. The overall discordance is 27.59%. The results of the regression analysis are given in Table 6. The model for prediction of achieving binocular vision was statistically significant at a significance level of 0.1 or 10%. Hereafter, we emphasize that given the significance of the variable-predictor in the model, the predictors showed as statistically obvious are: the size of the strabismus angle and the age of the subject, while the gender was not statistically significant. In case of an age increase in one year, possibilities of achieving binocular vision are reduced by about 20%. In case the strabismus angle is higher for one degree, the possibilities of achieving binocular vision are reduced by about 10%.

Table 5. Classification table for binocular vision

	Estimated presence of binocular vision			
Presence of binocular vision		Not	That	% correctly evaluated
	Not	31	5	86,11 %
	That	11	11	50,00 %
	Total stacking			72,41 %

Table 6. Regression analysis

Variables	B	S.E	Wald	df	Significance	Exp (B)
Sex	0,667	0,644	1,075	1	0,300	1,949
Age	-0,222	0,121	3,342	1	0,068	0,801
VOD	2,099	1,737	1,459	1	0,227	8,155
VOS	1,162	1,738	0,447	1	0,504	3,196
Angle strabismus	-0,098	0,059	2,780	1	0,095	0,907
Constants	0,748	1,956	0,146	1	0,702	2,113
χ^2 for the model						14,955
df (degree of freedom) for the model						5
significance for the model (p)						0,0106
Cox & Snell R^2						0,227
Nagelkerke R^2						0,309

The study conducted by Simonsz (2006) to investigate early surgeries of congenital strabismus in relation to late surgeries of congenital strabismus. Optimal age for surgery of congenital esotropia is different at many authors. Those for early surgery believe that further loss of binocular may be prevented by early surgery, while some of them believe that binocular vision can be maintained by early surgery. This study made a comparison between early and late surgeries. Children in early age (6-34 months), 231 children, and those aged 32-60 months, 301 of them, underwent a surgery. All of them were determined a strabismus angle, refraction anomaly, a degree of amblyopia and limited abduction. The children were re-examined at the age of 6, when a degree of binocular vision, strabismus angle and remaining amblyopia were determined. Refraction errors, amblyopia and limited abduction were equal in both groups of examinees, but a strabismus angle was a bit higher at younger examinees. At the age of 6, 13,5% of children who underwent a surgery at early group in respect to 3,9% of children in late group had a stereovision examined by Titmus test. Considering strabismus angle, there was no difference in both groups. When it is about acuity, early group had small, but significant advantage. In both groups there were children who had to be subjected to re-surgery at the age of 6. The authors think that children who underwent a surgery at early age had better acuity and stereovision at the age of 6 in comparison with children who underwent a surgery at later age. Similar results were reached by Shaouly and co. (1994) whose aim was to examine clinical characteristics and long-term post-surgical results at infantile esotropia. They classified 103 patients (206 eyes) with infantile esotropia and followed them for 8 years after the surgery from what four groups derived. 28 out of 103 patients (27%) has subnormal binocular vision, 24 (23%) had microtropia, 43 (42%) has a small deviation angle and 8 (8%) had a great deviation angle. Acuity of 20/30 or better was maintained at 174 out of 206 eyes. In the group of patients with subnormal binocular vision (28), the eyes kept horizontal position. However, 6 of 30 patients in the group with microtropia and 11 of 43 patients in the group with small deviation angle lost stability of horizontal eye position. They found out that two variables, latent and manifest nystagmus and persistent amblyopia at the time of surgery, had bad influence on post-surgical results. However, at patients with smaller pre-surgical deviation angle and at patients who underwent a surgery prior to the first year of life, it showed that it is possible to reach optimal results (subnormal binocular vision) or reach desired results (microtropia). Thus, the authors recommend a surgery after the treatment of amblyopia and whenever possible during the first year of life. Similar research was conducted by Krković and co (2003), whose aim was to establish how big is the influence of surgical correction of strabismus angle at children with esotropia on development of binocular vision, in relation to conservative treatment. The examinees were grouped in two groups, the first one with children treated by a surgery and then by orthoptic-pleoptic treatment, while the other group is composed of

children treated by conservative orthoptic method. Each group of children had 50 examinees – thus 100 examinees in total. Establishment of binocular vision was followed in the period of one year. Examinees were from 4 to 8 years old. After a year of treatment, it could be seen that the first group examinees reached far better results than those from the second group. In the first group, 1,8% of examinees reached simultaneous perception, 72% of them gained fusion along with simultaneous perception, and 20% of examinees reached all three levels, including stereovision. In the second group, 26% of examinees developed simultaneous perception, 28% of them developed fusion along with simultaneous perception, and all three levels were reached by 8% of examinees. It is important to say that 38% of examinees from the second group did not show improvement in treatment and after a year remained without binocular vision. It may be concluded that even at small values of strabismus angle it is recommended to make a surgery and establish orthophoria prior to conservative treatment because in that way qualitative binocular vision can be reached.

Conclusion

On the basis of obtained results of the research, the following conclusion may be reached: The functionality of binocular vision is influenced by the size of the strabismus angle, the age of the subjects, while gender and visual acuity have not shown statistical significance. It is important to point out that a lot can be done to further preserve and develop visual functions and sensory and motor cooperation in children from the earliest age using permanent diagnostics, medical therapy and tiflological rehabilitation. More thorough organization of work and education in primary and secondary health care in health institutions is needed, as well as the introduction of projects related to early detection of strabismus and amblyopia, starting with pediatric departments in the health centers, then preschool institutions to primary and secondary schools.

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GelcoPEP bioactive peptides play an important role on joint diseases: a 28-day observational study

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Abstract

The present prospective and single-center clinical study investigated the safety and efficacy of GelcoPEP bioactive peptides. The range of motion, general pain, and muscle strength were evaluated. The results indicated that administration of 10 g/day of hydrolyzed collagen for 28 days improved essential symptoms in individuals suffering from joint diseases, including range of motion, general pain, and muscle strength. These data support the view that hydrolyzed collagen may be administered to patients suffering from joint diseases. No adverse effects were observed during the trial. These data support its use for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis, and rheumatoid arthritis.

Key words: hydrolyzed collagen, joint diseases, range of motion, general pain, muscle strength.

Introduction

GelcoPEP, a bovine hydrolyzed collagen, is a dietary supplement that may be beneficial for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis, and rheumatoid arthritis. Its use in the treatment of degenerative joint diseases has increasingly gained support in medical community and among consumers[1].

Collagen is a major protein in the human connective tissue, while its concentration varies in different body parts: cartilages, cornea, arteries, and skin. Robust and inextensible, possessing a great tensile strength, it is the principal component of cartilages, tendons and articular joints, bones and teeth[1,2].

Also, collagen is the building material for the blood vessel walls—capillaries, veins, arteries. It imparts to them strength and energy, structure and flexibility for the effective transport of blood to each single cell. Collagen is of vital importance for muscle functioning. The collagen molecules provide the muscle fibers with power and structure, which are necessary for their functioning all day long. Collagen not only builds up the muscle fibers, but also the smooth muscles—the cardiac muscle, the bladder muscles and the genitalia muscles[2].

In preclinical studies, it has been demonstrated that orally administered hydrolyzed collagen is thoroughly absorbed by the intestine and circulated in the blood stream, remaining in the gastrointestinal tract. These studies also revealed that that a significant amount of hydrolyzed collagen derived peptides reaches joint tissue. In addition, it was exposed that treatment of cultured chondrocytes induced a statistically significant dose-dependent increase in collagen synthesis of the chondrocytes in cell culture experiments[3,4].

Based on the findings that hydrolyzed collagen is absorbed in its high molecular form, accumulating in cartilage, and is able to stimulate chondrocyte metabolism, it might be reasonable to use hydrolyzed collagen as a nutritional supplement to activate collagen biosynthesis in chondrocytes in humans, especially patients suffering from degenerative joint diseases. Thus, the aim of this prospective and single-center investigation is to extend these earlier findings with GelcoPEP.

Materials and methods

Study design

Subjects' complaints of joint discomfort were recorded using pre- and post-treatment questionnaires to evidence personal data and issues related to an individual's functional quality. A goniometer

was used to measure the range of motion[5], a pain scale (Borg) was applied to subjectively percept the pain[6], and a properly calibrated sphygmomanometer was utilized to evaluate muscle strength[7].

Urine was collected for a pregnancy test for women of childbearing potential. A blood sample was taken for determination of alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, blood urea nitrogen (BUN) and creatinine. Upon review of blood test results, eligible subjects were instructed to get an X-ray of the affected joints to confirm diagnosis.

A total of 64 subjects were recruited using the inclusion and exclusion criteria outlined in Table 1. At the first visit, selected subjects, properly informed by the Consent Term approved by the Scientific Committee of the Institute, were assigned to receive 10 g GelcoPEP (Gelco International, Pedreira, Brazil) daily. At the final visit, subjects were required to come to the clinical division for clinical assessment. A subject treatment diary was completed by each patient throughout the study period to determine product compliance, side effects, and supplementation use.

Statistical method

For comparing non-parametric values, the Wilcoxon's test was used, and for comparing parametric values, the variance analysis (ANOVA) test were used. A significance level of 5% was adopted in all comparisons and statistically significant results were marked with an asterisk (*).

Results

Baseline characteristics of patients are summarized in Table 2. Where applicable, values are expressed as mean ± standard deviation.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Males and females 45-60 years old
Females of childbearing potential must agree to use a medically approved form of birth control and have a negative urine pregnant test result
Disorder of the knee for more than three months
Able to walk
Availability for duration of study
Subject agrees not to start any new therapies during the course of the study
Able to give informed consent
Exclusion criteria
History of asthma, history of diabetes
Hyperuricemia
Hypersensitivity to NSAIDs
Abnormal liver or kidney function tests
Abnormal findings on complete blood count
Uncontrolled hypertension
History of allergic reaction to any ingredients in the test product
Hyperkalemia (potassium > 6.2 mmol/L)
History of cancer as well as gastrointestinal, renal, hepatic, cardiovascular, hematological, or neurological disorders
Anticipated problems with product consumption
High alcohol intake (>2 standard drinks per day)
History of psychiatric disorder that may impair the ability of subjects to provide written informed consent
Use of concomitant prohibited medication (narcotics, NSAIDs)
Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

Table 2. Baseline characteristics of patients.

Characteristics of patients	Values
Age (years)	59.7 ± 8.72
Sex (male/female)	28/36
Height (cm)	168.3 ± 9.74
Weight (kg)	72.9 ± 16.3
Systolic blood pressure (mm)	121.8 ± 8.92
Diastolic blood pressure (mm)	80.6 ± 7.71
Heart rate (bpm)	69.4 ± 7.56

The results are presented in Table 3 and Table 4 listing values for average, and standard deviation for each analyzed variable. Statistically significant results are marked with an asterisk (*)

Table 3. Range of motion, pain and muscle strength

	Range of motion[5]		General pain[6]		Muscle strength[7]	
Treatment	Pre	Post	Pre	Post	Pre	Post
Average	103,37	163,73	8,69	2,54	55,39	101,03
Standard deviation	12,65	10,54	10,41	13,12	10,19	11,92
Standard error	3,93	4,17	4,82	5,14	4,58	5,13

Table 4. Pre- and post- treatment groups

Comparison	P
Range of motion	0,021*
General pain	0,007*
Muscle strength	0,005*

These results indicate that administration of 10 g/day of hydrolyzed collagen for 28 days improved essential symptoms in individuals suffering from joint diseases, including range of motion, general pain, and muscle strength. No adverse effects occurred during the 28-day trial period. The treatment was reported to be well tolerated by subjects.

Discussion

In the last few years, various nutritional supplements, including chondroitin, glucosamine, soybean unsaponifiables and diacerein have emerged as new treatment options for joint disorders[8]. The aim of this prospective and single-center investigation is to evaluate the safety and the efficacy of GelcoPEP, which is a complex structural protein that may provide strength and flexibility to connective tissues.

An observational study investigated the use of hydrolyzed collagen as a nutritional supplement to reduce symptoms of joint damage, with the hope that this change would reflect improvements in joint health. In that study, individuals were recruited who had not been diagnosed with degenerative joint disease but who complained about joint pain that both the treating physician and the subjects interpreted as being a result of stressful exercising. It was reported that 78% of individuals at the end of the study noticed substantial improvement of their joint symptoms, including range of motion, pain, and muscle strength[9].

The evaluation of muscle strength is an important technique to diagnose the etiology of the disease, and to define rehabilitation strategies. The muscle weakness, which was observed in our study during the pre-treatment assessments, is directly associated with knee joint pain and joint disability[10].

Osteoarthritis results in changes that affect not only intracapsular tissue, as well as periarticular tissues, such as ligaments, capsules, tendons and muscles. Osteoarthritis patients compared to healthy individuals of the same age had muscle weakness, reduced knee proprioception, reduced balance and position sense[11].

The presence of joint effusion, even in small amounts, is a potent inhibitory mechanism reflex muscular activity of the joints. A reduced reflex muscular activity causes hypotrophy and weakness early, with the resultant associated mechanical damages, such as decreased range of motion[12].

Muscle strength declines rapidly during the detention of a member by decreasing the size of the muscle and stress per unit of the muscle cross-sectional area. The largest absolute loss of muscle mass occurs at the beginning of hypotrophy process. The pain inhibits reflex muscular activity, causing atrophy, and muscle weakness. The painful process is prior to the muscular weakness[13].

This single-center investigation suggests that GelcoPEP bioactive peptides may be beneficial for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis, and rheumatoid arthritis.

Conclusion

The purpose of this study was to determine whether administration of 10 g of avian sternal collagen type II hydrolysate daily would reduce joint pain in patients suffering from joint diseases. The design of the clinical trial was appropriate to reveal that collagen type II hydrolysate as a nutritional supplement ingested over 28 days was safe and efficacious in reducing symptoms of joint discomfort. The results of the trial provide data supporting the view that hydrolyzed collagen may be administered to patients suffering from joint diseases. Further research will elucidate additional benefits from hydrolyzed collagen.

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Instructions for the authors

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Abstract

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

Key words: Camera ready paper, Journal.

Introduction

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

Instructions for the authors

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

Table 1. Page layout description

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

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

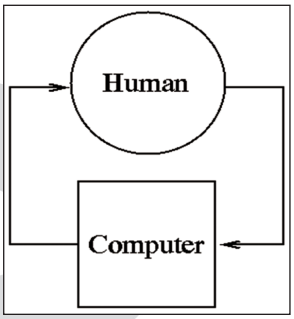

Figure 1. Text here

Conclusion

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

Acknowledgements (If any)

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

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