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Examining Of The Relationship Between Waist To Height Ratio, The Dietary Habits And Body Mass Index (BMI) Of Students In A Vocational Health High School

Rabia Kecialan[1], Rengin Kosif[2]

[1] Abant Izzet Baysal University Bolu Health School, Department of Pediatric Nursing, Bolu, Turkey,
[2] Abant Izzet Baysal University, Medical Faculty, Department of Anatomy, Bolu, Turkey.

Abctract

Introduction: The aim of the study is to assess the relationships between the WHtR, the dietary habits and the BMIs of students in adolescences. The WHtR is a criterion in obesity. Dietary habits are closely associated with gaining weight.

Methods: It was conducted with 127 students. Their anthropometric measurements, dietary habits and BMIs were recorded.

Results: Of the students, 11% were found to be slim, 12.6% to be obese and 3.1% to be slightly obese. The measurement of WC in the students showed that of them, 66.1% were below the 90th percentile, while 33.9% were above the 90th percentile. The WHtR mean of the female's was 0.43, male's was 0.45. The WHR was found to be 0.74 for female's and 0.83 for male's. Positively correlation was found between WHtR and BMI (r=0.693). Positive correlation (r=0.204) was found between the WHR and BMI, while a positive correlation (r=0.543) was found between the WHR and the WHtR. Of them, 63.8% were found to have breakfast, 86.6% lunch and 95.3% dinner. Positively correlation (r=0.226) was found between acid-containing beverages and the WHR.

Conclusions: The WHtR was found to be correlated with the WHR and BMI for the age group with whom this study was conducted. Of the students, 37% were found to consume acid-containing beverages. The WHR of these students was found to be higher.

Key words: Adolescence, WHtR, BMI, Nutrition, Relationship.

Introduction

Being overweight is caused by excessive fat accumulation in the body, and is a distinctive disease with a body structure often observed in the first impression and the health problems and risks it carries. Observations and statistics have demonstrated that the rate of being overweight is increasing in children and adolescents just like adults.[1] The most important reasons behind this increase are the limited mobility of children arising from developing technology and changes in their nutritional habits and foodstuff choices.[2] In recent years, a dramatic increase in the prevalence of obesity among children has been noted in many countries.[3]

According to the 2010 data from the World Health Organization, 43 million preschool children are overweight or obese, and a 4,2% increase can be seen when compared to the year 1990.[4] A city based study performed in Turkey has shown that in children, the rate of obesity is around 5% and the rate of being overweight is about 15%, with rapidly growing rates.[5] As a matter of fact, according to 2009 data from the World Health Organization, the prevalence of obesity in children in Turkey is 16.1%. When examined according to gender, the prevalence of obesity were found to be 15.6% in boys and 23.9% in girls.[6] Childhood obesity, as an important risk factor of type II diabetes, hypertension, and cardiovascular disease in adultlife, has increased remarkably worldwide in the past decades.[7] Obesity in children has been associated with subsequent morbidity and mortality in adulthood.[8] Studies in adults have shown that it is possible to identify not only those with normal weight having an adverse cardiometabolic risk profile but also those with overweight/obese condition having normal metabolic risk profile.[9]

Overconsumption of food is an important problem not only in adulthood but also in childhood and adolescence.[10] Fast food habits becoming widespread and physical activity lessening to

be replaced by sedentary lifestyles are among the reasons behind the increase in obesity prevalence, which is one of the most important results of an unbalanced diet.[11]

Nutritional habits are closely related to weight gain. Body Mass Index (BMI) is widely used as a measure to evaluate the impact of obesity on cardiovascular and metabolic risk factors, both in children and adults.[12] BMI is mainly used as an index for obesity, but has also been proposed as a standard reliable index of nutritional state after infancy.[13] Studies on BMI and fat mass in children and adolescents indicate that factors such as age, sex, pubertal stage and race are important in predicting adiposity.[14]

Waist to height ratio (WHtR) is a criteria that has recently been rising in use, and increases risk for cardiovascular diseases.[15] WHtR has been proposed as an easily measurable anthropometric index for detection of central obesity and to assess associations between cardiometabolic risk factor variables and central intra-abdominal obesity.[16] Even though body mass index is a diagnostic method used for overweight and obese children, it does not provide certain information on body fat ratio.[17] Waist circumferences (WC), WHtR, and neck circumferences can be measured for such reasons.[18] I many sources, it can be seen that waist/height ratios are very important in children and this ratio rising above 0.5 is related to an increase in fat ratio.[19]

Studies on WHtR and waist to hip ratios (WHR) in adolescents are increasing in number in our country.[20, 21] In this study, we aimed to evaluate the waist/height ratio and nutritional habits of health vocational school students, and determine their body mass indexes to evaluate with regard to obesity.

Materials and Methods

This study was planned as a descriptive study. The universe of the study consisted off all of the 11th grade students studying at the Izzet Baysal Golyuzu Vocational Anatolian High School. Permission from the AIBU Clinical Ethics Board was taken for the study. The study was performed with 74 female and 53 male students, for a total of 127 students. Those who were absent from school when the study was conducted, those who didn't agree to participate in the study, and those with chronic diseases such as diabetes mellitus were excluded from the study. In the questionnaire prepared for data collection, the socio demographic statuses, nutritional and meal habits, consumed food groups, consumed liquids, diet statuses, and chronic diseases of the students were questioned and their anthropometric measurements of the students were performed (Appendix 1). The performed anthropometric measurements were weight and height measurements, BMI, WC, hip circumference, WHtR and WHR. The free times allocated to the volunteering students were learned and data was collected in the classrooms through a data collection form. The anthropometric measurements were performed in an infirmary given by the school administration. Measurements were performed by a single person.

Height measurement: A simple mechanism formed of a moving head bar on a ruler fixed to a straight wall was used. Before measurement, the students took off their shoes and the posterior of the head, shoulders, gluteal area, back of the legs, and heels were given attention to touch the wall. A 90 degree angle was ensured between the sight direction of the students and the plane of the wall.[22]

Weight measurements were performed with school uniforms through a standard bascule after removing jackets and excess clothing[23] BMI was calculated as BMI=weight (kg)/height(m^2). After calculating BMI, the ≥85 percentile was defined as under risk of being overweight and the BMI ≥95 percentile was defined as overweight.[24] According to BMI and gender, students were classified as obese above the 95th percentile and overweight between the 85th and 95th percentile.[25] Table 1 shows the body mass index categories and the percentile intervals.

Table 1. BMI Categories and The Percentile Intervals

BMI Categories	Percentile Intervals
Underweight	BMI=<5.percentile
Normal	BMI= 5. percentile - < 85. Percentile
Overweight	BMI= 85.percentile - < 95.percentile
Obese	BMI = ≥95. Percentile

In the determination of the percentile intervals to which BMI values correspond, the table developed by Neyzi et al for children in our country

was used.[26] Waist and hip circumference measurements were performed with a non-flexible measure when the arms of the children were on the sides, feet were closed, and balance was equal on both feet.[27] WC was measured midway between the lowest rib and the top of the iliac crest at the end of gentle expiration.[28] For WC , values above the 90th percentile were accepted as abdominal obesity. We accepted the 90th percentile as a cut of.[31] After the measurements, waist/hip ratios were calculated. WHR>0.80 and WHtR≥0.50 were categorized as abdominal obesity.[32, 33] The Ashwell Shape Chart is also used in adults and children above 5 in waist/height evaluations. Accordingly, ≥0.4-<0.5 is classified as appropriate, values <0.4 and between ≥0.5-<0.6 are classified as warranting attention, and values ≥0.6 are classified as warranting action. In other words, risks present when the ratio is above 0.5 or below 0.4. The value being above 0.6 shows the necessity of taking action and an increased risk of chronic disease. (Table 2).[34]

Table 2. WHtR Intervals and Evaluation

WHtR	Evaluation
<4	Take Care
≥0.4 - <0.5	OK
≥0.5 - <0.6	Take Care
≥0.6	Action

Data obtained in the study was evaluated using the SPSS 17.0 statistics program. Normal distribution tests for mean values, t tests for the comparison of female and male students, and the one way Anova test for comparing quantitative continuous data in multiple independent groups were used. The Scheffe test was used as a complementary post hoc analysis method in order to determine the differences after the Anova test. In the analysis of other data, minimums, maximums, mean values, standard deviations, averages, and percentages were used. As non-parametric methods, in data analysis, the mann whitney u test was used to compare quantitative continuous data between two independent groups, and the kruskall whallis test was used to compare quantitative continuous data between multiple independent groups. The relationship between continuous variables was tested with the Spearman correlation analysis.

Results

Among the students who participated in the study, 51.2% were 16 years of age and 48.8% were 17 years of age. They were distributed as 58.3% female and 41.7% male. 79.5% of the students lived in houses and 20.5% lived in dormitories, with all of them (100%) having social security. When the education levels of the mothers were examined, it was seen that 63.7% had elementary education and below, 35.5% had middle or high school education, and 13.4% had college education. Among the fathers, 32.7% had elementary education and below, 49.6% had middle or high school education, and 23.6% had college education. While 66.1% of the students stated that they ate healthy, 33.9% stated that they thought they had an unhealthy and bad diet. The rate of those who stated that they were on a diet to lose weight was 6.3%, while the rate of those who didn't was 93.7%. While 94.5% of the students stated that they had no chronic diseases, 5.5% stated that they had asthma.

When the meal habits of the students were examined, it was seen that 63.8% had breakfast every day, 86.6% had lunch every day, and 95.3% had dinner every day. The rate of those who never had breakfast was 3.9% while the rate of those who never had lunch was 2.4%. All of the students (100%) reported having dinner regularly. 49.6% of the students never had a meal before bed. Those who regularly had brunch constituted 11%, those who regularly had midafternoon meals constituted 11% and hose ate before bed constituted 17%.

The anthropometric measurements of the students were given in the table below. Among the female students who participated in the study, the BMI average was 21,98±3.76 the WHtR average was 0.45±0.07, and the WHR average was 0.74±0.06. Among the male students who participated in the study, the BMI average was 21,81±3.22, the WHtR average was 0.45±0.04, and the WHR average was 0.83±0.05 (Table 3).

When the distribution of the students according to BMI was examined, it was seen that among female students 14.9% were thin, 64.9% were normal, 2.7% were mildly obese, and 17.6% were obese. Among male students 5.7% were thin, 84.9% were normal, 3.8% were mildly obese, and

Table 3. Anthropometric Measurements Mean by Gender

Gender	Height	Weight	BMI	WC	WHtR	HC	WHR
Female	162.64±5.96	58.35±11.18	21.98±3.76	74.97±11.92	0.45±0.07	99.16±13.05	0.74±0.06
Male	173.73±5.40	67.75±18.23	21.81±3.22	80.58±8.61	0.45±0.04	95.45±7.70	0.83±0.05

BMI: Body Mass Index, WC: Waist Circumfence, WHtR: Waist to Height Ratio, HC: Hip Circumference, WHR: Waist to Hip Ratio

5.7% were obese. No significant relationship between gender and WC was found (p=0.434).

Those with a waist value below the 90th percentile were found to be 64.9% among female students and 67.9% among male students, while those with waist values above the 90th percentile were found to be 35.1% among female students and 32.1% among male students (Table 4).

Table 4. Waist Circumference Distribution by Gender

		90 Percentile Under		90 Percentile and Above	
		n	%	n	%
Gender	Female	74	64.9	26	35.1
	Male	53	67.9	17	32.1

According to the results of the test performed in order to determine whether there was a significant difference with regard to WHtR and gender, no significant differences among the groups were found (p>0.05).

The WHtR results of the participants according to the Ashwell chart were given in Table 5. The cutoff value for waist/height was taken as 0.50. In females those in the ≥0.50 were found to be 24.3% while those in the appropriate interval were found to be 59.5%. In males, those in the ≥0.50 were found to be 17.0% while those in the appropriate interval were found to be 79.2%.

Table 5. Ashwell Chart WHtR Percentages

WHtR	Female (%)	Male (%)
Take Care <4	16.2	3.8
OK (≥0.4 - <0.5)	59.5	79.2
Take Care (≥0.5 - <0.6)	18.9	15.1
Action ≥0.6	5.4	1.9

WHtR: Waist to Height Ratio

The difference between the groups with regard to WHR averages was found to be significant (p=0<0.05). The WHR of females were found to be lower compared to those of males. The cutoff value for WHR was taken as 0.80. In females those in the ≤0.80 were found to be 85.1% while those in >0.80 interval were found to be 14.9%. In males, those in the ≤0.80 were found to be 24.5% while those in the >0.80 interval were found to be 75.5%.

Table 6. Anthropometric Measurements Correlation Relationship (n=127)

		BMI	WHtR	WHR
BMI	r	1.000		
	p	0.000		
WHtR	r	0.693**	1.000	
	p	0.000	0.000	
WHR	r	0.204*	0.543**	1.000
	p	0.021	0.000	0.000

BMI: Body Mass Index, WHtR: Waist to Height Ratio, WHR: Waist to Hip Ratio

The correlations between the anthropometric measurements were given in Table 6. Among anthropometric measurements, a positive significant relationship between WHtR and BMI was found (r=0.693; p=0.000<0.05). A very weak positive significant relationship between waist/hip ratio and BMI was also found (r=0.204; p=0.021<0.05). A medium strength positive significant relationship between waist/hip ratio and waist/height ratio was found (r=0.543; p=0.000<0.05).

When the WHR were examined according to the education status of the mother, university education was found to create a difference. The WHR averages of those whose mother had university education were lower than the averages of other groups (0.734±0.077).

When the WHR were examined according to the education status of the father, university education was found to create a difference. The WHR averages of those whose father had university education were lower than the averages of other groups (0.756±0.076).

The correlations between meals and anthropometric measurements were given in Table 7. A very weak negative significant relationship

between lunch and BMI was found (r=-0.207; p=0.019<0.05). The relationships between the other variables were not statistically significant (p>0.05).

Table 7. The Correlations between Meals and Anthropometric Measurements

Meals		BMI	WHtR	WHR
Breakfast	r	-0.026	0.009	0.172
	p	0.772	0.922	0.053
Lunch	r	-0.207*	-0.091	0.084
	p	0.019	0.310	0.348
Dinner	r	0.096	0.157	0.154
	p	0.285	0.078	0.085
Mid-morning meal (11 hours)	r	-0.022	0.048	-0.133
	p	0.806	0.590	0.135
Snack (4 hours)	r	-0.003	0.116	-0.028
	p	0.976	0.194	0.756
Night lying	r	-0.110	-0.112	-0.085
	p	0.217	0.208	0.339

BMI: Body Mass Index, WHtR: Waist to Height Ratio, WHR: Waist to Hip Ratio

In our study, a very weak negative significant relationship between yogurt and WHR was found (r=-0.225; p=0.011<0.05). A very weak negative significant relationship between dry legumes and BMI was found(r=-0.214; p=0.016<0.05). A weak negative significant relationship between bread and BMI was found(r=-0.254; p=0.004<0.05). A weak negative significant relationship between chocolate and WHR was found(r=-0.327; p=0.000<0.05). A very weak positive significant relationship between fizzy drinks and WHR was found (r=0.226; p=0.010<0.05). A very weak positive significant relationship between water and WHR was found(r=0.247; p=0.005<0.05). A very weak negative significant relationship between water and dry legumes was found(r=-0.187; p=0.035<0.05). The relationships between the other food groups were not statistically significant (p>0.05).

In our study, a strong positive correlation between the WHtR and BMI of the females was found(r=0.706; p<0.0001). A very weak positive significant relationship between WHtR and WHR was found (r=0.048; p<0.00001). A medium strength positive significant relationship between WHtR and BMI was found in male students (r=0.676; p<0.0001), and a medium strength positive significant relationship between WHR and BMI was found (r=0.426; p=0.001), while a strong positive significant relationship between WHtR and WHR was found (r=0.745; p<0.0001).

Discussion

Indices predictive of adolescent central obesity include WC, WHR and WHtR. WC is a highly sensitive and specific measure of upper body fat in young people and thus it is valuable for identifying overweight and obese adolescents at risk of developing metabolic complications. The same applies for risk factors of cardiovascular disease in children and adolescents, in whom WC and WHtR are better predictors than BMI.[35]

A regular and balanced diet is the basis of health.[36] Especially during adolescence, diet carries a lot of importance with regard to a healthy life, school success, and the prevention of possible future chronic diseases such as obesity, cardiovascular diseases, diabetes, and cancer. [37]

In our study, the majority of the students were found to regularly have the main meals of a day. When the meal habits of the students were examined, it was seen that 63.8% had breakfast every day, 86.6% had lunch every day, and 95.3% had dinner every day. The rate of those who never had breakfast was 3.9% while the rate of those who never had lunch was 2.4%. All of the students (100%) reported having dinner regularly. The consumption of dinner was more regular compared to other meals (95.3%). This may be explained by the positive effect off being alongside family in the evenings. In a study made in the city of Kocaeli with adolescents, it was seen that 83.7% had breakfast every day, 87.4% had lunch every day, and 95.9% had dinner every day.[38]

In our study, the BMI averages were found to be 21.98±3.76 in female students and 21.81±3.22 in male students. Among Greek adolescents between the ages of 12 and 17, BMI averages were found to be 21.4±3.4 in female students and 21.70±3.70 in male students.[35] A similar value of 22.23±0.25 BMI average was found in 16 year old girls in Brazil.[39] In a study performed by Bulduk et al with adolescents between the ages of 10-15, BMI averages were found to be 22.3±3.8 in females and 21.8±2.3 in males.[40] In a study performed by Adam et al with high school students between the ages of 16-19, the BMI averages were found to be 20.6±2.1in females and 24.5±4.1 in males.[41]

According to the BMI percentile values of the students that are specific to their age and gender, the rates of obesity among students at age 16-17 were found to be 17.6% in female students and 5.7% in male students Oner et al found the rates of obesity among students at age 12-17 were to be 2.1% in female students and 1.6% in male students.[42] In their study, Bundak et al reported the rate of mild obesity to be 25% and the rate of obesity to be 4% in 18 year old male students while those rates were 15% and 1% for 14 year old female students.[43] In our country, various studies performed on school children show the rate of obesity to rise above 10% in children and adolescents.[44]

BMI showing total fat but not giving information on the distribution of fat has lead researchers to alternative measurement methods. Especially fat accumulation in the abdominal area is important with regard to cardiovascular diseases. The methods that are suggested after the risk carried by central fat accumulation and increase in visceral adiposity are anthropometric measurements such as skin curvature thickness, mid arm circumference, WC, hip circumference, WHR, WHtR, and neck circumference.[29] WC is simple, yet effective alternative ways of measuring abdominal obesity in adults and children, and may be better predictors of disease risk than BMI in adults and children. In particular, WC is a better indicator of visceral fat than BMI in children n the current literature, children or adolescents with a WC >90th percentile were considered to have abdominal obesity.[45] In our study, the cut off point for WC was taken as the 90th percentile. Those with a waist value below the 90th percentile were found to be 64.9% among female students and 67.9% among male students, while those with waist values above the 90th percentile were found to be 35.1% among female students and 32.1% among male students.

In our study, the WC averages were found to be 74.97±11.92 in female students and 80.58±8.61 in male students. Among Greek adolescents between the ages of 12 and 17, WC averages were found to be 68.8±7.2 in female students and 75.45±8.4 in male students.[35] A similar value of WC average was found 73.97±0.67 in 16 year old girls in Brazil.[39] No significant relationship between gender and WC was found in our study. (p=0.434). In our study, those with a waist value below the 90th percentile were found to be 64.9% among female students and 67.9% among male students, while those with waist values above the 90th percentile were found to be 35.1% among female students and 32.1% among male students. In a study conducted with children between the ages of 6 and 18 in our country, the rate of those with a WC at the 90th percentile and above was found to be 2.5%.[46] In a study conducted by Dogru et al with children who had asthma, the rate of children with WC under the 90th percentile was found to be 60% and the rate of those with a WC above the 90th percentile were found to be 40%.[47] If the WC exceeds the 90th percentile according to age and gender, this shows abdominal obesity and is a basic criterion among IDF (International Diabetes Foundation) metabolic syndrome criteria.[48] Budak et al, found the BMI, weight and WC of adolescents with metabolic syndrome between the ages of 12 and 19 to be significantly higher compared to those who didn't have metabolic syndrome.[49]

The waist/height ratio was found to be 0.45±0.07 in female students and 0.45±0.04 in male students in our study. In a study performed by Bacopoulou et al with Greek adolescents, the average waist/height ratio in students of 16-17 years of age was similarly 0.43±0.05 in male students and 0.42±0.05 in female students.[35]

The cutoff value for waist/height was taken as 0.50 in our study according to the Ashwell chart. In females those in the ≥0.50 were found to be 24.3% while those in the appropriate interval were found to be 59.5%. In males, those in the ≥0.50 were found to be 17.0% while those in the appropriate interval were found to be 79.2%. In a cross sectional study performed in England with students at 16 years of age, the rates of students with a waist/height ratio above 0.5 were found to be 9.4% in females and 19.3% in males.[33]

According to the results of the test performed in order to determine whether there was a significant difference with regard to waist/height ratios and gender, no significant differences among the groups were found (p>0.05). The waist/hip ratio of females (x=0.749), were found to be lower compared to those of males(x=0.840). When 3rd and 4th year university students were compared with regard to BMI, WC, and WHtR, a significant difference was found only with regard to WC.[50] In a

study conducted with Chinese children at 16 years of age, BMI averages were found to be 20.2±3.3 in females, and 20.7±3.4 in males while WC was found to be 63.2±6.4 in females, and 68.8±8.2 in males, and the average WHtR was found to be 0.40±0.04 in females and 0.40±0.05 in males.[51]

In our study, the HC averages were found to be 99.16±13.05 in female students and 95.45±7.70 in male students. Among Greek adolescents of the same age, HC averages were found to be 97.15±8.40 in female students and 97.25±8.20 in male students.[35] In a study conducted in our country with adolescents between the ages of 10-15, average hip circumferences were found to be 98.1 in male students and 96.9 in female. Students.[40]

In our study, the waist/hip ratio averages were found to be 0.74±0.06 in female students and 0.83±0.05 in male students. Among Greek adolescents of the same age, HC averages were found to be 0.71±0.04 in female students and 0.77±0.06 in male students. The waist/hip average of the male students in our study is higher.[35] In a national study conducted with 240 female students attending their sophomore year at high school, the waist/hip ratio was found to be 0.74±0.4, which is compliant with our study.[52] A waist/hip ratio above 0.8 indicates that people are in the risk group with regard to abdominal obesity and have higher risks of chronic diseases.[53] The difference between the groups with regard to WHR averages was found to be significant (p=0<0.05). The WHR of females were found to be lower compared to those of males. The cutoff value for waist/hip ratio was taken as 0.80. In females those in the ≤0.80 were found to be 85.1% while those in >0.80 interval were found to be 14.9%. In males, those in the ≤0.80 were found to be 24.5% while those in the >0.80 interval were found to be 75.5%. In a study conducted by Aslan et al with high school sophomore female students at or above the age of 16, the WHR of 92.5% were found to be under 0.80 while the WHR of 7.5% were above 0.8.[52] Bulduk et al found the average WHR to be 0.80 ±0.32 in males and 0.77±0.72 in females in Turkish adolescents between the ages of 10-15.[40] These results are compliant with our study. In their study, Ayvaz et al found the average WHtR to be 0.43±0.03 in children between the ages of 12-16, and the average WHR ratio was found to be 0.82±0.05.[21]

In our study, no significant correlations between the education levels of the parents and the types of food consumed were found. In a study by Vereecken, differences in children's food consumption by mothers' educational level were completely explained by mother's consumption and other food parenting practices for fruit and vegetables but not for soft drinks.[54] In a study performed with adolescents between the ages of 11-13 in Ankara, BMI was found to be higher in the group with high socio economic status where the education level of the fathers were higher compared to the group with lower socio economic status.[55]

No studies explaining the relationship between waist/hip ratios and parental education were found. In our study, the waist/hip ratios of those whose parents had university education were found to be significantly lower in both gender groups compared to those with less parental education. This situation may be interpreted as parental education causing more nutrition with more awareness. In some studies, the prevalence of obesity was reported to increase with increasing parental education. A statistically significant linear relationship existed between maternal education and child weight in the middle socio-environmental group.[56]

In our study, a medium strength positive significant relationship between WHtR and BMI was found (r=0.693; p=0.000).

There is a very weak positive significant relationship between waist/hip ratio and BMI (r=0.204; p=0.021). As a matter of fact, in a study conducted with obese and non obese children between the ages of 7 and 18, a positive significant relationship between blood pressure and BMI was found whereas no relationship between WHR and blod pressure were found. The researcher claimed that the WHR was not an important indicator with regard to fat accumulation.[57] In a study conducted by Erguven et al with 31 obese adolescents, a positive significant correlation between WC and BMI was found.[58] In a study regarding being overweight in a kindergarten, it was found that waist and hip circumferences increased with BMI in both female and male students, with the difference being statistically significant.[27]

According to our study, the consumption of fizzy drinks increased the waist/hip ratio significantly, causing a widening especially in the WC. There are

many studies explaining the relationship of obesity with the consumption of sugary/fizzy drinks.[55] A study similar to ours regarding the relationship between WHR and fizzy drinks couldn't be found. The consumption of chocolate decreased the WHR, causing expansion in the hip circumference. The consumption of dry legumes decreased BMI.

WHtR is more closely linked to childhood morbidity than BMI and we suggest it should be used as an additional or alternative measure to BMI in children as well as adults. A simple public health message that is the same for adults and children of both sexes and all ages could be stated as `keep your WC to less than half your height.[33] Adolescence is a period where growth and development are fast. The provision of the nutritional elements, which increase in the amount necessary, is an important factor in the prevention of chronic diseases in adulthood. A healthy diet and healthy lifestyle habits are shaped during adolescence and become permanent. [38] Scans and anthropometric measurements regarding weight gain during adolescence are important in order to reduce risks. According to our study, fizzy drinks especially increase WHR and chocolate especially decreases it. A WHtR average study in the adolescent group at the 16-17 age interval according to gender hasn't been previously performed in our country. Our results show 0.45±0.07 in females and 0.45±0.04 in males. A positive correlation between WHtR and BMI and WHR was found.

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Corresponding Author
Rengin Kosif,
Department of Anatomy,
Abant Izzet Baysal University Medical Faculty,
Golkoy,
Bolu,
Turkey,
E-mail: rengink@yahoo.com

Data collection form

I. Socio-demographic data
1.Age:

2. What grade are you in :
Your Department Nursing ( ) Emergency medical technician ( ) Nurse assistant ( ) Other

3.Sex women ( ) Male ( )

4. I'm staying at home ( ) I'm staying in the dorms ( )

5. Social security: Medicare ( ) Social insurance institution ( )

6. Educational Status of the Mother

Literacy is not ( )

Literacy ( )

Primary ( )

Secondary school ( )

High school ( )

College ( )

7. Educational Status of the Father

Literacy is not ( )

Literacy ( )

Primary ( )

Secondary school ( )

High school ( )

College ( )

8. Your diet, how would you rate it ?

'I think I was a healthy and correct feeding' ( )

'I think I was wrong and unhealthy feeding' ( )

9. Do you have any diet to lose weight ?

Yes ( )

No ( )

10. Do you have any chronic disease? (Diabetes mellitus, hypo-and hyperthyroidism, Cushing syndrome, Addison, etc.)

Yes ( ) Please write ……

No ( )

II. Eating habits

11. The Habits of Meals (The table below please mark with an X the option that suits you.)

Meals	Daily /regular	A couple days a week	Never
Breakfast			
Lunch			
Dinner			
Mid-morning meal (11 hours)			
Snack (4 hours)			
Night lying			

12. Consumption of Food Groups

The table below please mark with an X the option that suits you.

Food Groups	Daily /regular	A week 3-4 times	A week 1 time	Pretty sparse	Never
Milk and milk products					
Milk					
Cheese					
Yogurt					
Meat eggs/ dry beans					
Egg					
Red meat					
Chicken					
The flesh of the fish					
Dry beans (Chickpeas, lentils, dry beans, etc.)					
Grains					
Bread					
Bagel /pastry etc.					
Rice					
Macaroni					
Burghul					
12. (Continue) The table below please mark with an X the option that suits you.					
Fruit / vegetable					
Seasonal fruits					
Seasonal vegetables					

Food Groups	Daily /regular	A week 3-4 times	A week 1 time	Pretty sparse	Never
Other Foods					
Cakes, bakery food, biscuits					
Potato chips					
Wafers, chocolate etc. sugary foods					
Fast food (Hamburgers etc.)					

Liquid foods

	Daily /regular	A week 3-4 times	A week 1 time	Pretty sparse	Never
Cola drink					
Juice ready					
Coffee					
Energy drink /an athlete drink (Red Bull, etc.)					

	From 10 cups more	5-10 cups	I consume less	Never
Water				

III. Anthropometric data

Height (cm):		
Weight (kg):		
Body Mass Index (BMI) (kg/m^2):		
Waist circumference (cm):	Height (cm)	Waist to height ratio: (cm/cm):
Hip circumference (cm):		
Waist-to-hip ratio:		

Training rounds: how to prepare good rounds

Hojjat Derakhshanfar, Kamelia Hemat

Emergency Medicine department, Shahid Beheshti University of medical sciences, Tehran, Iran.

Abstract

Backgrounds: training rounds are main aspect of medical education for both residents and medical students. In this regards, several investigations discussed about the rounds but the normal standards has not yet been defined. Therefore in the current paper we reviewed the environments of training rounds in Shahid Beheshti University of Medical Sciences associated hospitals.

Material and methods: The study population involved 15 attending physicians and 40 emergency medicine residents in the three hospitals, Imam Hussein, Lughman Hakim and shohadaye Tajrish of the Department of Emergency Medicine of Shahid Beheshti University of Medical Sciences in the academic year 2015 to 2016. Self-made questionnaire was used to compare the Ministry of Health and medical education standards with the outpatients' clinic in these centers.

Results: the results indicated that visit of a minimum of 1 and a maximum of 3 patients in each training session and the availability of Negatoscope drives and manometer during training rounds earned the most points. Average standard of educational round in total and in Imam Hossein, Lughman Hakim and Tajrish was 71.9%, 79.4%, 69.3% and 66.9 respectively.

Conclusion: the state of educational rounds in Luqhman Hakim, Imam Hussein and Tajrish Hospital were in acceptable situation considering Ministry of Health and medical education standards

Key words: Training, rounds, medical, education

Introduction

Ward rounds are aspect of medical education which can improve the patients care (1,2,3). Medical students experience ward round during their undergraduate and these rounds will elevate the quality of learning (4). In this regards studies reported that the less in ward round, the poorer outcome in surgical patients.(5) Moreover, main aspect of student in the lack of rounds and clinic do not have the opportunity for learning and practice (6 -10) studies have indicated that simple observations have little effect on trainees (7, 9–12).

In this regards, Lane et al (13) in their systematic review paper which was updated [14], discussed about ICU patient care rounds. The paper involved interprofessional rounds (physician, nurse, and pharmacist at minimum)[15–24]; standardizing practices[25–28]; defining roles for all participants[29–32]; considering a structured tool[33–44]; lessening the time spent on non-essential activities[26,27]; minimizing interruptions[45–47]; developing and documenting daily aims[31,40,48,49]; finding the best places for rounds (bedside vs. conference room) to optimize patient-centeredness and effectiveness [45,50–52]; and defining both an open and collaborative discussion environment[25,51–53].

As well as these practices, there are still many problems which influence the learning in medical education. Therefore in the current research we examined the environment of training rounds and compared it to Ministry of Health and medical education standards.

Methods and material

Research community

The study population included all faculty members (15 attending physicians and 40 emergency medicine residents in the three hospitals, Imam Hussein,Lughman Hakim and shohadaye Tajrish.) of the Department of Emergency Medicine of Shahid Beheshti University of Medical Sciences in the academic year 2015 to 2016.

Number of samples

All faculty members of emergency departments of Shahid Beheshti University of Medical Sciences, were enrolled in n the study.

Sampling

For Sampling and census methods we used available participants. This study was a cross-sectional study with a sample taken by the Department of Emergency Medicine faculty members in the academic year of Shahid Beheshti University of Medical Sciences.

The assessment tool was a self-made questionnaire that included the Ministry of Health and medical education in which clinical education standards in the training rounds were asked questions.

Content validity was used to determine the validity of the analysis and the questionnaire was finalized with expert advice and medical education reform. Cronbach's alpha was used to determine the reliability of the questionnaire.

The questionnaires delivered to faculty members and after voluntary completing, were collected. The questionnaire used the options "Yes" and "No" to questions. Questions were in five areas including Outpatient clinics, educational rounds, morning reports, journal clubs and clinical skills centers. When the answer was "Yes" rating 1 and each answer "no" was considered zero.

Total responses of faculty members were evaluated and every question in every area was calculated.

The following scoring system was used:

- Very poor: 20-0%
- Poor: 40-21%
- Medium: 60-41%
- Good: 80-61%
- Excellent: 100-81%

At the end of the collected data was entered in SPSS software version 21 and was analyzed.

Inclusion criteria

Faculty members of emergency departments of Shahid Beheshti University of Medical Sciences hospitals.

Exclusion criteria

Lack of consent to participate in the study.

Design of study

The study was cross-sectional.

Tools and methods for data collection

Researcher-made questionnaire were used to collect data in this study. How it was gathered, was to field. The questions in the questionnaire were based on standards which were approved by the Ministry of Health and medical education.

Data analysis

The data were extracted from the forms using the statistical software SPSS version 21. We used descriptive and inferential statistics.

Statistical methods and statistical tests used

In this study, the prevalence and ratio was used to analyze qualitative variables. To compare the level of five areas of the Outpatient Clinic also, educational rounds, morning reports, journal clubs and clinical skills centers with Ministry of Health and Medical Education standards , we used χ^2 test. P value of less than 0.05 was defined as significant.

Results

Special forms designed according to Ministry of Health and Medical Education standards were send to 15 attending physicians and 40 emergency medicine residents in the three hospitals, Imam Hussein ,Lughman Hakim and shohadaye Tajrish. The level of five areas of the Outpatient Clinic also, educational rounds, morning reports, journal clubs and clinical skills centers were compared with Ministry of Health and Medical Education standards.

Training Rounds

The results of participants' views regarding the standards for educational drove based on the separation of the study centers were summarized in Table 1.

Silences of Location for educational rounds, consent from each patient before training rounds, introducing Master and his companions to the patients before training, use of words when discussing educational Rand to be understand for the patient's and the availability of textbooks, medical and educational guide for students in Emergency Medicine departments were in variance at existing standards.

While visit of a minimum of 1 and a maximum of 3 patients in each training session and the availability of Negatoscope drives and manometer during training rounds earned the most points.

Average standard of educational round in total and in Imam Hossein, Lughman Hakim and Tajr-

ish was 71.9%, 79.4%, 69.3% and 66.9 respectively. In Total, the state of educational rounds in Luqhman Hakim, Imam Hussein and Tajrish Hospital were in good condition.

There was no statistically significant difference between the average assessments of emergency training rounds among centers under investigation. (p=0.1) figure 1.

Discussion

Our study revealed that the state of educational rounds in Luqhman Hakim, Imam Hussein and Tajrish Hospital were in good condition.

Natalie Powell et al (54) indicated that due lack of undergraduate educations many doctors refuse to perform rounds. They provided evidences that

Table 1. Evaluation of emergency medicine education level of shahid Beheshti University of Medical Sciences hospitals based on Training Rounds in the outpatient clinic from faculty members and students

Teaching Round	Shohadaye Tajrish	Imam Hussein	Lughman hakim	Total
Presence of all Attending physicians of emergency medicine in clinical education workshop	60%	80%	50%	3/63%
Monitoring by Masters of departments	60%	70%	87%	70%
Class neighborhood with emergency	80%	100%	87%	89%
Presence of Attending physicians for clinical education workshop to correct educational rounds	60%	75%	50%	7/61%
Conducting educational rounds by a faculty member	100%	90%	87%	3/92%
Presence of Attending physicians at educational rounds on different days	80%	100%	75%	85%
Bedside rounds conducted training	80%	100%	70%	3/83%
Having educational rounds of silent places	0%	10%	25%	7/11%
Having enough light for space of educational rounds	40%	80%	56%	7/58%
The availability of tools involves pressure gauge when training	100%	100%	100%	100%
The availability of tools involves an otoscope during training rounds	70%	80%	60%	70%
The availability of tools including hammer reflex examination in an educational round	40%	67%	50%	3/52%
Availability Negatoscope in an educational round	100%	100%	100%	100%
Teachers and students spend less than 10 minutes after taking history and physical examination	80%	100%	100%	3/93%
Authorization from each patient before training rounds	60%	50%	50%	3/53%
Introducing the Master and his companions to the patients before training	80%	90%	80%	3/83%
Visit the patient at least 1 and up to 3 per session training	100%	100%	100%	100%
All examinations and the education explained to patients during rounds	40%	50%	55%	3/48%
Repeated painful examinations of the patient during training	100%	100%	100%	100%
Patient room separated by a curtain in the presence of other patients during the examination	90%	80%	90%	7/86%
Use of words understood by the patient during the training rounds	20%	30%	20%	3/23%
Thanks and respond to questions before leaving the patient's bedside	20%	30%	20%	3/23%
Respect between teachers and students during the round training	90%	90%	90%	90%
Teacher training rounds feedback to students after the educational process and without the presence of patients	70%	80%	70%	3/73%
To provide textbooks to students	40%	33%	38%	37%
Equipped educational sector to a computer with high-speed Internet connectivity	80%	100%	85%	3/88%
Average of positive cases	9/66%	4/79%	3/69%	9/71%

ward-round training is essential for all medical trainees and junior doctors.

Learning in medicine is based on working and learns from a senior clinician and in this regards feedback is very important [55, 56].

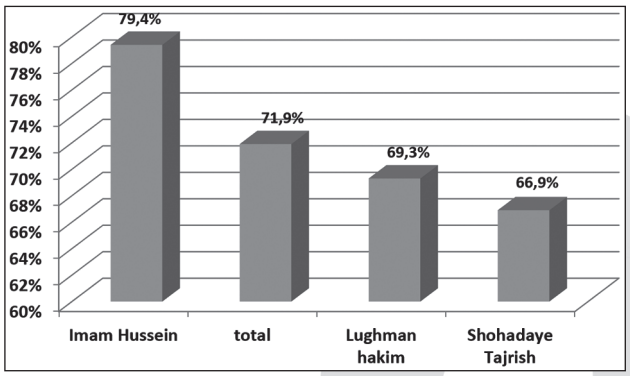

Figure 1. Comparison of the average of evaluation scores of emergency medicine departments of shahid Beheshti University of Medical Sciences hospitals based on Training Rounds in the outpatient clinic from faculty members and students

Feedback helps the trainee to find out him or herself whether is in good situation or not and how should improve his performance. [57–59]. Many researchers in this field concluded that feedback is a very essential in developing expertise [59, 60–65]. trainees in the health system accept that feedback can help them [66–69].

Studies have revealed that trainees spoke little, asked few questions, minimized self-assessment (if asked) and were not included in making decision what was talked about, explaining their perspective or planning ways to improve [70–75].

Investigations about feedback forms showed that students comments were often not specific, did not indicate what was done satisfactorily and what required improvement, and did not consist of an improvement plan [71, 76–78].

In this regards, Teacher training rounds feedback to students after the educational process and without the presence of patients scored high in three hospitals. Also, respect between teachers and students during the round training scored high.

Educational centers must consider the ways to encourage trainees to maximize the many feedback opportunities available to them. As a minimum, educators should remind students about their central role in the feedback process, and support them to develop confidence in meeting this role. Moreover, supervisors might require to develop the skills to shift the balance of responsibility and support students in precipitating feedback moments.(79)

Conclusion

The current article provided useful evidences about the training in educational hospitals. It has been demonstrated that learning environment is very important for learning and in this relation feedback between trainees and attending physician is important. Management of these environments is essential for improvement in leanings.

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Corresponding Author
Kamelia Hemat,
Emergency Medicine department,
Shahid Beheshti University of medical sciences,
Tehran,
Iran,
E-mail: thesis.irani@gmail.com

Expression of Bcl-2 and Bcl-xl as a predictor in achieving three-year survival in patients with diffuse large B cell lymphoma

Alma Sofo-Hafizovic[1], Adisa Chika[2], Lejla Ibricevic Balic[1]

[1] Clinic of Hematology, Clinical Center University of Sarajevo; Sarajevo, Bosnia and Herzegovina,
[2] Institute of Pathology, Clinical Center University of Sarajevo; Sarajevo, Bosnia and Herzegovina.

Abstract

Background: Programmed cell death has an important role in the formation and course of malignant diseases. The relation in the activities of proapoptotic and antiapoptotic factors determine whether and if the cell will start a process of apoptosis.

Material and methods: This was a retrospective-prospective study. Primary aim was to determine expression of apoptotic factors (bax, bad, bid, Bcl-2 and Bcl-xl) and their predictive significance in achievement of three-year overall survival of diffuse large B cell lymphomas.

Patients were enrolled into the study according to the following criteria: all newly diagnosed patients with DLBCL who had sufficient and adequate pathohistological material and who received immunochemotherapy as first-line treatment. Anti-CD20, anti-Bcl-2, anti-Bcl6, anti-CD10, IRF-4/MUM1, anti-CD138, Bcl-xl, bad, bax and bid were determined.

Results: The 60 patients de novo DLBCL were examined, median age was 45 year. GCB type was found in 65% of the patients. Median follow up was 47 months (from 3 to 91 months). Univariate analysis confirmed, in relation to the achievement of three-year survival, significant independent influence of Bcl-2 > 30% p=0.004 OR=3.41 (95% CI 1.49-7.8) and of bcl-xl expression > 9% p=0.003 OR=6.48 (95% CI 1.86-22.6) and of IPI>2 p<0.0005 OR=4.5 (95% CI 1.98-10.3). Multivariate analysis confirmed, in relation to the achievement of three-year survival, significant independent influence Bcl-2 > 30% p=0.01 OR=4.35 (95% CI 1.4-13.2) and IPI>2 p=0.002 OR=4.47 (95% CI 1.8-12.7), while Bcl-xl expression>9% has indicative influence p=0.051.

Conclusion: The expression of Bcl-2 has a significant impact while of Bcl-xl has indicative influence on the three-year overall survival in DLBCL.

Key words: DLBCL, blc-2, bcl-xl expression.

1. Introduction

WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues that was published in 2008 in Lyon[1] recognized, according to molecular, biological and clinical studies, different morphological, molecular and Immunohistochemical subgroups and entities within DLBCL. Significance in the IPI score in DLBCL was confirmed within numerous international studies conducted with a large number of patients.[2,3,4,5,6.] Two different molecular forms of DLBCL have been found using DNA mapping and Immunohistochemical staining: the first type with a better and the second with a worse prognosis.[7,8,9,10,11,12,13,14.] In the first type, the gene expression shows immature B cells in different stages of differentiation. This is a group that has immunophenotype similar to germinative center B cells and it is called GCB DLBCL, which has two distinct immunophenotype: bcl6+/CD10+/MUM1-/CD138- and bcl6+/-/CD10- /MUM1-/ CD138 -. The second type of the gene expression is seen in *in-vitro* activation of peripheral B cells (ABC) and it has characteristics of non germinative center (non GCB) with bcl6+/-CD10-/ MUM1+/CD138- immunofenotype.[15]

Data from Rosenwald, Hans, Hummel et Colomo studies emphasize that cases with CD10 expression are considered to be GCB type, as well as cases which have CD10-, bsl6+, MUM1- immunophenotype. All other cases are referred as non-GCB type.[12,13,14,15.]

Nowadays determination of immunophenotype is considered an important part of diagnostic procedure for DLBCL together with analysis of

molecular markings FOXP1 and GCET1 that is used in more recent studies.[16,17.]

The complex mechanisms of programmed cell death are determined by numerous oncogenes.

Apoptotic protein family regulates apoptosis by regulating mitochondrial permeability. Antiapoptotic proteins Bcl-2 and Bcl-xl are found in external mitochondrial wall where they inhibit release of cytochrome-c. Bax and bid move to mitochondria as a response to the lethal stimulation and in interaction with Bcl-xl and Bcl-2 (which they inhibit) affect voltage-dependent mitochondrial anion chain (VDAC). Result of this is releasing of cytochrome-c. Cytochrome-c binds to Apaf-1 after the release from mitochondria, and forms an activation complex with caspases (9.3) with caspasis 3 being an ultimate trigger of cells' death.

Internal and external pathways of apoptosis converge through activated caspase 3, and the results in activation of PARP (poli-ADP-ribose polymerase) and nucleosomal DNA fragmentation eventually leads to the death of the cell. Cells with bcl-2 gene alteration live longer and accumulate.[18,19,20,21.]

Treatment approach in DLBCL includes application of an anthracycline-containing regimen.

Salvage chemotherapy followed by high-dose therapy and autologous stem cell transplantation is a standard of the treatment for chemo sensitive relapses in diffuse large B-cell lymphoma.

More than 70% of patients will not benefit from standard salvage therapy, and continued progress is needed.[22.] Biological features of the tumor to a great extent determine its behavior, prognosis and result of the disease. These features may identify new target locations where new therapeutic options could be directed.

2. Material and Methods

This was a retrospective-prospective study. The primary aim was to determine in the examined group the response in terms of three-year overall survival (OS) in relation to expression of Bcl-2 and Bcl-xl. The secondary aim was to accomplish first complete remission (CR1) and Event Free Survival (EFS) where events were death in relation to the expression of Bcl-2 and Bcl-xl.

60 patients were analyzed as *de novo* DLBCL and they were treated at Department of Hematology of Clinical Center Sarajevo from 1 January 2002 to 31 December 2009. Median was 47 months (minimum 3, maximum 91 months).

Patients included in the study had the following characteristics: age >18 years, *de novo* DLBCL, *naive* patients that were not previously treated, that were planned to be treated with 4+4 cycles of R-CHOP or were treated according to this protocol, with an adequate biopsy material for analysis.

Non inclusion criteria

The study did not include patients with transformed low grade lymphomas into DLBCL, grey zone lymphomas, active hepatitis infection, and HIV infection.

Technical Information

Immunohistological staining was performed using the same biopsy material. Paraffin cubes were sliced and stained**.** Slices were incubated with the primary antibody including:

Anti-CD20 (1:150, clone L26, DakoCytomation, Glostrup, Denmark),

Anti-CD10 (1:150, clone 56C6, Novocastra Laboratories, Newcastle, Tyne, UK),

Anti-Bcl6 (1:40, clone PG.B6p, DakoCytomation, Glostrup, Denmark),

Anti-Bcl-2 (1:20, clone bcl-2/100/D5, Novocastra Laboratories),

CD138 (dilution 1:10, clone AM 411-10 M, BioGenex,CA USA,

IRF/MUM1 (dilution 1:40, clone sc 6059, Santa Cruz Biotehnology, INC, CA, USA),

Bcl-xl (dilution 1:25, clone 2H11, Zymed, Sauth San Francisco CA, USA),

Bad (C-7): clone sc-8044, starting dilution 1:200 Santa Cruz CA, USA,

Bax (dilution 1:40, code A 3533, Dako SA, Glostrup, Denmark) and

Bid (FL-195): sc-11423, Santa Cruz starting dilution 1: 200, CA, USA.

Visualization was performed using *EnVision® method* (DakoCytomation, Glostrup, Denmark) following manufacturers instruction. Adequate positive and negative controls were used. Expression of Bcl-2 was considered weak when it was ≤30%.

Expression of bcl6 and CD10 was quantified using *histo-score* system, using method described

by McCarty et al. Score system was adapted using the 40x lens microscope.

Positive expression for MUM1 and CD138 proteins was considered when it was positive in more than 25% of malignant cells. Expression of Bcl-xl, bax, bad and bid proteins was considered positive when there were more than 9% of positive malignant cells. Expression was rated into the three groups: 0-9%, 10-25% and> 25%. ZEISS Scope A1 microscope was used.

First line therapy was R-CHOP protocol, (rituximab day 1. 375mg/m^2 iv + CHOP/ day 1. cyclophosphamid 750 mg/m^2 iv, doxorubicin 50 mg/m^2 iv, vincristin max. 2 mg/ iv, day 1-5. Prednisone 100 mg p.o.). Radiotherapy was applied when there was bulky disease, extranodal presentation and in residual disease. Second line therapy was conventional chemotherapy DHAP (dexamethasone, cytarabine, and cisplatin) or ICE (ifosfamide, carboplatin, and etoposide).

Statistical analysis

When it comes statistical analysis we used univariate methods for evaluation of significant difference (X^2 test, binary logistic regression analysis). We assessed the overall survival with Kaplan-Meier methods and unstratified long-rank test. We used a multivariate backward Wald model to assess the significance for the efficacy variables and to establish the Odds ratio (OR) and 95% CI for each subgroup. $P < 0.05$ was considered as significant.

3. Results

This study included 60 patients with diagnosed de novo DLBCL, aged 18 to 72 years, with median age of 47 years and the highest number of patients with the disease aged 46-65. There were 31 (51.7%) male and 29 (48.3%) female patients. This can be seen in Table 1 which summarizes demographic properties of the studied patient groups.

This table also presents response to the therapy according to IPI. Discovered median time for

Table 1. Baseline demographics, response to therapy and overall survival according to International prognostic index (IPI), univariate analysis

Characteristics		n=60	CR n 47=, %	Sig X^2	OS n=44 3y %	sig. X^2	binary log.a
Age	60 years and Less than	50 (83.3%)	40 (80%)		37 (74.0%)		
	Over 60 years	10 (16.7 %)	7 (70.0%)		7 (70.0%)		p=0.903
Gender	male	32(51.7%)					
	female	29(48.3%)					p= 0.322
ECOG (*Eastern Cooperative Oncology Group)*	ECOG[8] 0,1,2/low	53 (88.4%)	44 (93.7%)	p=0.008	41 (93.2%)	P<0.05	p=0.002
	ECOG 3,4/high	7 (11.6%)	3 (6%)		3 (6.8%)		
Ann Anbor stage	I/II	26 (43.3%)	25 (96.15%)	P=0.012	25 (96.2%)	p<0.0005	
	III/IV	34 (56.7%)	22 (64.7%)		19 (55.9%)		
LDH (lactate dehydrogenase)	high	22 (63.3%)	12 (25.5%)	p=0.003	33 (75.0%)	p=0.002	p=0.005
	normal	38 (36.7%)	35 (74.5%)		11 (25.0%)		
Nodal site		27(45.0 %)	22 (81.0%)		21(77.8%)		
Total extranodal site		33 (55.0%)	25 (75.8%)		23 (69.7%)		
Extranodal site	>1	3 (5.0%)	0		0		
	=1	30 (50.0%)	25 (75.8%)		23(69.7%)		
International prognostic index (IPI)>2	Low risk + Low/ intermediate	22 (36.7%) 17(28.3%) Total 39 (65.0%)	36 (76.6%)		35 (79.5%)	p<0.0005	p<0.0005
	High/intermediate + High risk	15(25.0%) 6(10.0%) Total 21 (35.0%)	11 (23.4%)		9 (20.5%)		

overall survival in this study was 32.4 months, or 95% CI (29-36) months. By using univariate analysis (binary logistic regression), it was confirmed that the age of the examinees does not influence the overall survival p=0.903. Examinees gender also does not have influence on overall survival p=0.322 Statistically, ECOG>2 of the examinees significantly influence the survival p=0.002 OR=6.3 (95% CI 2.2-20.1). Statistically, increased LDH level significantly influence three-year survival p=0.005 OR 4.6 (95% CI 1.5-13.6). Influence of IPI >2 on three-year survival was examined. It appeared that IPI has statistically significant influence p<0.0005 OR=4.5 (95% CI 1.98-10.3). For each progression stage IPI chance of not surviving three years is increased by four times, or 95% CI (2 - 10 times).

We found significantly lower expression of anti apoptotic factor Bcl-2 p=0.01 and Bcl-xl p=0.032 in group GCB. More detailed data sample can be seen on Table 2.

Table 3. Presents how expression of apoptotic factors determines OS of the studied groups. It can be seen that influence of Bcl-2 expression (≤30%/ weak vs. >30%/ medium + strong) was examined on three-year survival of the examinees. It was shown that Bcl-2 has statistically significant influence p=0.004. OR=3.41 (95% CI 1.49-7.8). In the case that an examinee with ≤30%/weak expression Bcl-2 passes to the category >30%/ of medium-strong expression, the chance of not surviving three years is increased by 3 times, or 95% CI (1-8 times). The influence of Bcl-xl expression (0-9% vs. >9%) on three-year survival was examined and proved to have statistically significant influence p=0.003. OR =6.48 (95% CI 1.86-22.6). In the case that an examinee passes to the category of Bcl-xl >9% expression, the chance of not surviving three years is increased by 6 times, or 95% CI (from 2 to 22 times).

Table 3 also shows that examined influence of bax, bid and bad expressions (0-9% vs. >9%) on three-year survival of the examinees proved that bax p=0.264, bid p=0.936 and bad p=0.999 do not have statistically significant influence on three-year survival.

Figure 1. A shows differences of the examinees survival with expression Bcl-2≤30% (weak) and > 30% (middle and strong) is statistically significant p=0.004. Examinees with ≤ 30% Bcl-2 expression lived in average 37.3 months 95% CI (33.68-40.9 months), and examinees with > 30% Bcl-2 expression lived in average 28.3 months 95% CI (23.13-33.46 months).

Figure 1.B compares differences in survival between the examinees with Bcl-xl 0-9% and exam-

Table 2. Apoptotic protein expression in DLBCL n=60 divided in group GBC and non-GBC and impact on achieving CR1

		Immunohistochemical groups		GBC vs. non GCB p=X ²	CR1 p=X²
		Group GCB (n= 39)	Group non GCB (n=21)		
Bcl-2	≤30% (weak)	23(59.0)	5(23.8)	0.01	0.023
	>30% (medium +	12(30.8)	8(38.1)		
	Strong)	4(10.3)	8(38.1)		
Bcl-xl expression	0-9%	29(74.4%)	12(57.1%)	0.032	0.007
	10-25% +	9(23.1%)	4(19.0%)		
	>25%	1 (2.6%)	5 (23.8%)		
Bax expression	0-9%	25 (64.1%)	13 (61.9%)	0.977	0.821
	10-25% +	8 (20.5%)	5 (23.8%)		
	>25%	6 (15.4%)	3 (14.3%)		
Bad expression	0-9%	13 (33.3%)	9 (42.9%)	0.709	0.958
	10-25-% +	19 (48.7%)	8 (38.1%)		
	>25%	7 (17.9%)	4 (19.0%)		
Bid expression	0-9%	3 (7.7%)	0 (0%)	0.266	0.644
	10-25% +	13 (33.3%)	5 (23.8%)		
	>25%	23 (59.0%)	16(76.2%)		

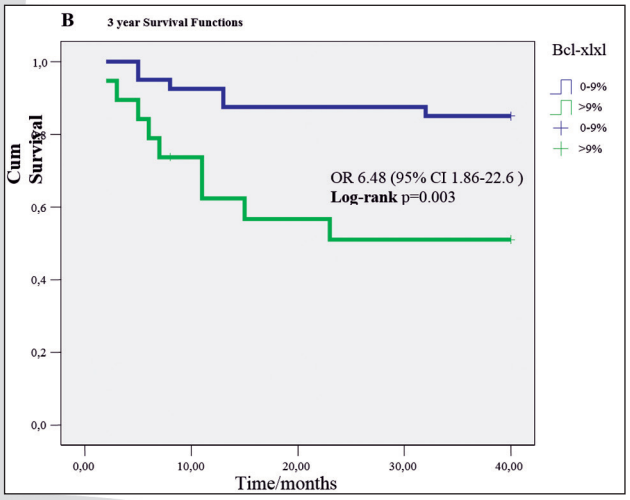
Table 3. Apoptotic protein expression in DLBCL n=60 pts and impact on achieving 3year OS

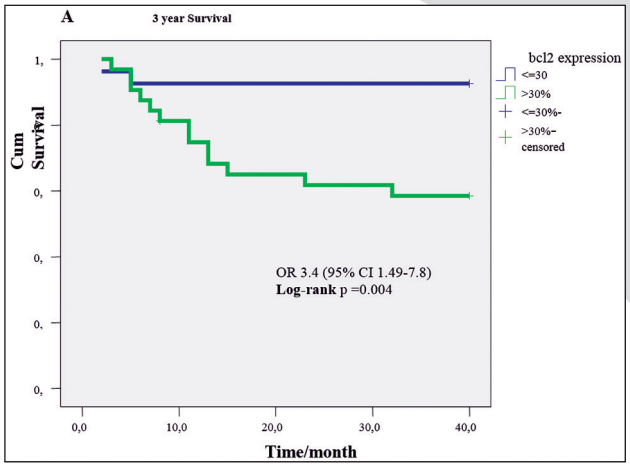
Apoptotic protein	expression	N =60	OS 3 y Sig. p=	Binar. OR 95% CI
Bcl-2	≤30% (weak)	28(46.7%)	0.004	3.4 (1.49-7.8)
	>30 % (Medium+	20(33.3%)		
	+strong)	12(20.0%)		
Bcl-xl expression	0-9%	41(68.3%)	0.003	6.48(1.86-22.6)
	10-25% +	13(21.7%)		
	+ >25%	6(10.0%)		
Bax expression	0-9%	38 (63.3%)	0.264	
	10-25%	13(21.7%)		
	>25%	9 (15.0%)		
Bad expression	0-9%	22(36.7%)	0.999	
	10-25-%	27(45.0%)		
	>25%	11(18.3%)		
Bid expression	0-9%	3 (5.0%)	0.936	
	10-25%	18(30.0%		
	>25%	39 (65.0%)		

inees with Bcl-xl >9% is statistically significant p=0.003. Examinees with Bcl-xl 0-9% in average lived 35.9 months 95% CI (32.6-39.12 months), and examinees with Bcl-xl >9 % lived in average 25.0 months 95% CI (17.7-32.8 months).

Figure 1. C illustrates differences in survival rate between examinees with IPI (0, 1, 2) and IPI (3, 4, 5) is statistically significant p<0.0005. Examinees with IPI≤2 lived in average 37.1 months 95% CI (34.33-39.87 months) and examinees with IPI>2 lived in average 23.8 months 95% CI (16.8-30.8 months).

All this data is summarized on the Table 4.





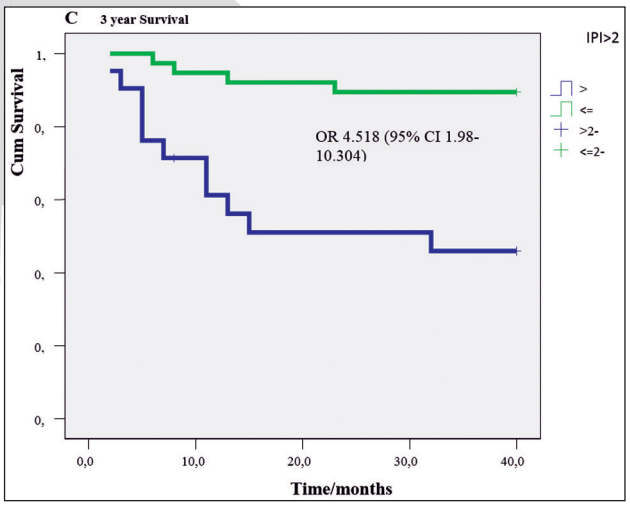


Figure 1. Kaplan-Meier curves for overall survival (3 year): A. expresion Bcl-2 (≤30% vs.>30%), B. expresion Bcl-xl (0-9% vs.>9%), C. IPI >2.

Table 4. End of study, 3.year overall survival N=60, the unstratified log-rank test.

Bcl-2	expression ≤30% n=28	expression >30% n=32		
	37.3 m 95% CI (33.68-40.9 m),	28.3 m 95% CI (23.13-33.46 m)	p=0.004	3.4 (1.49-7.8)
Bcl-xl	expression 0-9% n=41	expression >9% n=19		
	35.9 m 95% CI (32.6-39.12 m),	25.0 m 95% CI (17.7-32.8 m)	p=0.003	6.48(1.86-22.6)
IPI	≤2 n=39	>2 n=21		
	37.1 m 95% CI (34.33-39.87m),	23.8 m 95% CI (16.8-30.8 m))	p<.0005	4.518 (1.98-10.30)

Table 5. Univariate and multivariate analysis risk factor three-year overall surival

	Univariate Analysis		**Multivariate Analysis**	
Parameters	P value	OR 95% CI	p value	OR 95% CI
IPI>2	<0.0005	4.518 (1.98-10.304)	0.001	5.543 (1.943-15.814)
Bcl-2	0.004	3.4 (1.49-7.8)	0.01	4.35 (1.431-13.227)
Bcl-xl	0.003	6.484 (1.86-22.6)	0.051	5.345 (0.991-28.838)
bax	0.265			
bad	0.936			
bid	0.999			

Multivariate backward Wald methods were used to examine common influence of clinical variables/ factors contained in the International prognostic Index (IPI) and antiapoptotic factors Bcl-2 and Bcl-xl in relation to achieving three-year survival in DLBCL in the era of immunochemotherapy.

On Table 5 it is confirmed that statistically significant influence on three-year survival has IPI>2 p=0.001 OR=5.543 (95% CI 1.943-15.814), Bcl-2 (≤30% vs. >30%) p=0.01 OR=4.35 (95% CI 1.431-13.227). Bcl-xl ((0-9% vs. >9) p=0,051 OR 5.345 (95% CI 0.991-28.838) had indicative significant influence. It was confirmed that expression of Bcl-xl (0-9% vs. >9) has significant influence p=0.001 OR=0.48 (0.008-0.274) on event free survival (EFS) where event was death in three-year follow-up.

If an examinee moves from category I (0-9%) to category II (10-25%), the chance for death 95% in the population is increased from 72% to 99%. Between II (10-25%) and III category (> 25%) there is no statistically significant influence p=0.066.

4. Discussion

The primary aim of this study was to analyze prognostic influence of apoptotic factors in 60 patients with DLBCL treated with immunochemotherapy as first-line therapy on three-year overall survival (OS). Secondary aim was to achieve first complete remission (CR1) and event free survival (EFS) where events were death.

Immunohistochemical groups were determined according to sub classification of lymphomas according to 2008 WHO classification. (WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues). [1]

Clinical analysis

In our study there were 39 (65.0%) patients in group GBC.

Median follow-up was 47 months (3 - 91 months), which was sufficient because early relapse occurs mainly within first two years after the treatment. Median age was 45 years with the highest number of patients with the disease between the ages 46-65.

The data of median age of the diagnosis are comparable to epidemiological data in undeveloped countries published by AbidMB.[23.] This study states that the median age was 47.2, which is significantly different to the median age in developed countries. In developed countries age-specific incidence rates rise from around age 50-54 in the four main subtypes, with steeper rises for DLBCL and MZL as stated by the Cancer Research UK.[4] DLBCL occurs mostly in older people average age is mid-63 as published in the study Komrokji with the data of the US.[24]

IPI>2 influence on three-year survival was examined with binary logistic regression. It appeared that IPI has statistically significant influence p<0.0005 OR=4.5 (95% CI 1.98-10.3) Table

5. For each progression stage IPI chance for not surviving three years is increased by four times, or 95% CI (2-10 times).

Kaplan-Meier's survival curve is shown. Differences in survival between examinees with IPI>2 is statistically important p<0.0005. The examinees with IPI≤2 lived in average 37.1 months 95% CI (34.33-39.87 months) and the examinees with IPI>2 lived in average 23.8 months 95% CI (16.8-30.8 months).

Independent influence of IPI, Bcl-2 and Bcl-xl on three-year survival was examined with multivariate backward Wald methods. It was confirmed that statistically significant independent influence has IPI>2 p=0.001 OR= 5.543 95% CI (1.943-15.814).

Predicting role of clinical parameters contained in IPI was confirmed in 1993, in the era before immunochemotherapy, in the Shipp MA and colleagues study as well as in other numerous studies conducted until now. [2,3,4,5,6]

Analysis of the therapy response

We confirmed good clinical response to first-line therapy R-CHOP in achieving CR1 for 78.3 % of total patients, and data can be compared to results of referral centers. Study of Coiffier[25] confirms CR1 in 76.0% vs. 63.0% comparing first-line therapy R-CHOP vs. CHOP for treatment of DLBCL p= 0.005. In our study mainly patients with GCB immunophenotype were included.

The study confirmed that there were 8 (13.3%) patients with partial remission, and 5 (8.3%) with progressive disease in the first-line treatment. We had 16 (26.66%) deaths and 3 (5.0%) patients in early relapse. This data is summarized in the Table 6**.** The data related to partial remission and progressive disease correlate with data of Armitage J.O.[26], stating data of LNH-87-2 study.

Expression of Bcl-2

Bcl-2 is an antiapoptotic protein and belongs to a large family of proteins involved in the regulation of programmed cell death.[27] Strong expression of antiapoptotic protein Bcl-2 and weak expression of proapoptotic protein bax block apoptotic signal. Bax in interaction binds to bcl-2, preventing it in apoptosis suppression.[21] Expressions of Bcl-2 and bax that were found in this study, indicate inhibition of apoptosis and as such probably confirms one of the mechanisms of DLBCL development. Igbal J[s] study has demonstrated variable expression of the Bcl-2 in the two subgroups of DLBCL as well as a controversial role of Bcl-2 as a survival predictor in DLBCL. Significance of BCL-2 and other biomarkers should be assessed in the context of DLBCL subgroups in future studies. [28]

Our study determined Bcl-2 expression and its impact on disease course.

We confirmed significant difference in strong expression of bcl-2 in group GBC vs. group non GBC with X^2 p=0.01. In the GCB group the expression of bcl-2 was weaker. Achieved response to first-line therapy as a complete remission was significantly different X^2 p=0.023 in the group GBC vs. group non GBC. Prognostic importance of expression of Bcl-2 in non GBC was confirmed in Berglund[29] study as well as in Izidore S Lossos study which analyze the influence of 6 genes including Bcl-2 where it is confirmed that the measurement of six genes expression is sufficient to predict overall survival in diffuse large-B-cell lymphoma. The genes that were the strongest predictors were LMO2, BCL6, FN1, CCND2, SCYA3, and BCL-2..[30]

Expression of Bcl-2 (≤30% vs. >30%) influence was examined with binary logical regression on three-year survival of the examinees with the confirmation of statistically significant influence p=0.004 OR=3.41 95% CI (1.49-7.8). If an examinee with weak expression of Bcl-2 moves to the category of middle and high expression, the chance not to survive 3 years is increased by three times, or 95% CI (1-8 times). Kaplan-Meier curves survival are shown (Figure 1-A). Differences in survival between examinees with ≤30%/ (weak) and examinees with >30% (middle and strong) Bcl-2 expression: examinees with middle and strong Bcl-2 expression lived in average 28.3 months 95% CI (23.13-33.46 months), and examinees with weak Bcl-2 expression lived in average 37.3 months 95% CI (33.68-40.9 months)

C-myc rearrangement with DLBCL designates a subgroup with an extraordinary bad prognosis. The study of Perry AM[31] also confirmed in this DLBCL patient group high co-expression of c-myc. Bcl-2 was an independent predictor of poor survival, and could be used to stratify patients for risk-adapted therapies.

However, Nalan Akyurek in his study analyzed the influence of c-myc gene rearrangement with Bcl-2 and bcl6 on five-year survival with DLBCL, and identified high-risk group of patients with bad prognosis where prognostic importance of Bcl-2 rearrangement was not confirmed.[32]

Expression Bcl-xl

Antiapoptotic proteins Bcl-2 and Bcl-xl are found in external mitochondrial wall where they inhibit release of citochrome-c.[21]

We confirmed significant difference in expression of Bcl-xl (0-9 vs.10-25 vs.>25 %) p=0,032 X^2 in group GBC vs. group non GBC. Stronger expression of Bcl-xl was confirmed in group non GCB, and data can be comparable to the data of study by Bai M. In the study it was proved that the expression of Bcl-xl was significantly lower in the germinal center B-cell-like profile than in the non-germinal center B-cell-like profile (p=0.026).[33]

Expression bax, bad and bid

Relation of activities of apoptotic and proapoptotic factors determines if and when the cell starts an apoptosis.[18,19.]

We did not confirm significant difference in expression of proapoptotic factors by analysis X^2 test: bax p=0.977, bad p=0.709 and bid p=0.266 in group (GCB) vs. group non GCB.

In addition, binary logistic regression did not confirm statistically significant influence of bax, bid an bad expression (0-9% vs. >9%) on three-year survival of the examinees, and it appeared that bax p=0.264, bid p=0.936 and bad p=0.999 don't have statistically significant influence on three-year survival with DLBCL.

Results of multiple regression analysis: IPI, Bcl-2 and Bcl-xl

Multivariate backward Wald methods were used to examine independent influence of antiapoptotic Bcl-2 and Bcl-xl factors, as well as clinical features that are contained in the International Prognostic Index in relation to three-year survival in DLBCL in the era of immunotherapy.

It is confirmed that statistically significant independent influence on three-year survival has IPI>2 p=0.001 OR = 5.543 95% CI (1.943-15.814) and Bcl-2 (>30%) p=0.01 OR=4.35 95% CI (1.431-13.227). Expression of Bcl-xl (0-9% vs. >9%) has statistically indicative influence p=0.051 OR 5.345(95% CI 0.991-28.838) on three-year survival.

Results in our study, in relation with the impact of IPI, bcl-2 and OS, can be compared to the results of Berglound study. In Berglound's study, expression of Bcl-2 was associated with worse event-free survival and overall survival. In a multivariate analysis, a high international prognostic index score (3-5), non-GC phenotype and bcl-2 were independent adverse prognostic factors. [29]

In our examined group, a statistically significant influence of Bcl-xl expression (0-9% vs. >9%) is confirmed by univariate analysis p=0.003 OR=6.48 (95% CI 1.86-22.6).

Also, Bcl-xl expression (0-9% vs. >9%) of the examinees has statistically significant influence on EFS (death) p=0.001 OR=0.48 (95% CI 0.008-0.274). If an examinee moves from category I (0-9%) into category II (10-25%) the chance of not achieving three-year survival (resulted in death) is increased 72%-99% in 95% of the examined population. Between the categories II (10-25%) and III (> 25%) there is no statistically significant influence p=0.066. This result is probably the consequence of sample size. However, besides the small sample size, we have opinion that the influence of expression Bcl-2 is obviously stronger and as such overpowers Bcl-xl influence and doesn't enable an independent Bcl-xl influence.

This study included patients who received an immune chemotherapy R-CHOP as first-line therapy and who had biopsy adequate for immunohistochemical analysis. Second line therapy was conventional chemotherapy DHAP or ICE. In the study there were 16 patients who died in the period of 3 years after DLBCL was diagnosed. The study included 21 patients with non GCB and in this group 13 patient died. In first-line treatment there were 8 patients with PR, 5 patients with PB and 5 patients with an early relapse.

Moreover, Cristian Gisselbech reported in his study that patients with an ABC subtype or c-myc translocation responded poorly to the treatment. Early relapses and/or patients refractory to upfront rituximab-based chemotherapy have a poor response rate and prognosis. A better biological understanding of these patients and new approaches are warranted.[22.] There is still a large percentage

of patients (50-70%) whom besides applying standard therapy including HDT with ASCT still experience an unwanted flow of the disease.

It is realistic to assume that clinical characteristics, immunohistochemical factors and apoptotic factors have an important predicting significance, however, the molecular characteristics of DLBCL could be the target place for potentially new therapeutic options.[34,35.]

5. Conclusion

The results of this study confirmed that the patients with DLBCL and immunophenotype non GCB have significantly higher expression of Bcl-2 and Bcl-xl. We confirmed that there is significant impact of IPI>2, expression of Bcl-2, as well as indicative influence of Bcl-xl expression in relation to three-year survival using multivariate backward Wald method.

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Corresponding Author
Alma Sofo Hafizovic,
Hematology Clinic of the University Clinical Center of Sarajevo,
Sarajevo,
Bosnia and Herzegovina,
E-mail: almasof44@gmail.com

Immune mechanisms in recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF)

Elmira Hajder[1], Mithad Hajder[2], Milena Brkic[3], Ensar Hajder[4]

[1] PHI Institute of Human Reproduction, Obstetrics and Perinatal Medicine, "Dr. Hajder "Tuzla, Bosnia and Herzegovina,
[2] Department of Endocrinology, Internal Clinic, University Clinical Center, Tuzla, Bosnia and Herzegovina,
[3] Medical faculty, University of Banja Luka, Bosnia and Herzegovina,
[4] University Hospital, Essen, Germany.

Abstract

Recurrent pregnancy loss (RPL) and implantation failure (RFI) are common immune disorder during pregnancy. Numerous evidence of immune abnormalities in patients with RPL and RIF include autoimmune and alloimmune factors. Autoimmune factors are combined with: antiphospholipid syndrome, thyroid autoimmunity, polycystic ovary syndrome, anti-nuclear antibodies, celiac disease, anti-ovarian and anti-sperm antibodies. Alloimmune immunology factors are combined with: HLA-DQ phenotype, T and B blocking antibodies, decidual cytokines. Regulatory T cells, increased ratio CD4/CD8 of lymphocyte and NK cells. Recent study shows efficiency of immune therapy with intervene globulin (IVG) and granulocyte growth factor (G-CSF) in treating RPL and RIF, but there are no define risk factors which would direct towards efficient immune therapy.

Key words: autoimmune factors, Alogenic factors, PRL, RIF.

Introduction

ASRM define recurrent early pregnancy loss (RPL) as two or more unsuccessful pregnancy, which have been documented with ultrasound or histopathology (1). Pregnancy loss is the most common gynecology problem and has great physiology, psychology and financial implications. The great number of pregnancy loss occurs in healthy women during first trimester of pregnancy. Between 11 and 20% of all clinical pregnancies are lost before 20th week, 25% of implanted embryos are lost within 7-14 days (2). Pregnancy loss is defined as spontaneous loss of pregnancy within first 24 weeks of pregnancy. ''Early pregnancy loss'' is loss of pregnancy during the first trimester (less than 12 weeks of pregnancy) and it occurs in interval one on five. ''Late pregnancy loss'' occurs in second trimester (12-24 weeks of pregnancy), and is rare and occurs in 1-2% of pregnancy. Fetal death from 25th weeks of pregnancy and on is defined as stillborn (3). Recurrent pregnancy loss is growing problems in developed countries where women postpone giving birth till 30 or 40 years old. Research in Sweden population confirmed triple of risk of miscarriage of 8.7% at the age of 25, and it increases to 24.7% at the age of 35. At the age of 42 risk of pregnancy loss is 51%, and at the age of 45 risks is 74.7% (4). Recurrent implantation failure (RIF) relates on failure to achieve clinical pregnancy during transfer of at least four quality embryos (A or B) in at least three fresh or frozen cycles by women younger than 40 years old. Failure can be the result of embryo or mother (5).

Aetiology of miscarriage

Causes of miscarriage are still relatively unknown. However, around 50% of early pregnancy loss fetus shows chromosome aberration (aneuplodia), such as structural changes or abnormal number of chromosome (6). Increased risk of miscarriage are the age of both parents over the age of 35 or older (7), ethnic origin, psychological state of mother, very low or very high BMI before pregnancy, stress, using of non-steroid anti-inflammatory drugs, smoking and using of alcohol (8). Endocrine factors: PCOS high LH and testosterone (9), metabolic factors (valin, adip acid, L-lizin, creatine, ornitine, glycerol) with low values of serum eNOS (10), autoimmune disease of thyroid gland (11), hyperprolactinemia (12, 13), diabetes with insulin resistance (14) are common cause of RPL and RIF. Finally, numerous infections (viruses, bacte-

ria, parasitize) are connected with recurrent early and late miscarriage (15) and preterm labor (16). Namely, 15% of early miscarriage and 66% of late miscarriage are connected to infections (17). Uterine anomalies are also one of the causes of RPL (18). Women whose first pregnancy resulted in miscarriage have higher risk of second pregnancy loss (19). Large percent of PRL and RIF are autoimmune and aloimune causes (20).

Chromosomal abnormalities

Roughly, 15-20% of clinical pregnancy ends in spontaneous miscarriage during first trimester. Around 50% of all causes are aneuploidy and euploidy embryos that contain mtDNA (21). Chorionic causes of spontaneous miscarriage can be examined with different methods for research methods for research of etiology of RPL, especially genetic factors. Fetal chromosome abnormalities are primary causes of RPL and RIF, especially aneuploidy. In chromosome aneuploidy or polyploidy are included chromosome aberration X, Z, 13,16, 18, 21 and 22 chromosome. Conventional cario typisation is gold standard for diagnosing aneuploidy, with rate of failure of diagnosis in 21% of cases. Fluorescent hybridization in situ (FISH) is reliable methods for diagnostic of chromosome aneuploidy from choriocentesis. However, there are reports that show that there are no significant difference in aneuploidy rate between older (≥35) and younger aged women (<35). Sperm aneuploidy test (SAT) is diagnostic test for genetic analysis of sperm chromosome abnormality of aneuploidy. Over 60% of miscarriage in couples is related with increased incidence of chromosome abnormality. Test evaluate abnormal number on chromosome 13, 18, 21, X and Y sperm DNA fragmentation with fluorescent hybridization in situ (FISH). Indications for performing test are: male factor of infertility, recurrent pregnancy loss of unknown etiology, recurrent implantation failure and previous pregnancy with chromosome abnormalities (22). One of the causes of RIF is euploidy embryos. Mitochondrial DNA content (mtDNA) is a viability indicator in human euploidy embryos. Content of mtDNA, number of copies in euploidy embryos is relevant factor for implantation and sustainability of embryos. MitoScor is mitochondrial biomarker that uses mtDNA content and is the indicator of energetic embryo status. Increased amount of mtDNA in euploidy embryos show a weak implantation potential. On 3rd day of euploidy embryos value of MitoScore <34 gives implantation rate (IR) of 59%; MitoScore 34-52 gives IR of 44%; and one of 52-97 gives IR of 42%; and MitoScore >97 gives IR of 25%. In embryos with MitoScore >160 there is no implantation. In conclusion, increased amount of mtDNA in euploidy embryos is connected with poor implantation potential and can be an indicator of low metabolic fuel during growth of oocyte (21).

Immune profiling

Role of immune system in successful pregnancy is the key. While maintaining immune tolerance of half-allogeneic fetus, several component of immune system fulfill preparation for implementation of the fetus (23). Cells in NK, macrophage and dendrite cells are present in fetoplacental confrontation (24). Cytokine, like interleukin (IL-10), granulocyte colony-stimulating factor (G-CSF) and transforming factors of beta growth (TGF-beta) have favorably effect on implantation (25). Implantation causes local inflammatory reaction of decidue because of invasion of extravillous trophoblast in mother's tissue (26).

Table 1. Immune abnormalities in women with recurrent pregnancy loss and implantation failure

Autoimmune factors	Allogeneic factors
Anti-phospholipids antibodies	decidual cytokine (IL-1,IL-8, TNF-alfa)
Anti-thyroids antibodies	Regulatory T cells
Anti-nuclear antibodies	ratio CD4/CD8
Anti-ovarian antibodies	ratio TH1/TH2
PCOS	periphery NK cells
Increase level of IgM	in NK cells
IgA deficiency	HLA DQ phenotype
Endometriosis	Blocking T lymphocyte
Congenital thrombophilia	Blocking T lymphocyte

Numerous evidence of immune abnormalities in patients with RPL and RIF, include autoimmune and alloimmune factors (table 1). Autoimmune immunology disorders that are combined with recurrent pregnancy loss are: anti-phospholipids antibodies, anti-nuclear antibodies, anti-ovarian antibodies,

anti-sperm antibodies, antibodies for celiac disease and born and acquired thrombophilia. Alloimune immunology disorder are combine with RPL and RIF are: decidual cytokine, regulatory T cells, increased CD4/ CD8 of lymphocyte, increased ratio of TH1/TH2, pNK cell, HLA DQ haplotype and blocking antibodies T and B cells (20,4).

Autoimmune factors

Antiphospholipid syndrome (APS) is defined with recurrent pregnancy loss by thrombosis with the presence of antiphospholipid antibodies (aPL). Experimental data's show passive transfer of aPL results in clinical manifestation of APS, i.e., loss of fetus and thrombocitopheniom (27). AFS is one or more unexplained death in morphological normal fetuses after 10th week of pregnancy, difficult preeclampsia or eclampsia and insufficiency of placenta before 34th week of pregnancy and three or more unexplained consecutive spontaneous miscarriage before 10th week. (28). Target antigen are negative bunged up phospholipid and phospholipid that tied protein in serum. Syndrome appears during production of auto antibodies against phospholipid molecules that are integral element in cell membrane of syncytiotrophoblast. Antibodies on phospholipid molecule can damage inner wall of blood cell, causing creation of thrombosis and stenosis of lumen blood vessels, causing creation occlusion on maternal-placenta circulation. Beside strong connection between aPL and thrombosis, pathogenesis of APS in thrombosis development is not completely understood. The most common used serologic markers for APS are lupus anticoagulants (LA), anti-cardiolipin antibodies (aCL) and anti-beta 2 glycoprotein 1 antibodies (29). It is interesting that trophoblast can be directed towards aPL, especially on anti-phosphatidylserine antibodies (aPS). Cardiolipin is not present in membrane of trophoblast, but still aCL are involved in pathology of trophoblast. Maybe crossed reaction between aPS and aCL can up bring to this pathology (30). Animal in vitro experiment show that aPS and aCL antibodies can destroy trophoblast, inhibited chorionic gonadotrophic secretion (hCG) and limit invasion of trophoblast. In RPL positive aPL are between 8% and 42%. According to international consensus for classification and diagnosis, APS is present if at least one of the clinic criteria and one of the laboratory criteria (31, 28). Lab criteria include presence: Lupus anticoagulant, anticardiolipin IgG or IgM antibody, Anti-b2 glycoprotein and IgG or IgM antibody (28).

Polycystic ovary syndrome (PCOS)

PCOS is mostly connected with AFS. Prevalence of PCOS is 40% among women with RPL. Increased serum concentration of LH (>10 IU / I), and increase serum concentration of testosterone (>3 nmol / L) were connected to increased rate of RPL (9). Women with increase LH, have increase risk of abortion after spontaneous or IVF pregnancy. Insulin resistance as PCOS disorder has significant role in recurrent pregnancy loss. Women with history of RPL have increased risk for insulin resistance in first trimester of pregnancy (32). Recent meta-analysis concluded that insulin resistance is connected with RPL (33). Therefore, resistance on insulin can be one of the direct causes that lead to recurrent pregnancy loss (14).

Thyroid autoimmunity

Autoimmune thyroid disease (AITD) is the far most common cause of hypothyreosis in women of reproductive age. Prevalence in general public is 10-25% (34) while prevalence in AITD is 5-20% in normal pregnant women (35). There is lack of consensus regarding definition of upper limitation of TSH in pregnancy (<2.5 mIU/ml). The aim is to remain the level of THS <2.5 mIU/ml, and if the values are higher than <5mIU/ml it should be treated with 50 mg of thyroxin (11). Examined were two antibodies, anti-thyroid peroksidase (anti-TPOAb) and anti-thyroglobulin antibodies (anti-TGAb), together called anti- thyroid antibodies (ATA) and they are considered as marker of recurrent spontaneous pregnancy loss (36). Mother's immune answer, trophoblast function and thyroid function are in correlation. However, direct impact of ATA on trophoblast has not been researched, so it would need new research. Hypothyrosis is commonly connected to infertility, because thyroid hormones have direct influence on granules' of cell, lutheal cell and growth of oocyte. Meta-analysis Prummela at el., showed that positive anti-TPOAb were conjoined with double the

risk of miscarriage (37). Some researcher recommend empirical usage of thyroxin therapy (T4) in women with anti-TPOAb, even though there are no evidence for hypothyrosis (38). Women with present TPOAb will have difficulties getting pregnant in older age, and older women are pruner to pregnancy loss (39). That probably represents general activation of immune system, or it could be because of transplacental transfer of blocking antibodies on thyroid receptors (35). Several studies show that empirical T4 treatment in patients with TPOAb did not improve obstetric outcome (11). It can be assumed that infertile patients that have ATA present can be classified as reproductive syndrome of autoimmune failure (RAFS). Patients with RAFS should have immune examination on blocking antibodies T and B lymphocyte, ANA and APS panel, percentage of NK cells, DQ alfa haplotype and gene mutation on inherited thrombophilia. Thyroid disorder, especially Hashimoto thyroiditis (HT) and PCOS are conjoint. However, mechanism of this conjointment is not very clear. Polymorphism of gene PCOS is connected to fibrillin 3 (FBN3) can be involved in pathogenesis HT and PCOS. Fibrillin impacts on activities transforming factor of beta growth (TGFb). Namely, there has been found lower levels of TGFb on gene FBN3 in HT as well as in PCOS women. In conclusion, HT and PCOS are connected not only in frequency, but in etiology and clinical consequences (40).

Anti-nuclear antibodies (ANA)

ANA are specific class of antibodies that have the abilities to connect to specific structure within cell corn. At the moment ANA are characterized in two main categories; first include DNA and histone antibodies and the second antibodies on extraction of antigen (antibody on Smith's antigen, ribonucloprotein, SSA /Ro, SSB/La, SCL-70, Jo-1) (41). In retrospective studies, ANA are found five times more common in women with unexplained recurrent spontaneous pregnancy loss in comparison to women with successful pregnancy (42). Cubillos et al. found that the incidence of ANAs was 31.8% in patients with a history of miscarriages (110 patients), but only 5.7% in 35 healthy patients with proven fertility and no history of pregnancy loss or autoimmune disease (43).

Celiac disease

Celiac disease is chronic autoimmune enterophathy intercede with gluteus protein antigen from cereal. It follows type IV-Th2 of immune reaction. Prevalence is around 1% in general population while prevalence in infertile women is around 2.7%. Amenorrhea, recurrent miscarriage, prenatal death and early menopause are most common in groups of women with celiac disease. Celiac disease, especially untreated, seems to increase risk of RPL, premature birth, or decreased fetal growth. Beside, bad impact on mother with celiac disease is increased number of Caesarean -sections (44). By mothers with celiac disease autoantibodies conjoin directly on syncytiotrophoblast and prevent activities of trans-glutaminasis in tissue of placenta and compromise function of placenta (45). Significant increscent of common positive HLA-DQ2 /DQ8 haplotype is in women with celiac disease.

Antisperm antibodies (ASA)

Antisperm antibodies (ASA) are rare, but most common connected with hypofunction of ovaries. It is estimated that around 5% of immune infertility is connected with the presence of ASA in men and/or women. Prevalence of ASA in general population is from 0 to 2%. Prevalence of infertile men is significantly higher 7-26%. Risk factors for developing ASA in men are testis torsi, chickenpox, chryptohism, vasectomy and infection of genital tract. In retrospective study 3.2% on infertile couples were positive in cervical mucus on ASA, while 7% of infertile men were positive on ASA. Besides that, 0.4% of semen sample had spermatozoid covered with antibodies (47). In IUI study for treatment of unsolved infertility, it was found that 15% of sample of cervical mucus was positive with ASA. Authors suggest testing of cervical mucus for ASA should be done on patient with unexplained infertility, and intrauterine insemination can be efficient treatment to reach good rate of pregnancies (48).

Inherited Thrombophilias

There are more and more evidence that thrombophililas can affect on the outcome of the pregnancy. Different inherited thrombophilias such as activation of resistance of protein C, factor V

Leiden mutation (and homozygote and heterozygote), mutation of prothromb G20210A, lack of protein S, mutation of methilentethrahidrpholat reductasion (MTHFR) –C677T, hyperhomocistemie, or combination of above mentioned disorders are also connected with miscarriages in different states of pregnancies (49.50). Inherited thrombophilias cover group of genetic disorders of blood thickening, which brings to abnormal formation to blood clot (thromb). These disorders are shown in several studies as a cause of vascular complications which lead to spontaneous miscarriage, intrauterine fetus death. Preeclampsia (toxemia of pregnancy), and HELLP syndrome, which is a sever shape of preeclampsia characterized with hemolytic, increased level of liver enzyme and thrombocytopenia. Women who carry gene for inherited thrombophilias more commonly (2 to 14 times) have problem with clotting which leads to spontaneous miscarriage, in comparison with normal population (51). In women with RPL testing of Factor V Leiden (F5) and /or prothrombin G20210A (F2) it is necessary to identify candidate for anticoagulant therapy (52). The most common inherited thrombophilias is F5, with prevalence of 10% in white population (52). Mutation of the gene prothrombin (F2) occurs in 7.8% of women who had fetal loss because of blood clotting. Factor II is one of the major factors for blood clotting (52). Gene mutation MTHFR, will lead to accumulation of homocysteine in blood. This condition is called hyperhomocysteine, and the result is in creation of thromb and residue in arteries, even in childhood. Nutritive lack of vitamin B6, B12 and folic acid makes it worse. Women who had homozygote shape of mutation of MTHFR gene (both her allele that have mutation) have more than twice the risk of miscarriage (53).

Allogenic factors

DQ alfa haplotype

HLA antigen are connected with the level of immune response to specific antigen. DQ alfa genetypisation relates to specific type of HLA-II class, and is on the surface of blood cells. More HLA involves (HLA-A,B, DQ and DR) in repeated primary or secondary miscarriage (54). It is spotted that HLA-DQA1 compatibility of fetus with mother can lead to RPL. Researcher discovered that mothers which are HLA-DQA1 and/or DQB1 compatible with their fetus usually have high rate of miscarriage before eight week of pregnancy and progression of autoimmune process such as rheumatoid arthritis and antiphospholipid antibodies, during first trimester of pregnancy (54).

Blocking Antibodies

Early in pregnancy, mother immune system takes signal from fetus. Half of HLA fetus is inherited from mother, and the other half from father. When a woman gets pregnant, her immune system usually recognize father's HLA by fetus, and T and B cells in uterus produce protective, blocking antibodies which protects fetus from mother's uNK. If father's HLA is too similar to mother's, mother's B cells will not recognize the differences which are of vital significance for producing blocking antibodies. Women that have successful pregnancies and no miscarriage have high level of blocking antibodies, while those with RPL have low values of blocking antibodies. Couple with increased HLA and RPL have commonly lack of anti-paternal cytotoxic antibodies (APCA), anti-idiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf) (55).

Natural Killer cells (NK)

Immune system is combined with more than 30 types of white blood cells, including neutrophile, monocyte, lymphocyte and mastocyte. Lymphocyte, especially B-cells (producers of antibodies), T cells (CD4+ and CD8+) and pNK are in the middle of intensive research interest in the discipline ot reproductive immunology. Test of immunophenotypisation is the method for differentiation of these cells in blood. Special class of uterine (uNK) cells (CD3-, CD16-, $CD56^{bright}$) in placenta stimulate cell growth, beam molecules for growth of placenta and regulate mother's immune response on local level in mother/ placenta duel. Cells uNK which are isolated in early stage of pregnancy produce different citocine such as G-CSF, GM-CSF, M-CSF, TNF-alfa, IFN-gama, LIF and IL8. These citocine can play significant role in maintaining normal pregnancy (56). uNK cells have important role in ivastion of trophoblast and angiogenesis and make around 70% of immune cell decidue.

They are less toxic for throphoblast, increase receptor / gene expression of KIR, HLA-C, HLA –E and HLA-G (57). Results of Santillana et al. show that cut-off values >250 uNK cell 7h.p.f received by endometriosis biopsy are relevant and 50% of patients with these values have RIF in relation to control group, and cut-off for pNK is 13.9% (58).

Perifer NK cells (CD3-, CD16+, $CD56^{dim}$), when activated with citocine IL-2, become citotoxic for trophoblast. Same cell beam TNF –alfa which can destroy placenta. Women with pNK cell higher than 12% have risk of miscarriage despite immune treatment (immunization with father's leukocyte, prednisone, aspirin and heparin). In subgroup women that have more IVF failure, it is believed that TNG bens in amounts that unable implantation and early placenta formation. In women with recurrent miscarriage it has been confirmed that they have increased number of pNK cells in 52%. Increased activation can be triggered by semen or trophoblast antigen and can be activated before pregnancy or very early in pregnancy (59).

Regulatory T cells

T cells are characterized by expression of different phenotypes in production of different cytokine. T cells changes can be involved in pathogenesis or recurrent miscarriage. In periphery blood, CD3 level of T cell there is no difference in un-pregnant women with history of RPL in comparison to fertile women (60). However, level of CD3 cells in first trimester of pregnancies in women who recently had miscarriage is significantly lower to women who successfully carried pregnancy (61). RPL in first trimester is combined with CD3+ T cells and increased CD19 + B cells and /or CD56 +NK cells. Increscent in any of these cell populations is connected to pregnancy loss. Earlier it was suggested that successful pregnancy is conjoint with increased Th2 cytokine, and that Th2 cytokine are harmful for pregnancy such as increased ratio TH1/TH2 (62). Result of the study show that increased D4 T cells are present in women with RPL (62). Percentage of Th1 (CD3+ / CD4+ / TNF-alfa +, CD3+ / CD4 + / IFN-y +) and Th2 cell (CD3 + / CD4 + / IL-10 +, CD3 + / CD4 + / IL-4 +) in periphery blood determines in flow citometre**.** There is no significant statistic difference in percentage between Th1 and Th2 in women with RPL and control group, but the ratio Th1: Th2 are significantly higher in women with RPL than in control group (60). Level of suppressive T cells (CD3 + /CD8 +) in serum is not significantly different in women with RPL in correlation with control group (60). Ratio CD3+ / CD8 + / TNF-α through CD3 + / CD8 + / IL-10 cell has been significantly higher in RPL in comparison to normal pregnancy. T cells CD8 (+) 11b (-) were significantly higher in patients with RPL, while T cell CD8 (+) 11b (+) were lower in patients with RPL in comparison to control group (62). Over activation can be consequences of low level of citotoxine CD8+ T cells, which results in increased ration CD4/ CD8 T cells (4).

Results of Stricker et al., (2002) show the most common immunologic abnormality was the presence of antithyroid antibodies (53%), followed by antiphospholipid antibodies (36%), increased natural killer cells greater than 12% of total lymphocytes (35%), antinuclear antibodies (25%), increased IgM level (22%), increased CD4/CD8 T-cell ratio (14%) and antiovarian antibodies (11%). In addition, IgA deficiency was found in two patients, and seven patients had endometriosis. Patients with increased CD4/CD8 T-cell ratios had normal levels of CD4 T-cells but decreased CD8 T-cells (4).

Decidual cytokines

Decidual cytokines are small multifunctional protein, produces leucocite, and function is in first place immunomodulatory action, invasion of trophoblast and ingrowths of spiral arteries of mother in EVT fetus. While their deregulation can play role in etiology of RPL classic helper D4+ T ells are major producers of cytokine in subgroups Th1, Th2, Th17 and T regulatory lymphocyte. Main anti-inflammatory cytokine are: TNF alfa, IL-1, IL-6 and CXCL8 / IL-8, and anti-inflammatory cytokine are IL-10 and TGF-β. IFN-γ is relevant for successful pregnancy, and its key function is making spiral arteries and successful outcome of pregnancy. Decidual macrophage induces M-SF and IL-10, while the lack of IL-10 is connected with miscarriage.

Granulocyte factor of colony stimulation (G-CSF) is stimulating factor of granulopoesis, but it is quickly determine that it affects on expression of synthesis receptors, such as granulocyte-macrofage factor of stimulation of colonies (GM-

CSF), which are in different types of tissues, including reproductive cells. TNF-alfa as product of TH1 cells in increased in patients with RIF and RM, and blocks expression of G-CSF and negatively impacts on implantation of embryos. Function of G-CSF in reproduction is made through different mechanism:

- G-CSF induces embryo development, implantation and growth of trophoblast.
- G-CSF Inhibits NK cell activities and induce Th2 answer.
- G-CSF makes it useful in fixing implantation failure (RIF).
- G-CSF is useful for certain patients with RM.

Reduced decidual IL-6 and CXCL8 /IL-8, as well as increased TNF-α level can negatively impact on normal process in maintaining pregnancy by deregulation control of trophoblast invasion, as well as remodeling of spiral arteries and therefore contribute to etiology of early and late miscarriages.

Treatment of patients with RPL and RIF

Treatment of inherited thrombophilia

Combination of low dosage of aspirin and heparin of low molecule mass (NHM) is used to treating inherited thrombophilia, Treatments starts before pregnancy, and continues four to six week after the birth. Folic acid is given to patients with mutation of MTHFR gene.

Treatment of Antiphospholipid syndrome **APS** is treated with low dosage of aspirin and NMH. Heparin is large molecule and it does not go through placenta. Aspirin can penetrate through placenta, but the dosage that is used is so small that it has no influence on fetus. Impact of treatment is larger when administered before and continues during the pregnancy.

Treatment of patients with Anti-nuclear antibodies

Women with ANA and RPL are treated with prednisone. Prednisone does not penetrate through placenta so the fetus is exposed to the drug only in traces. Prednisone should start before pregnancy in dosage 2x10 mg/day.

Treatment of patients with alloimmune causes

Include immunization with father's leukocyte and /or intravenous application of immunoglobulin IVIG (66). Several studies show significant usage of IVIG treatment in patients with RIF that were preparing for IVF. Standard dosage is used (400-500mg/kg) or low dosage (200-250mg/kg) IVIG (63, 65).

Table 2. Laboratory testing for diagnosis in recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF).

Antiphospholipid antibodies (aPL)(panel)	Inherited Thrombophilias (panel)
Anticardiolipin antibodies (aCL) - IgG, IgM, IgA	Fibrinogen, Factor VIII
Antiphosphoglycerol antibodies - IgG, IgM, IgA	Factor IX, Factor XI, Factor XIII
Antiphosphoserin antibody - IgG, IgM, IgA	Homocysteine Factor V Leiden (F V)
Antiphosphoethanolamin antibody - IgG, IgM, IgA	Prothrombin G20210A allele (F II)
Lupus antikoagulans (LA)	Protein C, Protein S, Antithrombin III
Antiphosphoinositol antibody - IgG, IgM, IgA	5,10 MTHFR gene C677T mutations homozygosity
Actived partial thromboplastin tyme (aPTT)	Activated Protein C resistance (APC)
VDRL	PAI-1 heterozygous 4G/5G
Anti-prothrombin antibodies	**Allogenic factors**
Anticardiolipin/anti-DNA antibodies from SLE/APS	Haplotype: HLA-DQ2/DQ8 HLA-DQB1, HLA-DQB1
Anti-β2 glycoprotein-I antibody of IgG and/or IgM	Blocking Antibodies: T cell IgG, B cell IgG
Antinuclear Antibody Panel	T cell ratio CD4/CD8, pNK %
ANA titer, dsDNA, SSA, SSB, RNP, SM,	Th1: (CD3 + / CD4 +) TNF-alfa: IL-10
Antihistone Antibody	Th2 : (CD3 + / CD4 +) IFN-y: IL-10

Treatment with G-CSF (Neupogen))

Patients with RIF that have transfer of good quality G-CSF starts a role in process of oocyte and sperm maturation endometrial receptivity, implantation, embryo and fetal development. Result of newer studies show that after the therapy of G-CSF treatment there has been a statistically higher pregnancy rate and reduced statistic rate of miscarriage (67).

Role of mechanic damage in RIF

Mechanic damage of endometrial (biopsy / scratch or hysteroscopy) in cycles that precede ovary stimulation for IVF is suggested for improvement of implantation in women with RIF. Large control studies (four random and tree unrandom) have shown that local injury of endometrial induced in cycles that precede the stimulation of ovaried give 70% more possibilities to resolve in clinical pregnancies.

Conclusion

- RPL and RIF involves wide spectra of immune disorder connected to recurrent failure of natural or IVF induces pregnancy.
- RPL is defined as three or more consecutive spontaneous abortions.
- RIF is defined if there has not been any clinical pregnancies after three embryo transfer of good quality (grades A or B).
- In immune cause of RPL and RIF are included also autoimmune and alloimmune factors.
- Primary and secondary tromhbophilia is treated with combination of low dosage of aspirin and NHM.
- By secondary APS with present ANA with low dosage of aspirin and NHM we add prednizolon.
- IVIG treatment is safe and efficient in older women with alloimmune disorder. Treatment should begin before implantation in dosage of 30 mg and after that every 21 day administrates 20 mg all the way till the end of second trimester.
- Treatment with G-CSF is useful with patients with RIF and RPL and dosage is 1ug/kg/ day (Neupogen), and first dose is administered 6 days after ovulation or on the day of embryo transfer (ET), and after that continuous once a week till 12th week of gestation.

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Corresponding Author
Elmira Hajder,
PHI Institute of Human Reproduction,
Obstetrics and Perinatal Medicine "Dr. Hajder",
Tuzla,
Bosnia and Herzegovina.
E-mail: ensarh@bih.net.ba

Knowledge and attitudes of senior midwifery students towards medical malpractice

Birsen Karaca Saydam, Emine Serap Sarican, Gulbiye Dinc, Dilek Mamik Aktay, Aytul Hadimli

Department of Midwifery, Ege University Izmir Ataturk School of Health, Izmir, Turkey

Abstract

Objective: To evaluate knowledge and attitudes of senior midwifery students towards medical malpractice.

Methods: A total of 233 senior midwifery students (mean(SD) age: 22.7(1.2) years) were included in this descriptive questionnaire-based study conducted at School of Health/Faculty of Health Sciences from five universities in Turkey during 2012-2013 academic year. A 30-item questionnaire form was applied via face-to-face interview that composed socio-demographic items and items on knowledge and attitudes towards medical malpractice.

Findings: Medical malpractice was defined accurately by 85.0% of students, while erroneous medication (37.8%) was stated the most common type of malpractice. Overall, 88.8% of students were aware of medical malpractice law and 81.1% of the students stated that they will report a witnessed or suspected malpractice. Education was stated as the most likely measure to minimize the likelihood of malpractice (36.5%), while practicing in accordance with duty, authority and responsibilities of the profession was considered to be effective in preventing malpractice only by 10.7% of students. Similar rates identified for most likely responsible healthcare personnel (p=0.449), most common form of malpractice (p=0.381), awareness of law (p=0.083) and likelihood of reporting a witnessed or suspected malpractice (p=0.449) among students with versus without accurate definition of malpractice.

Conclusions: In conclusion, our findings revealed that majority of students had adequate knowledge of malpractice and aware of medical malpractice law. Although the theoretical background of our students regarding medical malpractice was at sufficient level, this seems not to be reflected in their attitudes and behavior towards malpractice.

Key words: Midwifery, students, medical malpractice, knowledge; attitude; education; legal

Highlights

- Majority of senior midwifery students had adequate knowledge of malpractice
- Attitudes and behavior toward malpractice should be improved
- Awareness of moral and legal issues regarding midwives' job description is crucial

Introduction

Medical malpractice refers to any act or omission during treatment of a patient that deviates from standard norms of medical practice and causes an injury to the patient and categorized as imprudence (a condition which leads to a complication due to carelessness), negligence (ignoring one's duty due to carelessness), lack of related skills (lack of needed scientific and technical qualifications to manage an activity) and disobedience of governmental regulations[1, 2, 3, 4].

In line with developments in medical standards of care increasing expectations from the medical profession along with improved public education and awareness, there is a growing increase in the number of claims and lawsuits for medical malpractice throughout the world as well as in Turkey[2, 5, 6, 7].

Surgery branches, general surgery and gynecology in particular, are more frequently involved in malpractice cases consistent with the fact that they require the performance of skill, equipment and team service at maximum level[8, 9, 10, 11].

Obstetrics and gynecology refers to a special area of medical practice that considers both maternal and fetal well-being, while malpractice cases are more commonly encountered in this field given the higher likelihood of complications[12, 13, 14]. Although

most malpractice claims and lawsuits occur against obstetricians and gynecologists[2, 5], midwives and nurses also carry a large risk of being accused for medical malpractice due to their active role in pregnancy, natal, and postnatal period[15, 16].

The specific position of midwifery in patients care service results from the fact that it is the first contact point between mothers and medical staff undertaking special responsibility of being directly in charge for looking after mother and embryo[17, 4].

There is no particular law regarding medical malpractices or malpractice in Turkey, yet medical practices and responsibilities are defined and regulated with the Law no 1219 on Performing Medicine and Medical Sciences, Regulation on Medical Deontology, Law no 2238 on Organ and Tissue Explanation Preservation and Implementation, Law on Private Hospitals, Regulation on Patient Rights, Business Ethics Rules of Medicine, law of Criminal Procedure, Turkish Penal Code (TCK) (TCK articles 83, 85, and 89) and several other regulations regarding the introduction of concepts such as 'eventual intent' (article 21, paragraph 1) and 'conscious negligence' (article 22, paragraph 3)10,[18,19,20]. Appealing to the High Council of Health is obligatory during the process of malpractice criminal suits in our country, while in addition to penalty responsibility; claim for damages can be filed against the healthcare personnel within the scope of the Law of Obligations and Civil Law[14, 19, 21]. Notably, data from analysis of malpractice cases appealed to Forensics Medicine Association's First, Second, Third, and Fifth Specialization Divisions or High Council of Health in past studies in Turkey revealed that most of the cases concluded to be malpractice were in the branch of gynecology and obstetrics and committed mostly by physicians as followed by midwives[2, 6, 9, 16].

The present study was therefore designed to determine knowledge and attitudes of senior midwifery students towards medical malpractice.

Methods

Study population

A total of 233 senior midwifery students (mean(SD) age: 22.7(1.2) years) were included in this descriptive questionnaire-based study conducted at School of Health/Faculty of Health Sciences from five universities in Turkey during 2012-2013 academic year. Senior students who were at school on the day of the survey was included based on their voluntary participation. Study centers were selected based on geographical distribution randomly (by drawing lots) amongst 32 universities with School of Health/Faculty of Health Sciences across Turkey to include Aegean region (Ege University İzmir Atatürk School of Health, n=69), Marmara region (Kocaeli University School of Health, n=63), Mediterranean region (Cukurova University School of Health, n=65) and Eastern Anatolia region (Atatürk University Faculty of Health Sciences, n=36) to represent geographical variability and entire population of midwifery students. No response was received from the Department of Midwifery (Nigde University Zubeyde Hanim School of Health) selected to represent Central Anatolia region.

An official letter was sent to schools regarding the conduction of the research, and written informed consent was obtained from each department and subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Research Committee of Ege University Faculty of Medicine. The survey form was sent via e-mail to each department and completed forms were asked to be returned to principal study center (Department of Midwifery, Ege University İzmir Atatürk School of Health) via mail.

Questionnaire data

A 25-item questionnaire form was applied via face-to-face interview to each student under the supervision of faculty members and composed socio-demographic items (n=7; age, health insurance, economic status and factors related to choice of midwifery profession) and items on knowledge and attitudes towards medical malpractice (n=18; definition and forms of medical malpractice, responsible healthcare personnel, legislations regarding medical malpractice, legal and medical consequences of malpractice regarding responsible healthcare personnel and patients, personal attitude towards reporting a witnessed or suspected malpractice and possible measures to minimize the likelihood of medical malpractice.

Statistical analysis

Statistical analysis was made using IBM Statistical Package for Social Science (SPSS) Statistics (IBM Corp. Released 2012, IBM SPSS Statistics for Windows, version 21.0. Armonk, NY). Questionnaire data were analyzed via descriptive statistics, while Chi-square (χ^2) test was used to compare attitudes towards malpractice according to knowledge of malpractice. Data were expressed as "mean (standard deviation; SD)" and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

Findings

Sociodemographic characteristics

Sociodemographic characteristics of midwifery students are summarized in Table 1. Most of students had moderate economic status (71.2%), 58.4% of students identified that they "willingly" preferred the department of midwifery, 57.0% considered midwifery as a carrier that suits their personality and 42.5% stated that they would prefer the same profession again (Table 1).

Table 1. Sociodemographic characteristics of midwifery students

Age (year), mean(SD)	22.7 (1.2)
Economic status of your family	
High	56 (24.0)
Moderate	166 (71.2)
Low	11 (4.7)
Health insurance	205 (88.0)
Choice of midwifery profession	
Willingly	136 (58.4)
Unwillingly	97 (41.6)
Would you choose midwifery again	
Definitely	99 (42.5)
Never	36 (15.4)
Not sure	98 (42.1)
Midwifery is a career that suits my personality	
No	100 (43.0)
Yes	133 (57.0)

Knowledge and attitudes of midwifery students on medical malpractice and related legislations

Medical malpractice was defined accurately by 85.0% of students. Medical malpractice was identified to be committed mostly by physicians (%37.3) and nurses (30.5%), while the erroneous medication (37.8%), overlooking symptoms and clinical findings (26.2%), and insufficient diagnostic work-up (15.9%) were noted as the three most common forms of medical malpractice. While 88.8% of students were aware of medical malpractice law, 73.4% of the students stated that the law does not protect midwives regarding medical malpractices. A punitive fine or imprisonment was identified by 31.8% of students as the criminal sanction against the person who commits malpractice. In total, 81.1% of the students stated that they will report a witnessed or suspected malpractice (Table 2).

Students' view on consequences of medical malpractice and possible preventive measures

Morbidity/mortality (32.2%) and decreased level of trust in healthcare system (38.6%) were the most commonly identified patient-related and social consequences of medical malpractice, respectively. Medical malpractice was stated to be related the increased violence against healthcare professionals by 57.1% of students (Table 3). Education was stated as the most likely measure to minimize the likelihood of malpractice (36.5%), while practicing in accordance with duty, authority and responsibilities of the profession was considered to be effective in preventing malpractice only by 10.7% of students. Only half (48.9%) of students considered graduate or post-graduate vocational education to be sufficient avoid malpractice (Table 3).

Attitudes towards malpractice according to knowledge of malpractice

No significant difference was noted in attitudes towards medical malpractice with respect to accurately knowing the malpractice definition with similar rates identified for most likely responsible healthcare personnel ($p=0.449$), most common form of malpractice ($p=0.381$), awareness of law ($p=0.083$) and likelihood of reporting a witnessed or suspected malpractice ($p=0.449$) among students with versus without accurate definition of malpractice (Table 4).

Table 2. Knowledge and attitudes towards medical malpractice and legislations

Definition of medical malpractice	**n(%)**
Accurate	198(85.0)
Wrong/ not know	35(15.1)
Committed mostly by	
Physicians	87(37.3)
Nurses	71(30.5)
Midwifes	12(5.2)
Laboratory staff	6(2.6)
Any healthcare personnel	46(19.7)
No idea	11(4.8)
Most common types	
Erroneous medication	88(37.8)
Overlooking symptoms and clinical findings	61(26.2)
Misdiagnosis due to insufficient diagnostic work-up	37(15.9)
Wrong-side surgery	25(10.7)
Insufficient patient information on discharge	15(6.4)
Bedside injuries (i.e. accidental falling) or insufficient nutrition	7(3.1)
Awareness about medical malpractice law	
Yes	207(88.8)
Not know	26(11.2)
Law protects the rights of midwives regarding medical practice	
No	171 (73.4)
Yes	62 (26.6)
Criminal sanctions against medical personnel	
A fine or imprisonment	74(31.8)
Suspension or dismissal	57(24.5)
Giving testimony	29(12.4)
Issue warning	10(4.3)
No idea	63(27.0)
Reporting a witnessed or suspected malpractice	
Yes, I do	189(81.1)
No, I don't	16(6.9)
No idea	28(12.0)

Discussion

Our findings in a cohort of senior midwifery students from universities located at four geographical regions across Turkey revealed that majority of students had adequate knowledge of malpractice (85.0%) and were aware of medical malpractice law (88.8%) and would report a witnessed or suspected malpractice (81.1%). Malpractice was identified to be committed mostly by physicians and nurses, to occur mostly due to erroneous medication and associated with patient morbidity/mortality and decreased level of trust in healthcare system, as well as a punitive fine or imprisonment as the criminal sanction against the responsible healthcare person. Education was stated as the most likely measure to minimize the likelihood of malpractice (36.5%), while practicing in accordance with duty, authority and responsibilities of the profession was considered to be effective in preventing malpractice only by 10.7% of students.

Surgery branches, gynecology and general surgery and in particular, are more frequently involved in malpractice cases consistent with the fact that they require the performance of skill, equipment, and team service at a maximum level[8,9,10,11].

Analysis of 707 medical practice error cases appealed to the 3rd Forensics Specialization Board in 2009 by Sanyuz et al. (2009) revealed that while

Table 3. Students' view on consequences of medical malpractice and preventive measures

Consequences on patients	**n(%)**
Morbidity-mortality	75(32.2)
Disease related complications	30(12.8)
Feeling of rage or distrust	26(11.2)
Prolongation of recovery	25(10.7)
No idea	65(27.9)
Other	12(5.2)
Social consequences	
Decreased level of trust in healthcare system	90(38.6)
Deterioration of health index	33(14.2)
Economic loss, decreased productivity, exclusion	15(6.5)
No idea	95(40.7)
Medical malpractice and increased violence against healthcare professionals	
Related	133(57.1)
Not related	30(12.9)
No idea	70(30.0)
Measures likely to minimize malpractice	
Education	85(36.5)
Working carefully	49(21.0)
Practicing in accordance with duty, authority and responsibilities of the profession	25(10.7)
Missing data	74(31.8)
Sufficiency of graduate or post-graduate education to avoid malpractice	
Sufficient	114 (48.9)
Not sufficient	119 (51.1)

Table 4. Attitudes towards malpractice according to knowledge of malpractice

	Defined malpractice		**p value**
	Accurately (n=198)	**Wrong or not know (n=35)**	
Committed mostly by			
Physicians	76(40.0)	11(32.4)	0.449
Other healthcare personnel[a]	114(60.0)	23(67.6)	
Not included[b]	8	1	
Most common types			
Erroneous medication or misdiagnosis	95(48.2)	18(51.4)	0.381
Negligence of disease findings	86(43.7)	12(34.3)	
Insufficient patient information on discharge	16(8.1)	5(14.3)	
Not included[b]	1	-	
Awareness about medical malpractice law			
Yes	179(90.4)	28(80.0)	0.083
Not know	19(9.6)	7(20.0)	
Reporting a witnessed or suspected malpractice			
Yes, I do	165(92.7)	24(88.9)	0.449
No, I don't	13(7.3)	3(11.1)	
Not included[b]	12	8	

Data are shown as n(%). [a]Nurses, midwifes, laboratory staff. [b]Data from students who marked "I have no idea" option were not included in the analysis. χ^2 test.

no evidence of malpractice was noted in 73.0% of the cases, amongst the remaining cases that concluded to be malpractice, most were in the branch of gynecology and obstetrics.

Data from retrospective analysis of approximately 680,000 files evaluated by the law courts and public defenders in the Forensic Medicine Association's First, Second, Third, and Fifth Specialization Divisions between the years 1990 and 2000 in Turkey by Buken et al. (2004) revealed that of 636 cases of medical malpractice, most (16.82%) were in the area of obstetrics and gynecology which increased to comprise 58% of cases from 1998 to 2000.

In a past study by Pakis et al. (2009) on autopsy profiles of 525 death cases with a medical malpractice claim in Turkey issued by the 3rd Specialization Board of Council of Forensic Medicine, it was reported that 167 of 525 cases were concluded to be medical malpractice, while most of them occurred in gynecology and obstetrics clinics (22.2%), committed by physicians (96.4%) followed by midwives (7.2%) and associated with failure to apply a treatment or mistreatment in most of cases.

In a retrospective analysis of 30 medical malpractice case submitted to jurisdiction and sentenced by the High Court of Turkey between 1978 and 2006 in Turkey by Can et al. (2011), erroneous treatment (47.0%), as followed by lack of care and attention (33.0%), negligence (13.0%) and misdiagnosis (7.0%) respectively, were concluded to be the most common causes of liabilities by judgement**.** Authors also analyzed the consequences of malpractice on patients and concluded that mortality was evident in 53.0% and morbidity in 47.0% of the cases[10].

In a descriptive-cross sectional study on causes of medical errors carried out in a single center over a period of 10 months by Jahromi et al. (2014), erroneous medication (87 out of 152 errors) was reported to be the most commonly encountered malpractice. Data from other studies also revealed failure to apply a treatment or mistreatment to be the most common cause of malpractice as followed by misdiagnosis and mistreatment6, [22,23,42].

Consistent with these findings, erroneous medication, overlooking symptoms and clinical findings, and insufficient diagnostic work-up were identified as the three most common forms of medical malpractice and were stated to be associated with the likelihood of patient morbidity/mortality by our students.

In accordance with high rate of young population in our country, the largest young population with 16.6 % compared to European countries (Turkish Statistical Institute, 2014)[24] and thus a need to invest more in schools (Demography and Labor Force, 2016)[25], significant proportion of students prefer to receive university education. Midwifery and Nursing was ranked fifth-sixth among graduate education preferences with ease of finding a job after graduation at a rate of 35.1% [26]. Accordingly, half of students in our cohort stated that they "willingly" preferred the profession of midwifery and would prefer the same profession again.

Such increase in the interest and demand for the profession should bring about the increase in the quality of education in parallel and all requirements must be met for this purpose. Given that gynecology and obstetrics has a high risk of medical malpractices in which midwives take active role in pregnancy and labor as required by their education[9], education of professional healthcare professions such as Nursing/Midwifery requires special rigor to reduce erroneous practices that may occur in patient care to a minimum. Accordingly, in our cohort education was stated as the most likely measure to minimize the likelihood of malpractice, while graduate or post-graduate midwifery education was considered to be adequate in this respect only by half of students. Similarly, in a past study on the knowledge, attitude, and behavior of physicians towards medical practice by Yildirim et al. (2009), inadequacy of education has been suggested to be the most frequent cause for medical malpractices and thus suggested to be complemented at scientific meetings such as congress/symposiums.

While majority of our students had accurate knowledge of medical malpractice, a non-negligible portion had also no idea on criminal sanctions against medical personnel who committed malpractice (27.0%), whether or not they would report a witnessed or suspected malpractice (12.0%) as well as patient-related (27.9%) or social consequences (40.7%) of medical malpractice. Besides, practicing in accordance with duty, authority and responsibilities of the profession was considered to be effective in preventing malpractice only by 10.7%

of students. Moreover, our analysis revealed that students with versus without a sufficient theoretical background on medical malpractice identified similar attitudes towards medical malpractice.

Hence, while the theoretical background of our students regarding medical malpractice was at sufficient level, this seems not to be reflected in their attitudes and behavior towards malpractice. The mismatch of theoretical background and attitudes towards medical malpractice in our cohort, orientates us to critically evaluate and modify the current education system via shifting it from the step of knowledge that represents the bottom step of the cognitive area that only requires memorization towards the steps of practice, analysis, synthesis and assessment[27].

Midwifery malpractice is amongst the common causes of complaints made by women in pregnancy services[17, 28]. Notably, disobedience of governmental regulations such as illegal abortions or administration of the procedures out of midwives' job description, rather than negligence and imprudence have been indicated as the most common type of malpractices committed by midwives[4, 17]. Likewise, retrospective analysis of cases evaluated by law courts and public defenders in the Forensic Medicine Association's First, Second, Third, and Fifth Specialization Divisions between the years 1990 and 2000 in Turkey by Buken et al. (2004) revealed that in 107 cases concluded to be medical malpractice there were 10 midwives and nurses accused and mainly due to notifying a physician about a case, delivering a risky birth at home, not monitoring progress of delivery carefully, leaving the patient during birth.

In a past study on litigation among midwives by Hood et al. (2010)[29], midwives are considered to be naïve about legal processes and to be unprepared and ill-equipped to deal with the consequences of working in an environment with high profile legal proceedings and thus to describe their work environment as becoming increasingly stressful since they experience fear of litigation.

Not only midwives, but most of the healthcare professionals do not have adequate information on laws, legislations, and regulations concerning themselves and their practices. Given that according to the article 44 of Turkish Penal Code, not knowing the law is not an excuse, it is important that healthcare professional must be aware of the circumstances that increase the risks of medical malpractice to be able to take necessary preventive measures[13].

Notably, practicing in accordance with duty, authority and responsibilities of the profession was considered to be effective in preventing malpractice only by 10.7% of our students, while 73.4% stated that the law does not protect midwives regarding medical malpractices. Hence it seems necessary for midwives not only to be properly trained to achieve improved skills but also to be aware of moral and legal issues regarding their implementation of technological advances in obstetric interventions and to learn their responsibilities included in the midwives' job description to not go beyond the bounds of their authority or competency and thus to minimize the risk of litigation[2, 17, 30, 31, 32].

In a recent analysis of closed claims of medical malpractice lawsuits involving midwives by McCool et al. (2015)[33], recommendations for improving clinical practice and avoiding litigation included the need for thorough and accurate documentation in practice, appropriate and timely consultation and collaboration, and having clinical skills matching the level of needed care.

In another study on the analysis cases of the Belgian, French and Dutch midwife on trial by Eggermont (2015)[34], juridical recommendations made for the midwife to avoid liability included achieving good skills and continuing lifelong education in assessing the necessity for and interpreting continuous fetal heart rate monitoring, immediately referring to an obstetrician in pathologic cases without any hesitation, working in a team with the obstetrician and other health-care professionals to provide quality care, practicing with good knowledge of the woman's medical history and update the record with her observations and actions and finally consciously choosing the appropriate type of medical intervention in urgent cases to prevent worse effects than doing nothing.

There has been a serious increase in the violence emerging in the field of healthcare in the recent years, while the factors such as negligence in medical intervention, dissatisfaction with treatment, insufficiency of the institution where the treatment is given, publications in the media, false

demands (such as report, medicine), and health policies have been suggested to be associated with the violence against the physician and healthcare professionals[35, 36, 37, 38, 39, 40]. In this regard, it should be noted that medical malpractice was stated to be related the increased violence against healthcare professionals by 57.1% of our students, while 73.4% stated that the law does not protect midwives regarding medical malpractices.

Certain limitations to this study should be considered. First, albeit it was planned to include senior students from the departments of midwifery of the universities from five regions selected by drawing lots, no data could be retrieved from central Anatolia. Besides, according to the Higher Education Statistics of the Republic of Turkey Student Selection and Placement Center (OSYM), the number of midwives graduated in 2012-2013 academic year is 1393 (Basara et al., 2013)[41]. In this case, only 16.7% of the population has been reached. Thus, relatively low sample size might prevent us to project results of the present study to the entire population. Nevertheless, despite these certain limitations, given the paucity of research available on this area in Turkey, our findings represent a valuable contribution to the literature.

In conclusion, our findings in a cohort of senior midwifery students revealed that majority of students had adequate knowledge of malpractice and aware of medical malpractice law. Although the theoretical background of our students regarding medical malpractice was at sufficient level, this seems not to be reflected in their attitudes and behavior toward malpractice along with no significant difference between students with versus without a sufficient theoretical background in terms of attitudes towards medical malpractice. Hence our findings emphasize that graduate midwifery education should target not only on gaining knowledge but also incorporate practice, analysis and synthesis steps of cognitive process as well as the awareness of moral and legal issues regarding responsibilities included in the midwives' job description. In order to reduce medical malpractices to a minimum, courses such as Forensics, Deontology, and Health Policies that aim at learning healthcare laws and regulations, ethical and legal dimension efficiently must be offered in all midwifery schools along with implementation of a life-long education via organizing in-service training programs and scientific organizations such as congress and symposiums.

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Corresponding Author
Birsen Karaca Saydam,
Department of Midwifery,
Ege University Faculty of Health Sciences,
Bornova,
Izmir,
Turkey,
E-mail: birsen.saydam@ege.edu.tr

Workplace Bullying and Health in Sub-Saharan Africa. What Do We Know?: A Descriptive Review

Gloria Macassa[1, 2]

[1] Department of Occupational and Public Health Sciences, University of Gävle, Sweden,
[2] Department of Health Sciences, Mid-Sweden University, Sweden.

Abstract

Workplace bullying is a growing public health problem worldwide. However research has mostly been carried out in developed countries. To date, research on the prevalence, nature and health consequences of workplace bullying is still a neglected area in sub-Saharan Africa. Thus, this descriptive review aimed to highlight the unfolding public health problem by reviewing studies that researched workplace bullying in sub-Saharan Africa. Results showed that workplace bullying exists in Sub-Saharan Africa across a variety of workplaces and professions. However there is still a dearth of studies addressing the prevalence and health consequences of workplace bullying, especially psychological health. More research is warranted regarding the psychological health impact of workplace bullying within the economic, social and cultural context of Sub-Saharan Africa.

Key words: Workplace bullying, Sub-Saharan Africa, health-consequences

Introduction

Workplace bullying has attracted research worldwide in recent decades and has been seen as a growing public health problem (1, 2). Although a matter of discussion still, many scholars agree that "Workplace bullying refers to a series of negative behaviours carried out frequently and over a prolonged period of time, usually against an individual employee by his or her colleagues or superior (3). Examples of such negative behaviours are as follows: excessive criticism of one's work; withholding of information, which affects performance; being assigned an unmanageable workload; spreading of rumours; and social isolation. Bullying is an escalating process in the course of which the person confronted ends up in an inferior position and becomes the target of systematic negative social acts (3).

Bullying bring tremendous consequences to the targeted as well to society in general (4). Research suggests that the majority of workers will be exposed to workplace bullying at some stage in their careers directly as targets or indirectly as by standards (3). Previous research also suggests a varying prevalence across different types of organizations, with some professions facing grate risk of exposure than others (4).

Prevalence, risk factors and health consequences of workplace bullying

The prevalence of workplace bullying worldwide varies according to the variability of accurate data from different countries and continents. In this, data availability has been the main reason for lack of accurate estimates. However the 2011 Monster Global poll conducted worldwide to a sample of 16517 found that overall 64 per cent reported being bullied, physically hurt or had their work performance affected while 36 per cent experienced bullying and 16 per cent saw it happen to someone else (5). Across the different continents 83 per cent of Europeans respondents reported to have been bullied as compared to 65 per cent in the Americas and 55 per cent in the Asian continent (5-7). Also workplace bullying varies across types of organization (or industries) with some professions being exposed to greater risks of bullying than others (7-8).

Overall, the literature suggests that bullying is a complex phenomenon as it has a variety of risk factors which usually interact with each other to trigger its occurrence. These factors include individual factors, social interaction, group dynamics, working environment, organizational, social and political contexts (9). In regard to individual factors evidence points to age, seniority and gen-

der as factors that increase the chance of being a victim or bully. For instance younger and older ages were found to be important (9-11), but results have been inconclusive regarding gender effects (10). On the other hand organizational factors related to workplace bullying include job stability, human resources practice, job design and type of occupation. For instance evidence suggests that the prevalence of bullying across organizations can reach 44 per cent (12) and that the occurrence is frequent among restaurant employees, teachers, business and university and health care professions, transportation workers and police (12). Also evidence shows that managers can be affected by workplace bullying (12,13). In relation to contextual factors, it is argued that some types of organizations enable increased bullying (e.g. health care sector) due to likely overload and specific internal organization system (14).

According to the European Agency for Safety and health at work, workplace bullying is an emerging psychosocial risk that negatively impact worker's health and well-being (15). For instance in a review of cross-sectional and longitudinal evidence, Verkuil and colleagues found a consistent and bi-directional association between workplace bullying and mental health outcomes such as anxiety, PTSD and other stress-related psychological complaints which could continue in an endless vicious cycle (16). Kivimäki et al study found that victims of bullying were at great risk of cardiovascular disease (17). Furthermore others studies point that exposure to workplace bullying is related to musculoskeletal disorders (MSDs) such as low back, upper back and neck , headache, backache, neckache, hand and foot pain (15,18); excessive alcohol use, drug use and sleep problems (19). Other consequences being decreased motivation among affected employees, lower productivity, high health costs as well as loss of human resources and for societies in general (19,20). Workplace bullying and its health consequences has been studied extensively in developed countries (3, 19, 21-26), but little research on the subject has been carried out in developing countries and specifically in Sub-Saharan Africa.

Aim

The aim of this study was to review studies that workplace bullying and its health consequences in sub-Saharan Africa.

Methods

This descriptive review was structured according to PRISMA guidelines and searches were performed using Medline/PubMed, Scopus and Google Scholar. Key words included workplace bullying in sub-Saharan Africa; prevalence of workplace bullying in sub-Saharan Africa as well as workplace bullying and health outcomes in Sub-Saharan Africa and workplace bullying and psychological health in sub-Saharan Africa. The inclusion criteria was a) studies carried out in sub-Saharan African countries, b) all types of documents such as peer reviewed articles, books and reports written in English with no restriction on year of publication. There was no limitation regarding year of publication.

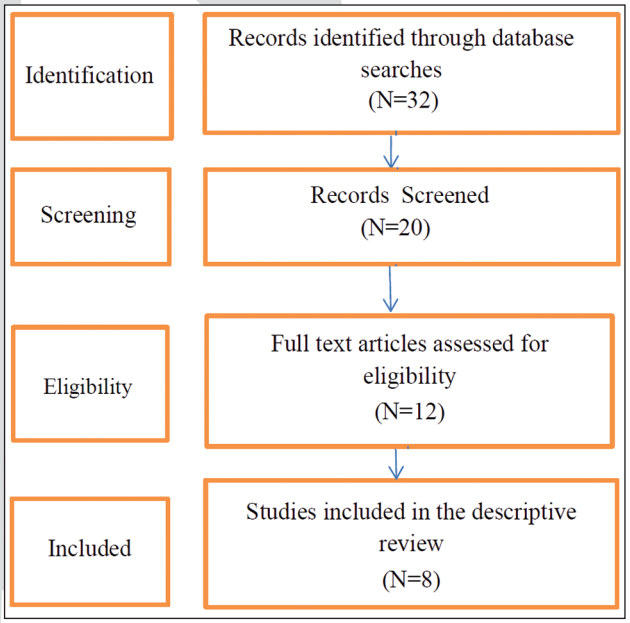

Figure 1. Flow diagram for review selection process

The process of the review started with abstract reading and exclusion of those who were not related to the study objectives. Letters and editorials were also excluded. In the first stage, the articles selected for review were considered for backward/forward assessment of their references and citations. In the next stage the full articles were reviewed by the author as well as one invited reviewer to ensure qual-

ity of the included studies. The review and selection process are displayed in Figure 1.

Results

The review found eight studies that have investigated workplace bullying in sub-Saharan Africa. In addition the studies were carried out in across different type's organizations and professions. However, the majority were within the areas of organization/management and only one study (of qualitative nature) carried out in Lesotho explored respondents experiences of health in relation to workplace bullying. Overall results for the different studies are presented in in Table 1.

The reviewed studies found that different types of workers (e.g. manufacturing, industry, teachers, and academic staff and hospital employees)

Table 1. Overview of studies included in the narrative review

Author/Year / Country/reference	Country	Study Design	N (sample)	Main Findings
Motsei et al, 2016 (37)	South Africa	Qualitative study	29 respondents in manufacturing and industry	Respondents experienced that their gender and ethnicity. Contributed for them being bullied in the workplace. Also that bullying in the workplace was influenced by political power shifts and changing identities
Obicci PA 2015 (20)	Uganda	Cross-sectional study	190 respondents of the public sector	62.5% of the respondents reported bullying in the past 6 months. Bullied respondents reported poor work performance
Adebayo et al 2014 (27)	Zimbabwe	Cross-sectional study	526 Teachers	Teachers experienced verbal workplace bullying (e.g. belittling remarks about their work or personal life.
Cunnift et al 2012 (30)	South Africa	Cross-sectional study	13 911 Workers from Mining, financial, government and academic research sectors	31% of respondents reported experiencing bullying in the workplace. Participants with high levels of SOC had low levels of workplace bullying
Fajana et al 2011 (31)	Nigeria	Cross-sectional study	313 human research practitioners	The study found gender differences in workplace bullying patterns. Women were more verbally abused than men
Ikyanyon D, 2013 (32)	Nigeria	Cross sectional	192 hospital employees study	Employees who experienced low workplace bullying had the highest job performance
Ilongo FN 2013 (dissertation thesis) (33)	Lesotho	Qualitative study Phenomenological analysis Participants	20 Academic staff	Bullied participants thought that it was lethal and damaging to their psychological wellness. Participants reported that bullying affected their emotional balance, intellectual stimulation , motivation, self-esteem family life, staff relations and general resilience
Steinman 2003 (35)	South Africa	Mixed method study (quantitative/qualitative study) interview	1014 (61qualitative interviews)	49% of participants reported incidents of verbal abuse, 20.4% mobbing, 22.3% racial harassment and 4.6% sexual harassment Patients and relatives were found to be perpetrators of the violence

reported being bullied in the workplace (27-35). Furthermore, Steinman study found high prevalence of workplace bullying among hospital workers. Regarding the relationship between workplace bullying and health, a study carried out in Lesotho found that academic staffs that were bullied reported experiences of poor psychological well-being which in turn affected their emotional balance and intellectual stimulation (33). Also, bullied personnel expressed that bullying affected their motivation, self-esteem and had negative impact on co-worker relations and family life (33).

Discussion

Results of this review showed that although few, some studies have tried to highlight the complex issue of workplace bullying in sub-Saharan Africa. However, all studies have been within areas of management or industrial relations and none within the area of Public health. This is of greater concern because as mentioned earlier, bullying in the workplace can lead to poor health outcomes, especially within the domain of psychological health (27-35). Psychological outcomes such as continuous stress, anxiety, depression, PSTD and physical outcomes like musculoskeletal disorders can potentially be costly for organizations and society across developing countries in sub-Saharan Africa. Also, companies can face reduced productivity as well as high rates of absenteeism and presenteeism (1-2, 15-17, 36, 37).

In the era of sustainable development as well as a future towards sustainable healthy workplaces, good psychosocial work environment for both employees and managers will be of greater importance. Thus, tackling workplace bullying is of central importance if organizations/ companies are to be more profitable (38).

Workplaces in Sub-Saharan Africa must move from focusing only in the physical safety to also promote protection and promotion of safe psychological environment for workers which in turn will be beneficial for communities and society at large. The concept of health and sustainable organizations must be seen along with employee well-being and profits as a central goal for organizations (38).

It is essential that public health scholars advance studies on the prevalence as well as health consequences of workplace bullying to be able to suggest strategies to tackle this growing problem. Also as suggested by Motsei et al (37), much attention should be paid to the different countries social context and its impact on the complex web of factors affecting the occurrence of bullying across different types of workplaces. On that regard, a study carried out in New Zeeland reported that Maori respondents experienced more bullying than their New Zeeland /European counterparts (39). However the Asian/Indian and Maori reported lower levels of psychologic strain (due to support of their supervisors). In addition, another study which addressed the acceptability of workplace bullying across six countries (and different cultural contexts) found that physically intimidating bullying was less acceptable than bullying related to work globally and within groups culturally similar (40). Furthermore, the study reported that in Asian countries work –related bullying was more acceptable than in Anglo, Sub-Saharan and Latin American Country clusters (40). The authors attributed some of the observed differences to cultural dimensions (such as human orientation, performance orientation and future orientation). Furthermore, the study reported that cultures with high performance orientation found bullying to be more acceptable as compared to those with high future orientation; and cultures with humane orientation was associated with less acceptability of work related bullying (40).

Limitations

The review only included studies that were published in English; therefore it might not have captured all available research in the field. However, it is less likely that this might have influenced the overall findings.

Conclusion

This review found that workplace bullying exists in Sub-Saharan Africa across a variety of workplaces as well across an array of professions. However, except one, the studies fell short to address the health consequences of workplace bullying, especially psychological health.

Findings of this literature inform the need for organizations and industries to acknowledge that

workplace bullying is an important issue to be addressed to make sure that it does not occur in the first place. Also, research is needed, specifically regarding the psychological health impact of workplace bullying within the economic, social and cultural context of Sub-Saharan Africa.

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Corresponding Author
Gloria Macassa,
Department of Occupational and Public Health Sciences,
University of Gävle,
Sweden,
E-mail: glamaa@hig.se

The Incidence of Occult Bacteremia and Serious Bacterial Infection in Children with Fever of Ages 3 months to 3 years

Hasan Yesilagac[1], Hayri Levent Yilmaz[2], Ayca Acikalin[2], Filiz Kibar[2], Deniz Hanta[3], Ozlem Karagun[1], Rana Disel[2], Akkan Avci[4], Betul Gulalp[1]

[1] Dept of Emergency Medicine, Adana Training and Research Center, Baskent University, Turkey,
[2] Dept of Emergency Medicine, School of Medicine, Cukurova University, Turkey,
[3] Newborn Intensive Care. Adana Metro Hospital, Turkey,
[4] Dept of Emergency Medicine. Adana Numune Training and Research Hospital, Turkey,

Abstract

Background: It was to determine the incidence, complications, and etiology of occult bacteremia and serious bacterial infections in Turkish children, ages between 3-36 months in Çukurova region of Turkey with fever of unknown origin.

Methods: The study included children aged 3-36 months that were admitted to Pediatric Emergency Clinic between November 1, 2006 and November 1, 2007 with tympanic temperature of greater than 38.0 ºC and fever of unknown origin. The complete blood count, peripheral smear, and C-reactive protein (CRP), and blood cultures were obtained in a detailed physical examination from patients.

Results: Among the 318 patients included in the study, 10 patients (3.1%) were found to have developed a serious bacterial infection. Occult bacteremia was detected in 4 (1.3%) patients, while pneumonia, urinary tract infection, meningitis and bacterial enteritis were found in 2 (0.6%), 2 (0.6%) patients, 1 (0.3%), and 1 (0.3%) patient, respectively.

S. aureus, S. pneumonia, and group B streptococci were isolated from the blood culture of patients that had occult bacteremia. The patients that had bacteremia were determined to have 11.65 ± 1.41 neutrophils/mm^3, 75% ± 5.8 granulocytes, 8.7% ± 3.0 mature neutrophils, and CRP value was 82.1 ± 31.7 mg/l, all were significantly higher than the values of patients without bacteremia (p = 0.028, 0.032, 0.002, and 0.006, respectively).

Moreover, patients that developed severe bacterial infection had 15.73 ± 2.69 leukocytes / mm^3, 12.31 ± 2.58 neutrophils / mm^3, 78.2 %± 7.7 granulocytes and 5.1% ± 4.7 mature neutrophils, were significantly higher (p 0.044, 0.000, 0.000, and 0.040) than the values of patients without severe bacterial infection.

While toxic granulation and fever lasting for 3-7 days were significant risks for occult bacteremia (p = 0.012, 0.01, and 0.007) and severe bacterial infections (p < 0.001, 0.002, 0.00), the gender, age, and the extent of fever were not significant.

Only 4 out of the 10 patients that developed serious bacterial infection had fever of greater than 39 °C and a leukocyte count of greater than 15.000/mm^3.

Conclusion: Occult bacteremia and serious bacterial infections should be considered in children aged 3-36 months that develop fever of unknown origin. The likelihood of serious bacterial infections increases when leukocytes are greater than 15.000/mm^3, neutrophils are greater than 10,000 / mm^3, or granulocyte percentage is greater than 70%.

Key words: Fever, serious bacterial infection, children, occult bacteremia

Introduction

Fever comprises 15.0% of child health and diseases outpatient visits and 10.0% of emergency department visits. Most of these patients are younger than 3 years old. In most cases, possible causes of the fever are identified after obtaining the history and performing physical examination. Self-limiting viral infections are present in most of the children with fever. However, backwards or occult bacterial infections can lead to serious bacterial infections (SBA) [1]. Torphy and Ray first described the occult bacteremia in a 1970 report of 12 cases[2]. This definition still represents the clinical problem frequently encountered in clinic to-

day. Despite the existing technology and evidence, there is no excellent test to detect all possible occult bacteremia cases. In children, it is difficult to distinguish occult bacteremia from a mild, self-limiting viral infection. Although occult bacteremia could recover spontaneously, in some cases, it can also cause a lot of serious bacterial diseases such as sepsis, pneumonia, osteomyelitis, septic arthritis, purulent pericarditis, and meningitis.

Ever since conjugated H. influenzae type b (Hib) vaccination has become a common practice, Streptococcus pneumoniae has been recorded for 90.0% of all occult bacteremia cases[1]. Haemopilus influenzae, Neisseria meningitidis, Salmonella, and Staphylococciis aureus are among other common etiological factors[3]. Rarely, group B st*reptococci* can also be responsible for occult bacteremia[4].

Physicians that evaluate children with fever often do not have an apparent infection source, and fall into a dilemma about: What kind of lab tests to order; Whether to start the patient on antibiotic treatment; If started, then what type of antibiotics to choose.

The purpose of this study was to determine the incidence and etiology of occult bacteremia and serious bacterial infections in Turkish children, had fever of unknown origin, aged between 3-36 months, residing in the Çukurova region of Turkey. Moreover, we aimed to determine the incidence of serious bacterial infections and their complications.

Materials and Methods

The study was conducted prospectively with the approval of the Cukurova University Clinical Researches Ethics Committee between November 1, 2006 and November 1, 2007 at Pediatric Emergency Medicine Unit of Çukurova University Faculty of Medicine and Pediatric Emergency Clinic of Adana Training and Research Hospital as a fulfillment of residency thesis.

The study group consisted of children between 3-36 months old that were admitted to Pediatric Emergency Clinic with fever of of greater than 38.0 °C (tympanic measurement), with no apparent origin of fever and blood cultures were taken.

According to the approval of Ethics Committee, there was only verbal informed and consent to examine the blood culture from primary parents of the patients. As consent was verbal, the explanation and question was; There was not found any focus defined the reason of fever on all systems physical examination of the patient, if you mentioned your verbal consent, blood culture would be evaluated with a sample of blood to enlighten and manage the treatment.

While obtaining the sample of regular blood tests in Emergency Department as blood count and basic biochemical tests (BUN,Cr, K, ALT). There was not another attempt to take blood sample. All participant consent replies were recorded on study forms of patients manually by the physicians. The ethics committee approved this consent procedure.

There was a total of 43 cases excluded; 27 cases whom their parents didn't give verbal consent and 16 cases had insufficient blood samples in spite of given verbal consent of parents.

Children whose origin of fever was identified while getting a medical history or during physical examination except for acute otitis media, had been immunized within 2-72 hours, received antibiotics in the last 7 days, with immune deficiency and chronic diseases, received immunosuppressive medications, had urinary catheters, had apparent viral disease findings as viral infection that leads to specific skin rash and flu-fever symptoms such as runny nose and sneezing, and had ill or toxic appearance were excluded from the study.

The patients' demographics characteristics and systemic examination findings were recorded. Yale Observation Scale (YOS) was applied.

The children had a good appearance with laughing, well nourished, crying strongly but calming down quickly, with no signs of dehydration, with good peripheral perfusion, YOS = 6 in spite of fever discharged from Emergency Department and followed with phone call at home. Parents were warned in new rashes on the skin, cyanosis of the skin, color changes such as mottling, cooling of hands and feet, disruption in eating behaviors, worsening relationships with family members, reduction of interest/curiosity, seizures, swelling of the fontanelle, continuous restlessness, crying or constant drowsiness, or if the family becomes anxious about the child's condition. The phone follow-ups were conducted every day for 3 days and on 7th, 14th, and 21st days. If the patients' prob-

lem persisted or if the patient's blood cultures were detected as positive the patients were invited to the hospital.

Statistical Analyses

The SPSS for Windows, version 15.0 was used for statistical analysis of the data[5]. The chi-square test was used for the group comparisons of discrete variables, the t-test was used for group comparisons of normally distributed continuous variables, and a Mann-Whitney test was used for group comparisons of non-normally distributed variable. The ROC analysis was used to evaluate the effectiveness of diagnostic tests. A p-value of 0.05 was considered significant.

Results

The study included 318 children that had fever with unknown origin (Figure 1), of which 127 (39.9%) were female, and 191 (60.1%) were male. Based on the medical histories, 307 (96.5%) patients had complete routine immunization, while 11 (3.5%) patients had missing vaccinations or have never been vaccinated. Ninety-eight patients (30.8%) had previously been immunized with the Hib vaccine, while 220 (69.2%) patients had not undergone Hib vaccination.

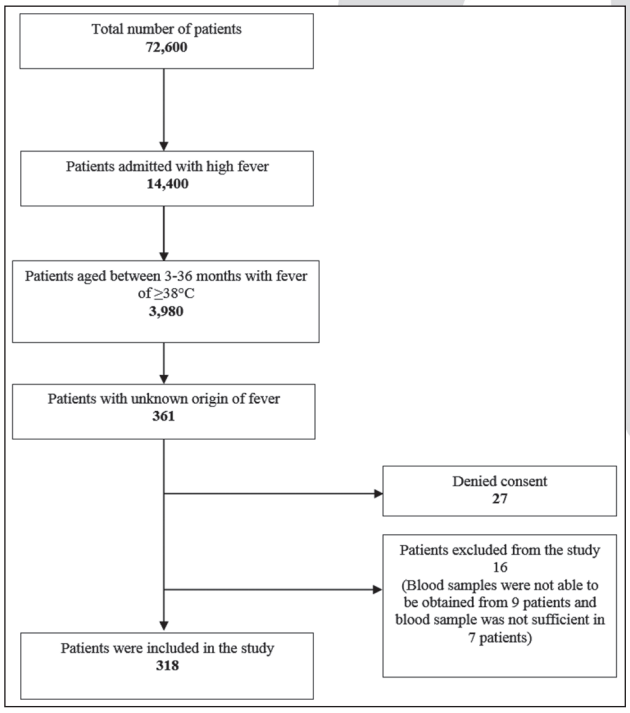

Figure 1. Flow chart illustrating the case selection

In patients with detected and undetected bacteremia, the p-values for gender, vaccines, Hib, 1st, 2nd, 3rd, 7th, 14th, and 21st day fever comparisons were 0.999, 0.132, 0.999, 0.999, 0.999, 0.01, 0.007, and 0.999, respectively.

The difference was detected only in fever at the 3rd and 7th days. While bacteremia was not detected in any of the 210 patients that did not have fever on the 3rd day, it was detected in 4 out of 98 patients (4.1%) who had ongoing fever on the 3rd day. Moreover, bacteremia was detected in 2 out of 297 patients (0.7%) that did not have fever on 7th day, while it was detected in 2 out of 11 patients (18%) that had continuing fever on 7th day.

The comparison of patients with detected bacteremia against patients with no bacteremia in terms of leukocyte count, absolute neutrophil count, CRP, granulocytes percentage and mature neutrophil percentage, and other variables are shown in Table 1.

The follow-ups of 308 out of 318 cases regarding the duration of fever on 1st, 2nd, 3rd, 7th, 14th, and 21st days were reached by phone.

The occult bacteremia was detected in 4 patients of 318 patients (1.3%). Among those 4 patients, S. aureus was the responsible factor in 2 patients (50%), S.Pneumonia in 1 patient (25%), and group B Streptococci in 1 patient (25%).

The serious bacterial infection (SBI) developed in 10 out of 318 patients (3.1%) (Figure 2).

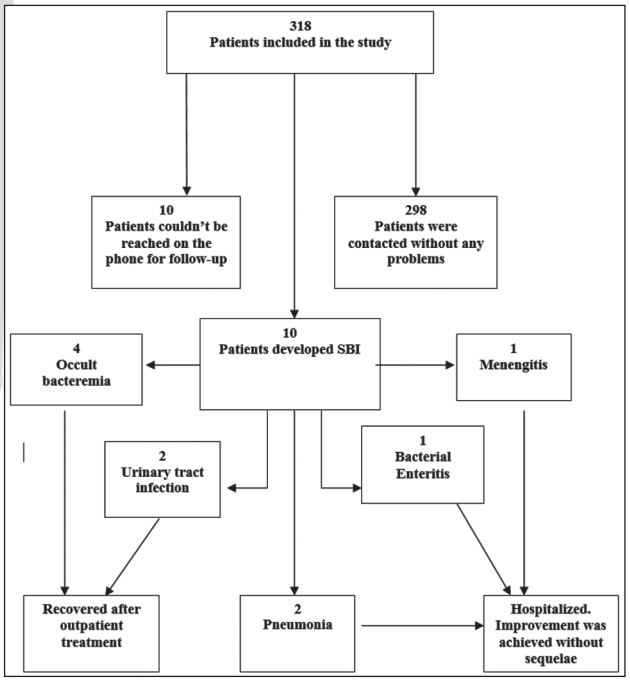

Figure 2. The outcomes of patients

Table 1. The comparison of investigated variables in patients with and without detected bacteremia

Variable	Bacteremia			P
	Absent Mean ± SD median (smallest-biggest value) n = 31 4		**Present** Ort ± SD median (smallest-biggest value) n = 4	
Age (months)	21.2±10.3 22 (3–36)		17.0±5.3 16 (12–24)	0.466
Fever (°c)	38.7±0.5 38.7 (38–40)		38.6±0.3 38.6 (38.4–39)	0.719
Leukocyte count (mm^3)	13.275±5160 12.750 (3500–40310)		15.552±1.656 15.905 (13.300–17.100)	0.219
Absolute neutrophil count (mm^3)	7.628±4.339 7.140 (420–28.210)		11.646±1.405 11.133 (10.640–13.680)	0.028
Granulocyte percentage (%)	56.3±20.1 60 (5–90)		75±5.8 75 (70–80)	0.032
Mature neutrophil percentage (%)	2.5 ± 4.0 0.0 (0–20)		8.7 ± 3 9.0 (5–12)	0.002
Crp (mg/l)	30.4 ± 33 19.8 (3.0–299)		82.1±31.7 81.4 (44.0–121.4)	0.006
Toxic granulation*	Present	211 (% 100)	0 (% 0)	0.012
	Absent	103 (% 96.3)	4 (% 3.7)	

** Due to the absence of numeric value the mean, distribution range ad median were not specified.*

Table 2. The comparison of investigated variables in patients with and without detected SBI

Variable	SBI			P
	Absent Mean ± SD median (smallest- biggest value) **N** = 308		**Present** Mean ± SD median (smallest- biggest value) **N** = 10	
Age (months)	21.2±10.3 22 (3–36)		22.4±9.2 21 (12–36)	0.658
Fever (°c)	38.7±0.5 38,7 (38–40)		38.8±0.5 38.7 (38–39.5)	0.711
Leukocyte count (mm^3)	13.225±5180 12.650 (3.500–40.310)		15.731±2690 16.455 (10.300–19.700)	0.044
Absolute neutrophil count (mm^3)	7.528±4.302 7.002 (420–28.210)		12.305±2.578 11.715 (8240–17.730)	0.000
Granulocyte percentage (%)	55.9 ± 20 60 (5–90)		78.2±7.7 80 (70–90)	0.000
Mature neutrophil percentage (%)	2.5±4.0 0 (0–20)		5.1±4.7 6.5(0–12)	0.040
CRP (mg/l)	30.5±33.1 19.8 (3–299)		48.8±42.1 32.9 (6.1–121.4)	0.171
Toxic granulation*	Present	210 (% 99.6)	1 (% 0.4)	P<0.001
	Absent	98 (% 91.6)	9 (% 8.4)	

** Due to the absence of numeric value the mean, distribution range ad median were not specified.*

The p-values of the comparisons of patients that developed SBI versus the ones that did not develop SBI in terms of gender, vaccination status, Hib, and fever on the 1st, 2nd, 3rd, 7th and 21st days were 0.745, 0.300, 0.999, 0.999, 0.999, 0.002, 0.00, and 0.32, respectively.

A significant difference was only detected in fever on 3rd and 7th days. The SBI developed in 2 out of 210 patients (1%) that did not have fever, and in 8 out of 98 patients (8.2%) that had fever on the 3rd day. Moreover, SBI developed in 3 out of 297 patients (1.0%) that did not have fever and in 7 out of 11 patients (63.4%) that had fever on the 7th day.

The patients that developed the SBI and those who have not were compared in terms age, fever, leukocyte count, absolute neutrophil count, CRP, granulocyte and mature neutrophil percentage is shown in Table 2.

When the granulocyte percentage limit value was set to 70 percent, SBI had not developed in any of the 200 patients with granulocyte percentage of less than 70. Meanwhile, the SBI had developed in 10 out of 118 patients (8.5%) whose granulocyte percentage was greater than 70. The threshold value's sensitivity was 100%, specificity was 65%, positive predictive value was 8.5%, and negative predictive value was 100% (p = 0.000).

In addition, SBI developed in 1 out of 229 patients (0.4%) whose absolute neutrophil count was less than 10,000 mm^3. Furthermore, we determined that the SBI had developed in 9 out of 89 patients (10.1%), whose absolute neutrophil count was greater than 10.000 mm^3. The threshold value's sensitivity was 90%, specificity was 74%, positive predictive value was 10%, and negative predictive value was 99.6% (p = 0.000).

When the patients were evaluated in terms of the leukocyte count, the SBI had developed in 3 out of 206 patients (1.5%) whose leukocyte count was less than 15,000 mm^3 and in 7 out of 112 patients (6.3%), whose leukocyte count was greater than 15.000 mm^3. The sensitivity, specificity, positive and negative predictive values for the 15,000 threshold value were 70%, 66%, 6.3% and 98.5%, respectively (p = 0.037).

Patients that had a fever of greater than 39 °C and a leukocyte count of greater than 15,000 mm^3 made up the empirical antibiotic therapy group. In this group, 4 out of 51 cases (7.8%) developed the SBI and the threshold values' sensitivity, specificity, and positive and negative predictive values were 40%, 85%, 8% and 98%, respectively (p = 0.059).

Lastly, we determined that the SBI has developed in 6 out of 261 patients (2.2%) whose fever was less than 39 °C or greater than 39 °C but, the leukocyte count was less than 15,000mm^3.

Discussion

The fever in young children without a toxic appearance has been still an issue. It has caused the most controversy and debates regarding the occult bacterial infections, and diagnostic methods for the prediction of bacteremia. Many guidelines related to evaluating infants and young children with fever have been published to help physicians in the decision-making process[6]. However, approaches applied in assessments and treatment of these children showed significant differences[7].

Vaccinations provide effective protection and have been indicated for the decrease in the rate of bacteremia and SBI. In the recent decade, conjugated vaccine against Haemophilus influenzae type b has been put into use, but the permission for conjugated pneumococcal vaccine has not been obtained yet. With the use of this H. influenzae vaccine, the frequency of occult bacteremia decreased to 2.0%[8], and in recent publications that frequency has been reported to drop even further to 1.1%. In our study, the rate of occult bacteremia was 1.3%, which was lower than the values prior to Hib vaccination, but consistent with the values after the Hib vaccination.

The most common causes of occult bacteremia are Streptococcus pneumonie (90%), Haemophilus influenzae, Neisseria meningitidis, Salmonella, and Staphylococciis aureus [1, 3]. In our study, the occult bacteremia was present in only 4 patients (50% S. aureus). Many studies have concluded that bacteremia did not show socio-economic, geographic, or national/ethnic tendency[9]. The gender does not affect the risk of bacteremia[10]. In our study, we also did not find any statistically significant difference between cases diagnosed with bacteremia in terms of the genders.

Although occult bacteremia can be encountered at any age, it is more common in children aged 3-36 months, when excluding children younger than 3

months[11]. While the risk of occult bacteremia in children aged 3-24 months is 2.5%, it is 4% in children aged 25-36 months[12]. Bacteremia is most frequently observed in children aged 3-24 months[13]. However, there are some studies that reported no significant relationship between age and the rate of patients with bacteremia[12]. In our study, the mean age of patients with detected bacteremia was 17.0 ± 5.3 months without any significant relationship between age and patients with bacteremia (p = 0.466).

The risk of occult bacteremia has been associated with the increase in body temperature[3]. The rectal temperature of 39.4 °C increases the risk of bacteremia from 4.6% to 7.2%[14]. High fever was found to increase the incidence of bacteremia[8]. In another study, it was reported that the rate of bacteremia increased in children with a fever of 41 °C and higher[15]. There has been no documented proof of bacteremia in children with fever of less than 38.9 °C; however, it has been stated that high fever alone should not be considered as a good indicator for bacteremia. In our study, in patients with bacteremia, the fever was 38.6 ± 0.3 °C and bacteremia was not associated with fever (p = 0.719).

In children with occult pneumococcal bacteremia, persistent fever was observed in 76.1% of patients who did not take any antibiotics and in 23.9% of patients that took oral or parenteral antibiotics[8]. The duration of fever is not a predictive value for bacteremia[16]. In our study, we found that a fever that persisted after the first two days to be significant and empirical antibiotic therapy may be responsible.

Leukocyte count is the most commonly used laboratory test to predict occult bacteremia. The leukocyte count of 15,000 / mm^3 is considered the threshold for bacteremia[17], with a sensitivity of 87.0%, specificity of 73.0%[18], and positive predictive value of 11.0%[19]. One study reported that leukocyte count was not a predictive factor for occult bacteremia[20]. In our study the leukocyte count of 15.55±1.65/mm^3 was not associated with occult bacteremia, which was consistent with the above mentioned study (p = 0.219). On the other hand, it may be associated with the lower number of bacteremia cases in our study.

Absolute neutrophil count and mature neutrophil count were independent predictors of bacteremia[21]. The absolute neutrophil count of 11,646 ± 1,405 / mm^3 (p = 0.028), granulocyte percentage of ± 5.8% (p = 0.032), mature neutrophil percentage of 8.7 ± 3.0%, and toxic granulation (p = 0.012, p = 0.012) supported the diagnosis of bacteremia.

In bacteremia, CRP had a 89.0% sensitivity and 88.0% negative predictive value[22]. The CRP might have a limited sensitivity for staphylococci, a pathogen common in tropics[23]. In our study, the CRP was determined to be significant with a value of 82.1 ± 31.7 mg / l (p = 0.006).

The sensitivity of a single blood culture for the diagnosis of bacteremia was only 45-70.0%[24]. Generally, approximately 4-12% of blood cultures were contaminated[25]. In our study, the occult bacteremia was detected in 4 patients (1.3%). The blood cultures of 34 patients (10.7%) were contaminated, while the blood cultures of 280 patients (88%) were sterile. Although the rate of contaminant bacteria in our study was higher than rates reported in the studies from United States, they were in conjunction with other studies[20].

Based on the literature, 1.4-13% of the 3-36 months-old children presenting to the hospital with fever had been identified to have SBI[26, 27]. In our study, this ratio was 3.1% and there were 4 cases of occult bacteremia; pneumonia, urinary tract infections, meningitis, bacterial enteritis. Although the rate of SBI in our study was consistent with other studies, the small amount of these patients and using empirical antibiotic therapy could be reasons of reduced rate of SBI. Four patients that had occult bacteremia improved without any complications, while 6 patients with SBI did not have bacteremia, and it was an unexpected result.

In our study, similar to other studies, we set the threshold value of absolute neutrophil count for possibility of SBI at 10,000 / mm^3. Some studies have suggested that CRP was a better predictor of the risk for development of SBI compared to absolute neutrophil count and leukocyte count[16]. However, we did not find CRP significant in distinction of patients that developed and did not develop SBI in our study.

The absolute neutrophil count and the absolute number of mature neutrophils have been reported to be more sensitive than leukocyte count, granulocyte percentage and mature neutrophil percentage. In addition, absolute neutrophil count of 10,000 / mm^3 and/or presence of 500/mm^3 mature neutrophils would constitute an 80.0% chance for the presence of SBI with sensitivity of 75.0%[28]. In

our study, the granulocyte percentage in peripheral blood smear was more effective in determining the presence of SBI than absolute neutrophil count and leukocyte count, while toxic granulation was found to be significant in distinction of patients that developed SBI from the patients.

Eight different clinical identification rules for detection of serious infections in children with fever had been compared and were only effective, moderately[29]. Although the patients had fever of greater than 39°C and leukocyte count of greater than 15.000 mm^3 in their clinical examination and follow-up results were treated with empiric antibiotic treatment, 7.8% of these cases developed SBI in our study. Meanwhile, 2.2% of patients that had a fever of less than 39 °C or greater than 39 °C, but a leukocyte count less than15,000 developed SBI. Detailed studies are needed to determine the indications for the empirical use of antibiotics and the reasons for why the empirical treatment is not sufficient.

In conclusion, children aged 3-36 months with fever of unknown origin; a combination of leukocyte count of 15,000 / mm^3; a mean absolute neutrophil count of 10,000 / mm^3; and a granulocyte percentage of 70.0% could constitute a high probability for occult bacteremia and serious bacterial infections. However, patients without these criterias could have bacteremia, also. The antibiotic treatment at an early stage might reduce the risk of SBI development in high-risk patients. However, a very extensive series of studies and effective markers are needed for early identification of patients that will be treated with empirical antibiotic treatment.

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Corresponding Author
Hasan Yesilagac,
Baskent University,
Turkey,
E-mail: docbetul@yahoo.com

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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.

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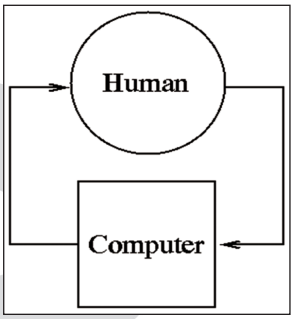

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Conclusion

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

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Corresponding Author
Name Surname,
Institution,
City,
Country,
E-mail: