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# Neutrophil-to-Lymphocyte Ratio: A Predictor of Cardiometabolic Complications in Morbid Obese Girls

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***Abstract*****—**Obesity is a low-grade inflammatory state. Childhood obesity is a multisystem disease, which is associated with a number of complications as well as potentially negative consequences. Gender is an important universal risk factor for many diseases. Hematological indices differ significantly by gender. This should be considered during the evaluation of obese children. The aim of this study is to detect hematologic indices that differ by gender in morbid obese (MO) children. A total of 134 MO children took part in this study. The parents filled an informed consent form and the approval from the Ethics Committee of Namik Kemal University was obtained. Subjects were divided into two groups based on their genders (64 females aged 10.2±3.1 years and 70 males aged 9.8±2.2 years; $p \geq 0.05$). Waist-to-hip as well as head-to-neck ratios and body mass index (BMI) values were calculated. The children, whose WHO BMI-for age and sex percentile values were > 99 percentile, were defined as MO. Hematological parameters [haemoglobin, hematocrit, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width, leukocyte count, neutrophil %, lymphocyte %, monocyte %, eosinophil %, basophil %, platelet count, platelet distribution width, mean platelet volume] were determined by the automatic hematology analyzer. SPSS was used for statistical analyses. $P \leq 0.05$ was the degree for statistical significance. The groups included children having mean±SD value of BMI as 26.9±3.4 kg/m$^2$ for males and 27.7±4.4 kg/m$^2$ for females ($p \geq 0.05$). There was no significant difference between ages of females and males ($p \geq 0.05$). Males had significantly increased waist-to-hip ratios (0.95±0.08 *vs* 0.91±0.08; p=0.005) and mean corpuscular hemoglobin concentration values (33.6±0.92 *vs* 33.1±0.83; p=0.001) compared to those of females. Significantly elevated neutrophil (4.69±1.59 *vs* 4.02±1.42; p=0.011) and neutrophil-to-lymphocyte ratios (1.70±0.71 *vs* 1.39±0.48; p=0.004) were detected in females. There was no statistically significant difference between groups in terms of C-reactive protein values ($p \geq 0.05$). Adipose tissue plays important roles during the development of obesity and associated diseases such as metabolic syndrom and cardiovascular diseases (CVDs). These diseases may cause changes in complete blood cell count parameters. These alterations are even more important during childhood. Significant gender effects on the changes of neutrophils, one of the white blood cell subsets, were observed. The findings of the study demonstrate the importance of considering gender in clinical studies. The males and females may have distinct leukocyte-trafficking profiles in inflammation. Female children had more circulating neutrophils, which may be the indicator of an increased risk of CVDs, than male children within this age range during the late stage of obesity. In recent years, females represent about half of deaths from CVDs; therefore, our findings may be the indicator of the increasing tendency of this risk in females starting from childhood.

***Keywords*****—**Children, gender, morbid obesity, neutrophil-to-lymphocyte ratio.

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## I. Introduction

THE childhood obesity is a multisystem disease, which requires the attention of health professionals, because it is associated with severe complications and potentially negative results. In pediatric age group, obesity leads to hypertension, dyslipidemia, chronic inflammation, increasing tendency in blood coagulation, endothelial dysfunction and hyperinsulinemia, all of which may be considered as important risk factors for CVDs [1].

Obesity is a clinical condition, which is associated with low-grade inflammation. Subclinical inflammation is a central component of cardiometabolic disease risk in obese subjects. White blood cells (WBCs) may be associated with early derangements in metabolism and preclinical signs of cardiac damage and may be effective tools during the investigation of obese children [2].

Hematological parameters and indices are greatly affected by gender in childhood. Since this may cause significant differences, gender must be considered while the clinical parameters are evaluated. In this respect, there is not sufficient information related to differences, which may be caused by obesity.

It is emphasized that gender differences may affect the associations between some physiological events and leukocyte count and also may cause alterations in inflammatory markers as well as neutrophil counts [3], [4]. It is also reported that the severity of some chronic diseases may differ between the genders [5].

Many studies report that complete blood cell count (CBC) including WBCs have been associated with many diseases including obesity and metabolic syndrome (MetS) [2], [6]-[9]. However, the studies performed examining detailed profile of hematological parameters as well as indices among MO children based upon gender difference are rare.

The aim of this study is the evaluation of hematological parameters from the gender point of view in MO children due to the increasing rates of morbid obesity detected in pediatric age group.

## II. Patients and methods

### A. Patients

The study population consisted of MO children, whose ages vary between 06-18 years. Out of 134 children included in the study, 64 were females and 70 were males. These groups were constituted using age- and sex- dependent BMI percentile tables created by World Health Organization [10]. Normal weight group comprised the children, whose values vary between $15^{th}$ -$85^{th}$ percentiles. Those having the values above $99^{th}$ percentile were included in the remaining two MO groups.

Written informed consent forms were taken from the parents of the children prior to the study. The study protocol was approved by Namik Kemal University, Faculty of Medicine Ethical Committee. Weight, height, waist circumference (C), hip C, head C and neck C of the patients, who were consulted to Namik Kemal University, Faculty of Medicine, Outpatient Clinics of Pediatrics Department, with the obesity complaint, were measured and recorded. BMI values as well as waist-to-hip C and head-to-neck C ratios were calculated.

### B. Measurements

Anthropometric measurements were performed as described elsewhere [11]. Weights were measured to the nearest 0.1 kilogram using an electronic scale. Children were weighed without shoes and wearing underclothes only. Standing heights of children wearing light clothing and without shoes were measured with a portable stadiometer to the nearest 0.1 centimeter. Waist C values were determined with a tape measure as a horizontal line at the uppermost lateral borders of the hip crest followed by a normal expiration. Hip C was measured starting at suprapubic region passing through inferior area of gluteus to complete the horizontal line. Head C was determined by measuring around the head from the middle of the forehead to the occipital area by placing the tape measure above the ears. The largest C of the head should be measured. Neck C was performed by taking the measurement horizontally passing through the thyroid cartilage while the child is looking forward.

### C. Obesity Criteria

Children, whose values were above 99, based upon age- and sex- dependent percentiles introduced by WHO and also approved by Republic of Turkey, Ministry of Health for Turkish children were included into the scope of study. Exclusion criteria were the presence of chronic diseases and/or severe congenital anomalies.

### D. Laboratory Analyses

Blood samples were obtained after an overnight fasting. Basic hematological parameters were determined by the automatic hematology analyzer; Pentra DX-Nexus (Horiba Medical ABX SAS, Japan) as described elsewhere [12].

In both genders of MO children CBC analyses including indices of red blood cells (RBCs), WBCs and platelets (PLTs) [hemoglobin (HGB), hematocrit (HCT), RBC count (RBC), mean corpuscular volume (MCV), mean corpuscular HGB (MCH), mean corpuscular HGB concentration (MCHC), RBC distribution width (RDW), WBC count (WBC), polymorphonuclear leukocyte (neutrophil) (PMNL (N) %), lymphocyte (L) %, monocyte %, eosinophile %, basophile %, PLT count (PLT), PLT distribution width (PDW), mean PLT volume (MPV)] were performed by automatic hematology analyzer. N-to-L ratios were calculated.

High sensitive C-reactive protein (*hs*CRP) levels were determined by immunological test system in a Roche COBAS C-501 chemistry analyzer.

Fasting blood glucose and insulin concentrations were determined by spectrophotometric hexokinase assay and electrochemiluminescence immunoassay, respectively [12].

The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index was calculated according to the formula: HOMA-IR= fasting glucose (mg/dL) * fasting insulin (μIU/ml) / 22,5*0,0555 [12,13,14].

The potential differences between gender groups were investigated. The disciplines and members of the study were informed about the instructions to obey. Analyses were performed according to the ethical rules guided by World Medical Association Helsinki Declaration, Good Clinical Practice and Good Laboratory Practice.

### E. Statistical Evaluation

Data were analyzed statistically using SPSS 20 statistical package program for Windows. Shapiro-Wilk test was used to check normality of data distribution. Differences between the groups were estimated by either Student's t or Mann Whitney U tests depending upon whether the data allows for parametric or non-parametric statistics. Values obtained for variables were presented as frequency, percentage, x±SD. The degree for statistical significance was accepted as $p \leq 0.05$.

## III. Results

Out of children divided into two different groups based upon their genders, mean age±SD value detected for female children was 10.2±3.1 years. The corresponding value in the group of male children was 9.8±2.2 years.

BMI values of female and male children were calculated as 27.7±4.4 $kg/m^2$ and 26.9±3.4 $kg/m^2$, respectively. Any statistically significant differences were not noted in terms of ages as well as BMI values of the groups ($p \geq 0.05$).

Waist-to-hip C ratios (0.95±0.08 *vs* 0.91±0.08; $p \leq 0.01$) were significantly higher in male children than those found for female children.

Head-to-neck C ratios (1.65±0.11 *vs* 1.69±0.14 in male and female children, respectively) did not differ between two genders ($p \geq 0.05$).

Hematological parameters in MO male children and MO female children were summarized in Tables I-III.

Statistically significant differences were observed for MCHC values (33.6±0.92 *vs* 33.1±0.83; $p \leq 0.001$) between different genders of MO children. Values detected in male children were significantly higher than those found for female children (Fig. 1).

TABLE I
MEAN±SD VALUES OF TOTAL AND DIFFERENTIAL LEUKOCYTES COUNTS AS WELL AS THEIR INDICES IN MO FEMALES AND MO MALES

| Parameters | MO Female Children | MO Male Children | p |
|---|---|---|---|
| ***WBCs*** | | | |
| wbc | 8.500±1.983 | 7.956±2.020 | 0.119 |
| neutrophils* | 4.69±1.59 | 4.02±1.42 | 0.011 |
| lymphocytes | 2.91±0.77 | 2.97±0.79 | 0.617 |
| monocytes | 0.68±0.19 | 0.69±0.21 | 0.515 |
| eosinophils | 0.196±0.108 | 0.240±0.181 | 0.095 |
| basophils | 0.031±0.021 | 0.026±0.015 | 0.094 |
| Nly* | 1.70±0.71 | 1.39±0.48 | 0.004 |

* p≤0.05
nly-neutrophile to lymphocyte ratio

TABLE II
MEAN±SD VALUES OF RBCS COUNTS AS WELL AS THEIR INDICES IN MO FEMALES AND MO MALES

| Parameters | MO Female Children | MO Male Children | p |
|---|---|---|---|
| ***RBCs*** | | | |
| Rbc | 4.92±0.31 | 5.00±0.33 | 0.100 |
| hgb | 12.85±0.89 | 13.53±3.32 | 0.120 |
| Htc | 38.86±2.42 | 39.66±4.96 | 0.238 |
| mcv | 79.38.±6.15 | 77.56±7.49 | 0.128 |
| mch | 26.25±2.20 | 26.28±1.46 | 0.916 |
| mchc* | 33.1±0.83 | 33.6±0.92 | 0.001 |
| rdw | 14.26±1.30 | 14.09±1.63 | 0.530 |

* p≤0.05

TABLE III
MEAN±SD VALUES OF PLTS COUNTS AS WELL AS THEIR INDICES IN MO FEMALES AND MO MALES

| Parameters | MO Female Children | MO Male Children | p |
|---|---|---|---|
| PLTs | | | |
| PLT | 350.3±62.2 | 336.4±71.1 | 0.232 |
| Mpv | 8.16±0.85 | 8.26±0.55 | 0.634 |
| Pdw | 13.29±1.92 | 13.41±3.39 | 0.876 |

* p≤0.05

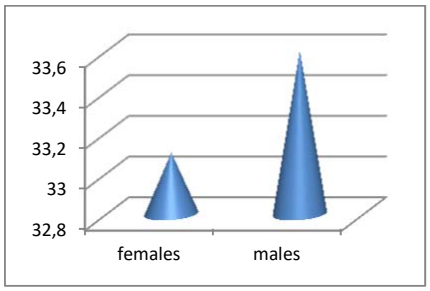


Fig. 1 MCHC values in male and female groups

Significantly increased N percentages (4.69±1.59 *vs* 4.02±1.42; p≤0.05) and N-to-L ratios (1.70±0.71 *vs* 1.39±0.48; p≤0.01) were detected for female children compared to male children (Fig. 2).

No significant differences were observed between *hs*CRP (3.66±3.23 in male children and 4.04±3.37 in female children) and HOMA-IR (2.6±2.4 in male children and 2.9±1.9 in female children) levels of the groups (p≥0.05).

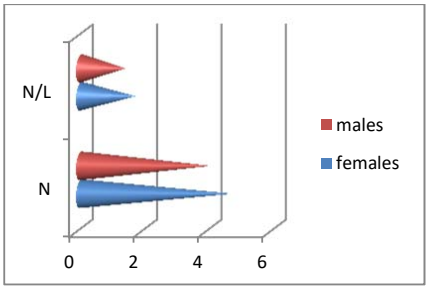


Fig. 2 Variations of neutrophile percentage and neutrophile-to-lymphocyte ratios based upon gender

## IV. DISCUSSION

Adipose tissue is known to play important role in the development of obesity and obesity-associated diseases such as MetS and CVDs [15]. Aside from different alterations, several CBC parameters also are subjected to variations in such diseases. These alterations gain even more importance during childhood period of life. It is reported that obese adolescents exhibit higher WBC counts in comparison with those having the normal weight and this reflects the chronic proinflammatory stage in obese children. A positive correlation between this parameter and adiposity and a negative correlation with cardiorespiratory health were reported in male children [16]. Increased WBC count as well as N and L percentages were also observed in adult male patients with Met S. These parameters appear to be concordant with the number of MetS components. The increases in WBC counts observed only in male gender during obesity and MetS may be related to the vascular protective effects of estrogens. In general, it is discussed that morbidity and mortality caused by CVDs are much higher in male gender than in females. This is explained by the suggestion that estrogens play some roles to protect women from atherosclerosis by decreasing inflammatory cell adhesion as in the case of increased vasodilatation or nitric oxide synthesis [15], [17]. In some studies, significant correlations were found between CVDs and N as well as eosinophil counts [15], [18].

The findings obtained in our study have emphasized the importance of considering gender factor in clinical studies. Our study confirms that male and female children may exhibit different WBC trafficking profiles during inflammatory states as in the case of morbid obesity.

It has been introduced that gender exhibits significant effects upon the alterations in N percentages, one of the subsets of WBCs. In the late stages of obesity, it has been detected that female children have higher circulating Ns than male children as the indicator of more depressed state of immune system.

In spite of the tendency that heart disease is generally thought of as a "man's disease", it is understood from the recent data that around the same number of men and women have lost their lives due to heart diseases each year. It has been demonstrated that women with diabetes exhibit much higher CVDs mortality when compared with diabetic men. Women suffered from heart crisis have been detected to exhibit higher

mortality than men of the same age [19], [20]. It is reported that CVDs affect not only men but also affect women in even worse in some clinical conditions. Since the disease differs in its presentation, progression or in its clinical consequences in different genders and also in women due to the fact that it is less understandable than in men, diagnosis or treatment options may be insufficient. This may be more risky particularly in low- and middle-income countries compared to that in developed countries due to less access to effective health care services [20], [21]. Within this context, women represented 49.7 % of deaths from CVDs in 2013 according to the 2016 year statistics [22].

The lack of a significant difference between two genders in terms of *hs*CRP concentrations makes differences in N percentages as well as N-to-L ratios more meaningful. This study proves the importance of the association between gender and N recruitment.

## V. Conclusion

In conclusion, elevated N percentages were observed in MO female children in our study. Data, which are being recorded against female gender in recent years, can be evaluated as the extrapolated form of this finding observed during childhood period. In comparison with males, women are associated with much less physical activity, higher total cholesterol, triglycerides, low density lipoprotein cholesterol concentrations. Smoking rates have already been equalized in both genders. Due to all of these factors as well as the several others the risk observed in both genders has already been balanced. One could think that this risk may increase among women in future years. It is remarkable to observe some supporting findings related to this notion among pediatric population in this study. This may be evaluated as a finding concerning the thought that the tendency towards the increased risks related to CVDs has even been observed starting from the early periods of life.

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