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Hematologic Inflammatory Markers and Inflammation-Related Hepatokines in Pediatric Obesity

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***Abstract*—**Obesity in children particularly draws attention, because it may threaten the individual's future life due to many chronic diseases it may lead to. Most of these diseases including obesity itself altogether are related to inflammation. For this reason, inflammation-related parameters gain importance. Within this context, complete blood cell counts, ratios or indices derived from these counts have recently found some platform to be used as inflammatory markers. So far, mostly adipokines were investigated within the field of obesity. Metabolic inflammation is closely associated with cellular dysfunction. In this study, hematologic inflammatory markers and cytokines produced predominantly by the liver (fibroblast growth factor-21 (FGF-21) and fetuin A) were investigated in pediatric obesity. Two groups were constituted from 76 obese children based on World Health Organization criteria. Group 1 was composed of children, whose age- and sex-adjusted body mass index (BMI) percentiles were between 95 and 99. Group 2 consists of children, who are above 99th percentile. The first and the latter groups were defined as obese (OB) and morbid obese (MO). Anthropometric measurements of the children were performed. Informed consent forms and the approval of the institutional ethics committee were obtained. Blood cell counts and ratios were determined by automated hematology analyzer. The related ratios and indexes were calculated. Statistical evaluation of the data was performed by SPSS program. There was no statistically significant difference in terms of neutrophil-to lymphocyte ratio, monocyte-to-high density lipoprotein cholesterol ratio and platelet-to-lymphocyte ratio between the groups. Mean platelet volume and platelet distribution width values were decreased ($p < 0.05$), total platelet count, red cell distribution width (RDW) and systemic immune inflammation index values were increased ($p < 0.01$) in MO group. Both hepatokines were increased in the same group, however increases were not statistically significant. In this group, also a strong correlation was calculated between FGF-21 and RDW when controlled by age, hematocrit, iron and ferritin ($r = 0.425$; $p < 0.01$). In conclusion, the association between RDW, a hematologic inflammatory marker, and FGF-21, an inflammation-related hepatokine, found in MO group is an important finding discriminating between OB and MO children. This association is even more powerful when controlled by age and iron-related parameters.

***Keywords*—**Childhood obesity, fetuin A, fibroblast growth factor-21, hematologic markers, red cell distribution width.

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I. Introduction

OBESITY is a worldwide health threatening clinical problem, because it has a great potential to be complicated with many severe chronic diseases. Its association with low-grade inflammatory state increases the number of investigations performed related to the diagnostic efficacies of many biochemical, hematologic or immunologic parameters on varying degrees of obesity as well as metabolic syndrome (MetS). The studies performed on pediatric population are particularly important, because childhood obesity may be a preparatory stage for severe chronic diseases during the adulthood period of the child. There are many studies comparing parameters in OB state with the normal body mass index (N-BMI) state [38], [39]. These studies try to demonstrate possible differences between children with N-BMI and the groups comprising overweight, OB, or MO children. Some studies have focused on the deviations from the normal in children with MetS [1], [2]. So far, numerous parameters were considered within this context. Aside from traditional markers, such as leptin or adiponectin, new generation obesity markers such as spexin or adipolin have also been investigated. Variations between groups or deviations from the healthy normal have been reported for such parameters [3], [4].

Within the scope of hematologic parameters, complete blood cell count analysis (CBCA) is the set of tests, which are routinely performed. They primarily give diagnostic information about the type and nature of anemia as well as the other hematologic diseases. In recent years, it was discovered that members of CBCA and particularly the indexes as well as some ratios may be useful from an inflammatory point of view and may be related to insulin resistance (IR) [5], [6]. It was suggested that some hematologic indices may be associated with systemic inflammation [7], [8]. Systemic immune-inflammation (SII) index and ratios calculated from CBCA members are also important [9]. Within this context, the possible uses of these indices and their potential clinical applications have been examined in the diagnosis and risk stratification of carotid artery disease, given the inflammatory nature of the atherosclerotic process [10]. RDW is introduced as a prognostic marker in multiple clinical studies [11]-[15].

Hepatokines are synthesized by the liver. FGF-21 and fetuin A were introduced as inflammation-related hepatokines. Along with other compounds synthesized by the liver, their behavior was examined in many physiological as well as clinical states both in adults and children. Hyperenergetic,

high-fat feeding state, physiological pregnancy, gestational diabetes mellitus, type 2 diabetes, obesity, and MetS are the conditions, during which hepatokine measurements have already been performed. Hepatokines are also introduced as the molecular transducers of exercise [16]-[21].

Metabolic functions of the hepatic hormone FGF-21 have been recognized for more than a decade. Responses to nutritional stresses have been analyzed [22]. FGF-21 is a stress-inducible hormone that has important roles in regulating energy balance and glucose as well as lipid homeostasis. The role of FGF-21 as a potential biomarker and its therapeutic potential are being discussed in various disorders such as obesity, diabetes, non-alcoholic steatohepatitis [23].

Fetuin A, also termed alpha-2-Heremans Schmid-glycoprotein, is being studied in various clinical states such as obesity, diabetes, gastrectomy, in vitro fertilization, insulin sensitivity, Alzheimer's disease model [24]-[31].

The aim of this study was to detect possible differences between OB and MO children in terms of hematologic-inflammatory markers as well as two inflammation-related hepatokines such as FGF-21 and fetuin A. Within this context, the available evidence regarding the role of common CBCA indexes, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-high density lipoprotein cholesterol ratio (MHR), platelet-to-lymphocyte ratio (PLR), SII index, mean platelet volume (MPV), platelet distribution width (PDW), and RDW was investigated and their possible associations with FGF-21 as well as fetuin A were assessed from the differential diagnosis view of obesity and morbid obesity.

II. Patients and Methods

A. Study Population and Groups

The study was performed on pediatric population. 76 OB individuals participated in the study. Groups were composed of 30 OB (Group I) and 46 MO (Group II) children. Written informed consent forms were filled out by the parents of the children. The study design was approved by the Ethics Committee of the Namik Kemal University, Faculty of Medicine.

B. Measurements

Anthropometric measurements including waist, hip, head neck circumferences as well as body weight and height were taken. BMI values were calculated.

C. Classification of Obesity

BMI percentiles adjusted for age and sex of the children [32] were used to classify obesity. The children, who were within 95th and 99th percentiles were defined as OB. Children, whose percentiles were above 99 were considered MO.

D. Hematologic Measurements and Laboratory Analysis

Routine biochemical analyses and tests within the scope of CBCA were performed by autoanalyzers. Erythrocyte, leukocyte, platelet counts, counts of leukocyte subgroups, RDW, MPV, PDW were recorded. NLR, MHR, PLR were calculated. Values for SII index were calculated by:

Platelet count*(neutrophil count-to-lymphocyte count)

Concentrations of hepatokines (FGF-21 and fetuin A) were determined by enzyme-linked immunosorbent assay.

E. Statistical Evaluation of the Data

The software of statistical package for social sciences was used for the statistical calculations. Mean ± standard deviation or median values were tabulated depending on the type of the data distribution. Where appropriate, t or Mann-Whitney U test were used to determine statistically significant differences. Bivariate and partial correlation analyses were performed. Graphs for the correlations between RDW and FGF-21 were constituted. The statistical significance value was accepted as $p < 0.05$.

III. Results

There was no statistically significant difference between the ages of OB and MO groups. Mean age ± SD values of OB and MO groups were 12.0 ± 3.1 and 10.7 ± 3.3 years, respectively.

Within the scope of hematologic inflammatory markers, the values measured for RDW, total platelet count (TPC), MPV, and PDW were shown in Table I. Table I also listed NLR, MHR, PLR and SII index values calculated from neutrophil, lymphocyte, monocyte, platelet counts and high-density lipoprotein cholesterol concentrations.

Out of parameters related to iron metabolism, concentrations related to serum iron, ferritin and hematocrit were 69.5 ± 25.9 µg/dl, 36.9 ± 22.5 ng/ml, 38.5 ± 2.6% in OB group and 67.9 ± 24.3 µg/dl, 45.6 ± 22.2 ng/ml, 38.6 ± 3.3% in MO group, respectively. No statistically significant differences were observed between two groups in terms of iron-related parameters ($p > 0.05$).

TABLE I
HEMATOLOGIC INFLAMMATORY MARKERS

Groups	Group I OB (x ± SD)	Group II MO (x ± SD)	p
RDW	14.3 ± 1.1	15.1 ± 1.4	0.008
NLR	3.3 ± 1.1	3.6 ± 1.0	NS
MHR	0.014 ± 0.020	0.017 ± 0.029	NS
PLR	119.5 ± 40.3	122.8 ± 34.8	NS
SII index	911 ± 344	1213 ± 405	0.001
TPC	287 ± 72	336 ± 74	0.004
MPV	9.0 ± 1.0	8.6 ± 0.7	0.036
PDW	15.2 ± 2.4	13.4 ± 3.4	0.006

NS = not significant

Concentrations of FGF-21 and fetuin-A, as the inflammation-related hepatokines, were given in Table II.

TABLE II
INFLAMMATION-RELATED HEPATOKINES

Groups	Group I OB (m)	Group II MO (m)	p
FGF-21 ^{m} (ng/L)	152	183	NS
Fetuin-A ^{m} (mg/L)	325	366	NS

NS = not significant, m = median.

The only association found among the parameters investigated in both groups was the correlation between RDW and FGF-21 ($r = 0.295$; $p = 0.047$) in MO group (Fig. 1). This correlation was not detected in the other group.

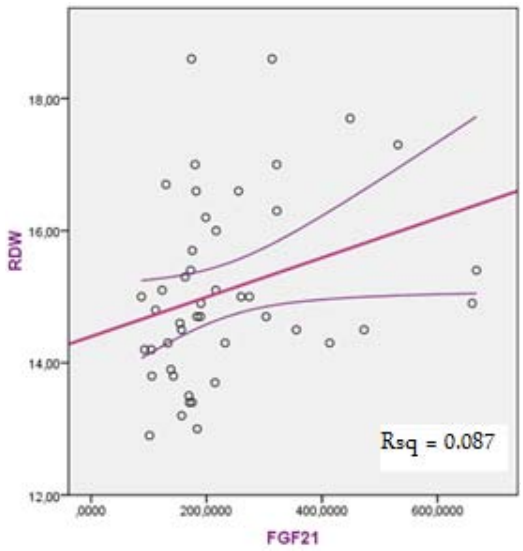

Fig. 1 Bivariate correlation between RDW and FGF-21 in MO group.

Statistically significant bivariate correlations were not observed between FGF-21 and age, iron, ferritin or hematocrit values. The same was true for the associations between RDW and the above parameters.

Partial correlation calculated between FGF-21 and RDW when adjusted for age was found as $r = 0.316$; $p = 0.034$. Partial correlation calculated between FGF-21 and RDW when adjusted for iron-related parameters (iron, ferritin, hematocrit) was $r = 0.326$; $p = 0.033$. However, partial correlation detected between FGF-21 and RDW when adjusted for age, iron, ferritin, hematocrit was calculated as $r = 0.425$; $p = 0.005$. This strong association was shown in Fig. 2.

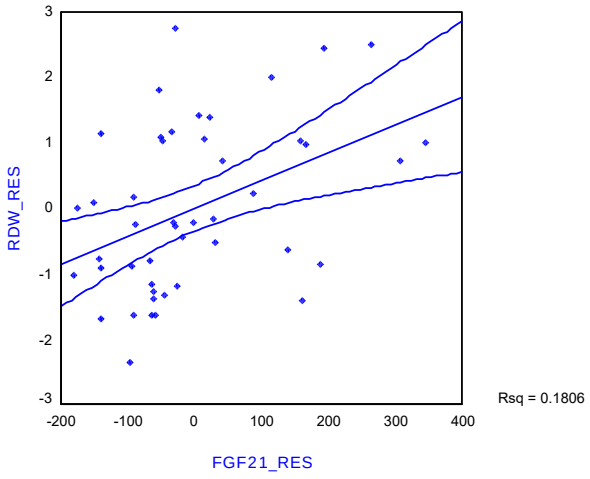

Fig. 2 Partial correlation between RDW and FGF-21 in MO group controlled by age, hematocrit, iron, ferritin

IV. Discussion

In this study, hematologic inflammatory markers were evaluated and their associations with FGF-21 as well as fetuin A were investigated in children with obesity. Both hepatokines are known for their inflammation-related natures, however, this has not been cleared yet due to much controversial data. RDW is a parameter closely associated with iron deficiency. High levels indicate iron deficiency status in the individuals. In recent reports, its relation with inflammatory processes has also been declared [7], [8], [13], [14]. Obesity, being a low-grade inflammatory state, may closely be related to these parameters. In this study, the characteristic features and possible differences were investigated between OB and MO children related to the matter. Morbid obesity is the advanced form of obesity; therefore, some expectations may come true.

Lower hemoglobin, lower mean corpuscular volume, lower mean corpuscular hemoglobin, higher RDW are associated with higher prevalence of microcytic, normocytic anemia [7]. RDW is available in the standard CBCA. Besides its traditional use in the differential diagnosis of anemias, RDW values reflect abnormalities in erythropoiesis and red blood cell metabolism-related to aging, sex, ethnicity, systemic inflammatory state, and oxidative stress. Thus, higher RDW values are common findings in several acute clinical conditions and chronic diseases. Increasing evidence suggests a prognostic role of higher RDW levels in many cardiovascular diseases [12], [33]. In a similar manner, higher RDW levels in patients with acute heart failure suggest that this parameter, which is the most important mortality predictor, is independently associated with systemic inflammation in these patients [13]. Association of RDW with MetS was also reported [34]. The strong correlation of RDW and chronic inflammation suggests that this parameter may be defined as an unspecific and general "chronic disease prognostic marker" [14].

Concerning the pathophysiology of these relationships, some reports have suggested that inflammation affects the hepatic production of the iron regulatory peptide hormone (hepcidin), causing abnormal iron absorption, thereby affecting RDW [8], [35].

In this study, significantly higher values for RDW, TPC and SII index and significantly lower values for MPV and PDW were detected in MO children compared to the values obtained for the children in OB group.

Fetuin A, a hepatocyte derived protein, serves multifaceted functions [24], [28]. Fetuin A, one of the proinflammatory cytokines, was related to IR in children. Increased levels were observed in OB children [26], [27]. In another study, fetuin-A was found to be correlated with MCP-1, TNF-α, and IL-6. However, it was introduced as the only inflammatory marker positively correlated with BMI in children [25].

In our study, increased fetuin A concentrations were observed in MO group. In this group, levels higher than the levels found in OB group ($p > 0.05$) can be evaluated as the indicator of a higher level of inflammation in MO group.

In a study performed with resveratrol, an antioxidant phytochemical, it was reported that resveratrol

supplementation caused significant reductions in tumor necrosis factor alpha and FGF-21 levels whereas significant elevations were observed in adiponectin concentrations [36]. Another study reported FGF-21 as one of the inflammation-related factors and found that aerobic exercise significantly OB body weight and serum FGF-21 levels in OB+exercise group compared to OB group [37]. In our study, also a statistically insignificant increase was observed for FGF-21 levels in MO group in comparison with OB group ($p > 0.05$).

Although they are within the normal limits, the observation of relatively higher concentrations of ferritin in MO group compared to those in OB group ($p > 0.05$) is in line with the increasing obesity degree in MO group of children.

Considering the studies reporting that both FGF-21 and RDW are dependent upon age of the individuals [40], [41], the association of each parameter with age was calculated. None was obtained in groups.

The associations of each parameter with iron-related parameters (iron, ferritin, hematocrit) were calculated. In a similar manner, none was obtained in groups.

Despite these findings, the correlation between FGF-21 and RDW ($r = 0.295$; $p = 0.047$) calculated in MO group was turned out as a much stronger correlation ($r = 0.425$; $p = 0.005$) when controlled by age, iron, ferritin, hematocrit. This finding has pointed out that this statistically significant correlation in MO children is affected by age as well as iron-related parameters.

V. Conclusion

The existence of correlation between RDW and FGF-21 in MO group may be taken into account in further studies and considered as a possible potential marker of adverse events in this advanced form of obesity. Age- and iron-related parameters-dependency of this association may provide some links between these parameters and iron as well as energy metabolisms during the severe form of obesity.

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