

The Evaluation of New Generation Cardiovascular Risk Markers in Childhood Obesity

Mustafa M. Donma, Sule G. Kacmaz, Ahsen Yilmaz, Savas Guzel, Orkide Donma

Abstract—Obesity, as excessive fat accumulation in the body, is a global health problem. The prevalence of obesity and its complications increase due to easy access to high-energy food and decreased physical activity. Cardiovascular diseases (CVDs) constitute a significant part of obesity-related morbidity and mortality. Since the effects of obesity on cardiovascular system may start during childhood without clinical findings, elucidating the mechanisms of cardiovascular changes associated with childhood obesity became more important. In this study, we aimed to investigate some biochemical parameters which may be involved in obesity-related pathologic processes of CVDs. One hundred and seventy-seven children were included in the study, and they were divided into four groups based upon WHO criteria and presence of the metabolic syndrome (MetS): children with normal-BMI, obesity, morbid obesity, and MetS. High-sensitive cardiac troponin T (hs-cTnT), cardiac myosin binding protein C (cMyBP-C), trimethylamine N-oxide (TMAO), soluble tumor necrosis factor-like weak inducer (sTWEAK), chromogranin A (CgA), multimerin-2 levels, and other biochemical parameters were measured in serum samples. Anthropometric measurements and clinical findings of the children were recorded. Statistical analyses were performed. Children with normal-BMI had significantly higher CgA levels than children with obesity, morbid obesity, and MetS ($p < 0.05$). Cardiac MyBP-C levels of children with MetS were significantly higher than of children with normal-BMI and OB children ($p < 0.05$). There was no significant difference in hs-cTnT, sTWEAK, TMAO and multimerin-2 between the groups ($p > 0.05$). These results suggested that cMyBP-C and CgA molecules may be involved in the pathogenesis of obesity-related CVDs.

Keywords—Biomarker, cardiovascular diseases, children, obesity.

I. INTRODUCTION

CARDIOVASCULAR diseases (CVDs) are severe health problems encompassing a group of heart and blood vessel disorders. Obesity is epidemiologically associated with CVDs such as coronary arterial diseases (CADs), atrial fibrillation, ventricular arrhythmias, heart failure, cerebrovascular diseases, and sudden cardiac death. The effects of obesity on the cardiovascular system (CVS) possibly start much before the emergence of clinical signs and symptoms. The evaluation of CVDs risk-related biomarkers just before the symptoms come out is important from the point of view of early diagnosis and prevention of cardiovascular complications caused by obesity in children.

Ischemic heart disease and stroke are responsible from a total of 56 million deaths in 2015 [1]. Obesity affects CVS leading

to hypoventilation syndromes e.g. obstructive sleep apnea [2], [3]. A 10 kg increase in body weight increases CAD risk by 12%, SBP 3 mm. Hg, DBP 2.3 mm. Hg [4], [5].

Studies performed on young deaths caused by non-CVDs have shown that, in case of obesity and visceral adiposity, atherosclerosis develops much before the emergence of cardiovascular symptoms [1], [6].

Adipokines secreted by adipose tissue play roles in the development of CVDs in obesity by way of various mechanisms [7]. Adipose tissue accumulating around heart in obese individuals causes functional disorders in heart both through mechanical and metabolic effects. It has been shown that epicardial fat deposits are associated not only with CADs but also with coronary arterial calcification and coronary plaque sensitivity [8].

Obesity is also associated with hypertension. Body mass index (BMI) and weight gain is positively correlated with hypertension. Weight loss has been shown to decrease hypertension risk. Particularly, widened waist circumference is known to be a risk factor for hypertension development in obesity [9], [10].

Cardiovascular risk factors are used for the identification of individuals with CVDs risk and to decrease morbidity and mortality by taking early preventive measures [11]. Cardiovascular markers are molecules, which play roles in physiologic or pathophysiologic periods in CVS and those that can be measured objectively and therefore, are used during diagnosis, prognosis or treatment of CVDs. For a molecule to be a marker, it is not necessary to participate in the pathophysiologic process of the disease [12], [13].

In this study, some new generation markers, which are known to be related to CVDs and are thought to be associated with cardiovascular complications of obesity were considered. In this context, high sensitivity cardiac troponin T (hs-cTnT), cardiac myosin binding protein C (cMyBP-C), trimethylamine N-oxide (TMAO), soluble tumour necrosis factor like weak inducer of apoptosis (sTWEAK), chromogranin A (CgA) and multimerin 2 levels were evaluated in children with normal BMI, obesity, morbid obesity and metabolic syndrome (MetS). At the same time, anthropometric measurements as well as clinical and biochemical parameters were determined and their correlations with new generation markers were investigated.

The aim of this study was the interpretation of some new

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generation cardiovascular risk markers, which may contribute to early recognition as well as good understanding of CVDs-development mechanisms in obesity to be able to lead to early diagnosis of cardiovascular complications in childhood obesity, taking related preventive measures, initiation of treatment if necessary and contributing to further clinical studies.

II. MATERIALS AND METHODS

A. Selection and Description of the Cases

This is a controlled, prospective study performed on children admitted to ambulatory clinics of Tekirdag Namik Kemal University, Faculty of Medicine, Department of Pediatrics between the dates of August 2020 and May 2021.

The study population was composed of cases with normal-BMI and obese children according to anthropometric measurements. The families were informed about the details of the study. Written and oral informed consent forms were taken from the families and children, respectively. Children and families, who accepted to join in this research, were included in the scope of the study. Those with systemic diseases affecting body functions, leading to alterations in biochemical parameters or using medicines such as corticosteroids, levothyroxin, metformin were excluded from the study.

One hundred and seventy-seven children, who constitute the study population was divided into four groups; control (N-BMI), obese (OB), morbid obese (MO) and MetS groups.

The study is designed as controlled and prospectively. The study protocol was approved by Tekirdag Namik Kemal University, Medical Faculty, Non-Interventional Clinical Studies Ethics Committee.

B. Anthropometric Measurements

Body weight, height, waist circumference, hip circumference, head circumference, neck circumference of children, who were admitted to the study were measured using the following technics after the physical examination and a detailed history taken from the parents. Digital balance, stadiometer, and a flexible, non-elastic tape were used during the measurements.

- **Body weight:** Body weights of the shoeless children with thin-issued clothing were measured positioned to his/her feet on a digital balance.
- **Height:** A stadiometer fixed to the wall was used for height measurement of the individual without shoes. The sliding horizontal rod of a stadiometer consisting of a vertical ruler was positioned on the top of the head. The child must stand keeping feet flat, the back straight and the chin parallel to the floor.
- **Waist circumference:** The tape was placed horizontally around the waist. Tape must not compress the skin. Measurement was taken as a horizontal line at the midpoint of the upper limit of the iliac crest and the lower rib just above the individual's hipbones followed by a normal expiration.
- **Hip circumference:** This is the distance measured around the widest part of the buttocks. The measuring tape was

positioned parallel to the floor. It was placed around the hip just above the hip bone and kept at the maximum point where the width was the greatest.

- **Head circumference:** This is the widest possible circumference of the child's head. This line passes from the forehead above the eyebrows, above the ears and through the most prominent part of the back of the head.
- **Neck circumference:** The measurement was performed with the participant standing with the back straight and head in the Frankfurt horizontal plane, while the child is looking forward with neck in an upright position. The tape was positioned in the lower neck circumference just above the laryngeal prominence passing through the most prominent part of the thyroid cartilage.

C. Obesity and Metabolic Syndrome Criteria

Body mass index values were calculated from body weight in kg divided by the square of body height in meters. These values were used to find the percentiles from age- and sex-adjusted BMI tables prepared by WHO [14].

Children with BMI values above 99th percentile and without MetS criteria were included in MO group. Those with BMI values above 99th percentile with at least two of MetS criterias were defined as MetS group. Obese group was composed of children, whose BMI values were between 95th-to-99th percentiles. Children, whose BMI values were between 15. and 85. percentiles constituted the group with N-BMI.

Children in MetS group had at least two of the following three MetS criteria in addition to their BMI values above the 99. percentile [15].

1. Fasting blood glucose concentration equal to or above 100 mg/dL
2. Serum triglyceride concentration equal to or above 150 mg/dL or serum high density lipoprotein-cholesterol concentration equal to or below 40 mg/dL
3. Systolic blood pressure higher than 130 mm Hg or diastolic blood pressure higher than 85 mm Hg

Children, who had only one of the above three criteria were included into MO group.

D. Data Collection

Histories as well as anthropometric measurements of the participants, who applied to Tekirdag Namik Kemal University, Faculty of Medicine, Ambulatory Clinics of Pediatrics were taken prior to physical examination. Those, who met the study inclusion criteria were selected. Biochemical data obtained from the peripheral blood samples were examined for the clinical evaluation related to the present application. Under the light of these data, patients with the appropriate findings were recorded.

E. Evaluation of Insulin Resistance

Values for Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values were calculated by the following formula.

"HOMA-IR = Fasting Blood Glucose (mg/dl)* Insulin (μ IU/ml) * 0,0555/22,5"

F. Methods for Biochemical Analysis

Concentrations of the parameters included in the research protocol of the study were determined in peripheral blood samples drawn from children in Tekirdag Namik Kemal University, Medical Faculty, Department of Medical Biochemistry Laboratory. Blood samples taken from the participants were put into test tubes with red cap and gel separator. Tubes were centrifuged at 1000 rpm for 10 minutes and sera of the samples were separated. Collected samples were stored in -80 °C until the assays were performed. Sera samples were analyzed at room temperature during the day of analysis. All parameters were analyzed by kits working with ELISA (Enzyme-Linked Immunosorbent Assay) principle. Analysis of CgA (catalog no: E1730Hu), TMAO (catalog no: E4733Hu), hs-cTnT (catalog no: E4862Hu), multimerin-2 (catalog no: E4585Hu), cMyBP-C (catalog no: E3757Hu), sTWEAK (catalog no: E3823Hu) were performed by Bioassay Technology Laboratory (Shanghai Korain Biotech Co., Ltd. China) ELISA kits. Intra-assay CV and Inter-assay CV of the study kits were < %8 and < %10, respectively.

G. Statistical Analysis

Statistical analysis of the study data was performed by SPSS software. Shapiro-Wilk test was used to determine the normality of the study data. Analysis of variance (one-way ANOVA) test was used to determine whether or not there is a statistically significant difference between the means of four independent groups for normally distributed data. In order to find out exactly which groups are different from each other a post-hoc Tukey test, which compares the mean between each pairwise combination of groups, was conducted. When normality of the distribution was not satisfied, nonparametric Kruskal-Wallis test was used for comparisons among four groups. This was followed by post-hoc testing using Mann-

Whitney U test. Correlations between variables were determined by using Pearson or Spearmans rank correlation tests, where appropriate. Values of the parameters were tabulated as percentage, mean ± standard deviation and median. A two-sided p value less than 0.05 was accepted as statistically significant.

III. RESULTS

A total of 177 children were recruited. Four groups were constituted from the study population. First group was composed of children with normal BMI. Obese children were divided into three groups. There were OB and MO children in Group 2 and Group 3, respectively. Morbid obese children with MetS were included into Group 4. The number of cases in Group 1, Group 2, Group 3 and Group 4 were 44, 43, 45 and 45, respectively. Mean age ± standard deviation (standart error) values of the children in groups were 10.9 ± 4.2 (0.6) years for children with normal BMI, 12.1± 3.2 (0.5) years for OB children, 10.6 ± 3.5 (0.5) years for MO children and 12.1 ± 2.6 (0.4) years for children with MetS. Any statistically significant difference was not observed between any two groups (p>0.05). Female-to-male ratios were 1.1, 1.2, 1.1 and 0.9 for Groups 1, 2, 3, and 4, respectively. No difference was observed among the groups in terms of gender difference (p>0.05).

Body mass index, waist circumference, hip circumference, head circumference, neck circumference, systolic blood pressure, diastolic blood pressure, fasting blood glucose, fasting insulin, HOMA-IR, total cholesterol, TRG, LDL-C, HDL-C values were tabulated in Table I.

The values for some current and potential cardiovascular markers measured in groups of the study population were shown in Table II.

TABLE I
ANTROPOMETRIC MEASUREMENTS AS WELL AS CLINICAL AND BIOCHEMICAL PARAMETERS IN CHILDREN WITH N-BMI, OBESITY, MORBID OBESITY AND METABOLIC SYNDROME

| | | Group 1 N-BMI x±SD | Group 2 OB x±SD | Group 3 MO x±SD | Group 4 MetS x±SD |
|-------------------|-------------------|--------------------------|-----------------------|-----------------------|-------------------------|
| BMI | kg/m ² | 17.6±3.0 | 23.7±3.4 | 27.6±5.3 | 30.3±4.7 |
| Waist C | cm | 64.8±11.8 | 80.9±8.4 | 88.2±14.2 | 97.1±10.4 |
| Hip C | cm | 77.4±14.7 | 93.2±11.9 | 97.0±16.2 | 104.7±14.3 |
| Head C | cm | 53.1±2.7 | 54.5±2.0 | 55.1±2.3 | 55.4±2.2 |
| Neck C | cm | 29.3±3.8 | 32.9±3.7 | 33.6±3.8 | 35.7±3.2 |
| SBP | (mm Hg) | 102±10 | 115±17 | 113±12 | 126±16 |
| DBP | (mm Hg) | 69±8 | 74±11 | 72±10 | 85±11 |
| FBG | (mg/dL) | 92.5±5.9 | 94.9±9.3 | 90.6±5.5 | 97.9±8.3 |
| FI ^m | (µIU/mL) | 9.76 | 14.94 | 19.33 | 33.01 |
| HOMA ^m | | 2.37 | 3.32 | 4.30 | 7.87 |
| TC | (mg/dL) | 156.3±29.9 | 159.8±28.0 | 156.1±28.3 | 177.6±34.6 |
| TRG | (mg/dL) | 84.6±43.1 | 101.7±46.0 | 90.8±40.8 | 165.9±89.0 |
| LDL-C | (mg/dL) | 83.1±28.7 | 89.5±27.6 | 86.3±24.4 | 99.9±27.4 |
| HDL-C | (mg/dL) | 56.2±12.9 | 52.8±12.1 | 51.5±8.5 | 46.8±10.0 |

BMI = body mass index, N= normal, OB= obese, MO= morbid obese,

MetS= metabolic syndrome, C=circumference, SBP=systolic blood pressure, DBP=diastolic blood pressure, FBG=fasting blood glucose, FI=fasting insulin, HOMA-IR=homeostatic model assessment for insulin resistance index, TC=total cholesterol, TRG=triglycerides, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol

[BMI 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-3 <0.001, 2-4 <0.001, 3-4 <0.05 waist C 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 2-3 <0.05, 3-4 <0.01 hip C 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 2-3 <0.001, 3-4 <0.001, head C 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-3 <0.001, 2-4 <0.001, 3-4 <0.001, neck C 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 3-4 <0.05 SBP 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 2-3 <0.001, 3-4 <0.001, DBP 1-4 <0.001, 2-4 <0.001, 3-4 <0.001, FBG 1-4 <0.001, 3-4 <0.001, 2-3 <0.05 FI 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 3-4 <0.001, HOMA-IR 1-2 <0.001, 1-3 <0.001, 1-4 <0.001, 2-4 <0.001, 3-4 <0.001, TC 1-4 <0.01, 3-4 <0.01, 2-4 <0.05 TRG 1-4 <0.001, 2-4 <0.001, 3-4 <0.001, LDL-C 1-4 <0.05 HDL-C 1-4 0.01 m median

TABLE II

HIGH SENSITIVE CARDIAC TROPONIN T, MULTIMERIN-2, CHROMOGRANIN A, TRIMETHYLAMINE N-OXIDE, SOLUBLE TUMOUR NECROSIS FACTOR LIKE WEAK INDUCER OF APOPTOSIS AND CARDIAC MYOSINE BINDING PROTEIN-C LEVELS IN CHILDREN WITH NORMAL BODY MASS INDEX, OBESITY, MORBID OBESITY AND METABOLIC SYNDROME

| | | Group 1 N-BMI median | Group 2 OB median | Group 3 MO median | Group 4 MetS median |
|------------|---------|----------------------------|-------------------------|-------------------------|---------------------------|
| hs cTnT | (ng/L) | 31.5 | 32.1 | 30.4 | 33.3 |
| Multimerin | (ng/mL) | 2.36 | 2.12 | 2.14 | 2.30 |
| CgA | (ng/L) | 843 | 691 | 688 | 677 |
| TMAO | (ng/ml) | 5.37 | 5.10 | 4.72 | 5.07 |
| sTWEAK | (ng/L) | 351 | 325 | 309 | 367 |
| cMyBP-C | (ng/mL) | 3.79 | 4.02 | 4.26 | 4.50 |

N=Normal, OB=Obese, MO=Morbid Obese, MetS=Metabolic Syndrome, BMI=Body Mass Index, hs cTnT=high-sensitive cardiac troponin T, CgA=chromogranin A, TMAO=trimethylamine N-oxide, sTWEAK=soluble tumor necrosis factor-like weak inducer of apoptosis, cMyBP-C= cardiac myosin binding protein C, NS=not significant

[hs cTnT^{NS}multimerin-2^{NS}CgA^{1-2 <0.05, 1-3 <0.05, 1-4 <0.05}TMAO^{NS}sTWEAK^{NS}cMyBP-C^{1-4 <0.05, 2-4 <0.05}]

Upon evaluation of the study population (n=177), statistically significant negative correlations were found between multimerin and BMI (r= - 0.195; p<0.01) as well as waist C (r= - 0.219; p<0.01).

Negative correlations were observed between CgA and DBP in MO (r= - 0.308; p<0.05) and MetS groups (r= - 0.346; p<0.05).

cMyBP-C was positively correlated with DBP in OB group (r= 0.487; p<0.001) (Fig. 1).

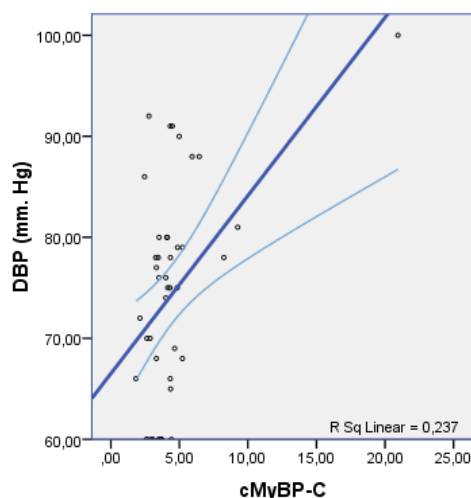


Fig. 1 Bivariate correlation between diastolic blood pressure and cardiac myosin binding protein-C with linear regression line with 95.0% mean prediction interval in obese children

None of these correlations related to DBP was detected in children with N-BMI.

In MO group, correlations were calculated between cMyBP-C and some lipid parameters (cMyBP-C vs TRG (r= 0.316; p<0.05) and cMyBP-C vs HDL-C (r= - 0.369; p<0.05).

IV. DISCUSSIONS

In recent century, easier food availability, reduced physical activity, increasing use of medicines such as antidepressants leading to obesity development, make obesity a global health problem with increasing prevalence both in adults and children [16]-[21].

Under the influence of inflammatory events, abnormal secretion of adipokines, increased fatty acids in obesity, the prevalence of morbidity- and mortality-causing diseases such as CVDs, type 2 diabetes mellitus, dyslipidemia, and cancer increases particularly in adults [22], [23]. Since obesity cases are being observed in earlier ages and with higher prevalences than previous years, complications such as hypertension, dyslipidemia and insulin resistance formerly mostly detected in adults are being detected much more common in children. In spite of having normal body weight during their adulthood, individuals with the history of pediatric obesity meet with CVDs more frequently [24], [25].

In our study, serum CgA levels were significantly lower in OB, MO and MetS groups compared to children with N-BMI. Besides, serum CgA levels of MO and MetS children were inversely correlated with DBP values. This finding agrees with the studies performed on patients with myocardial infarction (MI) as well as acute coronary syndrome [26], [27]. However, when the elevated CgA levels observed in CVDs, hypertension and insulin resistance associated with obesity were considered, this finding has become an unexpected one. This finding makes one think that CgA in children may act by different mechanisms other than those in adults and therefore, may introduce CgA as a valuable parameter in childhood obesity.

In our study, decreases detected in OB, MO and MetS groups in comparison to the group with N-BMI pointed out the CgA-induced “antiinflammatory ability” and protection of the endothelial barrier against TNF-alpha-induced vascular permeability [28], [29].

It was also reported that physiological CgA levels can work as an endothelial barrier regulator and as an angiogenic switch triggered by proteolytic cleavage [30], [31].

We wanted to assess the function and utility of full-length CgA, because in a recent report, the physiological role of full-length CgA was introduced as a circulating stabilizer of endothelial integrity and in protection against myocardial injury [32].

Full-length CgA inhibits cell adhesion, depress myocardial contractility and relaxation. Full-length CgA induces the production of protease nexin-1. Antiangiogenic protein nexin-1 is a potent inhibitor of two proteolytic enzymes (plasmin and thrombin). These enzymes can cleave CgA. The inhibitor,

nexin-1, might also serve to prevent the cleavage of CgA. Circulating full-length CgA exerts inhibitory activity on angiogenesis. Therefore, nexin-1 preserves the antiangiogenic activity of CgA [33]. It is reported that antiangiogenic agents provide a novel therapeutic option for prevention and treatment of human obesity and its related disorders [34].

In our study, in all obese groups (OB, MO, MetS) CgA levels were depressed compared to control group. Under the light of above information, this finding confirms the anti-inflammatory and antiangiogenic aspects of full-length CgA.

Some studies confirm our findings in an indirect manner [26], [27]. In the first study, CgA levels were evaluated after acute coronary syndrome. CgA levels were found to be inversely correlated with BMI [27]. In another study, patients after MI were examined. Patients, whose CgA levels were above the average value had a lower BMI values than patients with CgA levels below the average [26].

In our study, neither obesity nor MetS caused any significant difference in the concentrations of TMAO, sTWEAK and multimerin in children.

High sensitive-cTnT is a new generation marker used for prediagnosis, diagnosis and prognosis of CVDs. In a study, elevated levels were observed in children with MetS compared to non-OB and OB children without MetS [35]. In another study, no difference was found between OB and control groups [36]. Our findings agree with the results of the latter study.

In recent years, the number of studies related to risk evaluation, diagnosis, and prognosis of CVDs showed a significant increase [1], [4], [12], [13]. Cardiac MyBP-C functions in contraction and relaxation of cardiac muscle as well as timing of systole. For this reason, functional impairment of this protein may cause impaired systolic and diastolic functions. Since it increases much more rapidly starting from the early phases of MI, this parameter may potentially be a more informative marker than serum troponins during early diagnosis of MI [37]-[39]. Besides, it may be helpful for the early diagnosis of heart failure and may be included in the organization of the treatment protocols [40], [41]. Serum cMyBP-C levels are elevated during cardiac stress, most probably due to cardiac protective activity of this parameter. Therefore, levels may be valuable for the determination of CVDs severity and prediction of future cardiovascular events [42].

In our study, cMyBP-C levels of children with MetS were significantly higher than children with N-BMI and obesity. In terms of this parameter, there was not a significant difference between MO children and those with MetS. In MO group, cMyBP-C was correlated with elevated DBP, elevated TRG and depressed HDL-C, which are well-accepted MetS criterias. Cardiac MyBP-C levels correlated with MetS and MetS criteria emphasize the association of this parameter with CVDs risk in obesity.

The lack of a significant difference between MO and MetS group may point out that cardiovascular pathology may begin in some MO children, even if MetS criterias have not been met.

The finding of elevated cMyBP-C levels in MetS make someone think that cMyBP-C may be a valuable marker in the

evaluation as well as early diagnosis of the effects of obesity on cardiovascular system.

Cardiac MyBP-C is a cardiac-specific protein and its expression is restricted to the heart but is at least twice as abundant as cTnT/TnI [43], [44].

Cardiac-MyBP-C has potential as an “ultra-early biomarker” for the diagnosis of heart attack, but still needs further investigation. It was introduced as a new blood test detecting heart attacks more quickly and it could lead to faster treatments. It was suggested as a protein released to the blood stream by damaged heart muscle [45].

In a study performed on adults, cMyBP-C levels were reported as 0.95 ± 0.34 ng/ml in control group whereas 227 ± 50 ng/ml in patients with MI. Circulating cMyBP-C is a sensitive and cardiac-specific biomarker with potential utility for the accurate diagnosis of MI. cMyBP-C released in the circulation very early following a cardiac injury may point out the onset of MI [46], [47].

In our study, hs-cTnT and cMyBP-C, two protein-based biomarkers used for the evaluation of CVDs, were considered during the evaluation of different pediatric obesity stages. While hs-cTnT did not exhibit any statistically significant difference among the groups, statistically significant differences were observed for cMyBP-C concentrations between MetS group and N-BMI as well as OB groups. Elevated levels of cMyBP-C observed in MetS group was a valuable finding in children in our study.

A. Study Limitations

Aside from full-length CgA measured in this study, the determination of its specific fragments, vasostatin 1, serpinin and catenastatin at the same time would make possible to compare their obesity-reducing as well as cardioprotective effects.

V. CONCLUSION

In conclusion, to the best of our knowledge this is the first study to point out elevated cMyBP-C levels in children with MetS, emphasizing the feature of being an ultra-early biomarker in heart attacks. The detection of elevated cMyBP-C levels in MetS group suggests the initiation of potentially life-threatening metabolic events such as cardiac injury during childhood. The previous levels of cMyBP-C may be helpful for the prevention of CVDs. Keeping this parameter within the normal range during early periods of life may reduce the risk of developing CVDs over the course of the lifetime. This may point out that cMyBP-C may be involved in the pathogenesis of obesity-related CVDs.

ACKNOWLEDGMENT

This study was supported by Tekirdag Namik Kemal University Rectorate, Scientific Research Projects Coordination Unit. Project No: NKUBAP.02.TU.20.278.

REFERENCES

- [1] H. C. H. Ho, E. Maddaloni, and R. Buzzetti, “Risk factors and predictive biomarkers of early cardiovascular disease in obese youth,” *Diabetes Metab. Res. Rev.*, vol.35, no.4, pp.e3134, 2019.

- [2] D. Segula, "Complications of obesity in adults: a short review of the literature," *Malawi Med. J.*, vol.26, no.1, pp. 20-24, 2014.
- [3] P. Poirier, T. D. Giles, G. A. Bray, Y. Hong, J. S. Stern, F. X. Pi-Sunyer, and R. H. Eckel, American Heart Association; Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism, "Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism," *Circulation*, vol.113, no.6, pp. 898-918, Feb. 2006.
- [4] I. Csige, D. Ujvárosy, Z. Szabó, I. Lőrincz, G. Paragh, M. Harangi, and S. Somodi, "The impact of obesity on the cardiovascular system," *J. Diabetes Res.*, vol.2018, pp.3407306, Nov. 2018.
- [5] M. Bastien, P. Poirier, I. Lemieux, and J.P. Després, "Overview of epidemiology and contribution of obesity to cardiovascular disease," *Prog. Cardiovasc. Dis.*, vol.56, no.4, pp.369-381, 2014.
- [6] H. C. McGill Jr, C. A. McMahan, E. E. Herderick, A. W. Zieske, G. T. Malcom, R. E. Tracy, and J. P. Strong, "Pathobiological determinants of atherosclerosis in youth (PDAY) research group. Obesity accelerates the progression of coronary atherosclerosis in young men," *Circulation*, vol.105, no.23, pp. 2712-2718, Jun. 2002.
- [7] M. A. Alpert, J. Omran, and B.P. Bostick, "Effects of obesity on cardiovascular hemodynamics, cardiac morphology, and ventricular function," *Curr. Obes. Rep.*, vol.5, no.4, pp.424-434, 2016.
- [8] A. Smekal, and J. Vaclavik, "Adipokines and cardiovascular disease: A comprehensive review," *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, vol.161, no.1, pp. 31-40, 2017.
- [9] I. Janssen, P. T. Katzmarzyk, and R. Ross, "Waist circumference and not body mass index explains obesity-related health risk," *Am. J. Clin. Nutr.*, vol.79, no.3, pp.379-384, 2004.
- [10] N. Katsiki, V. G. Athyros, and D. P. Mikhailidis, "Abnormal peri-organ or intra-organ fat (APIFat) deposition: An underestimated predictor of vascular risk?," *Curr. Vasc. Pharmacol.*, vol.14, no.5, pp. 432-441, 2016.
- [11] D. Ural, "Kardiyovasküler risk belirlenmesi ve tabakalandırılmasının kılavuzluğuyla yapılan tedavi yaklaşımı: Öngör, önlenebilir ve bireyselleştir," *Anadolu Kardiyol. Derg.*, vol.11, pp.551-556, 2011.
- [12] M. Thiriet, "Cardiovascular risk factors and markers," in *Vasculopathies. Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems*, vol. 8, M. Thiriet, Ed. Cham: Springer, 2018, pp. 91-118.
- [13] M. N. Lyngbakken, P. L. Myhre, H. Røsjø, and T. Omland, "Novel biomarkers of cardiovascular disease: Applications in clinical practice," *Crit. Rev. Clin. Lab. Sci.*, vol.56, no.1, pp.33-60, 2019.
- [14] World Health Organization (WHO). The WHO Child Growth Standards. 2016 June. Access: <http://www.who.int/childgrowth/en/>
- [15] P. Zimmet, K. G. Alberti, G. Kaufman, N. Tajima, M. Silink, S. Arslanian, G. Wong, P. Bennett, J. Shaw, S. Caprio, and IDF consensus group, "The metabolic syndrome in children and adolescents-an IDF consensus report," *Pediatr. Diabetes*, vol.8, no.5, pp. 299-306, 2007.
- [16] E. H. Zobel, T. W. Hansen, P. Rossing, and B. J. von Scholten, "Global changes in food supply and the obesity epidemic," *Curr. Obes. Rep.*, vol.5, no.4, pp. 449-455, 2016.
- [17] B. Srouf, L. K. Fezeu, E. Kesse-Guyot, B. Allès, C. Méjean, R. M. Andrianasolo, E. Chazelas, M. Deschasaux, S. Hercberg, P. Galan, C. A. Monteiro, C. Julia, and M. Touvier, "Ultra-processed food intake and risk of cardiovascular disease: prospective cohort study (NutriNet-Santé)," *BMJ*, vol.365, pp.11451, May 2019.
- [18] B. Swinburn, G. Sacks, and E. Ravussin, "Increased food energy supply is more than sufficient to explain the US epidemic of obesity," *Am. J. Clin. Nutr.*, vol. 90, no.6, pp.1453-1456, 2009.
- [19] U.S. Department of Health and Human Services: Physical Activity Guidelines for Americans, (2nd ed). Washington, DC: U.S. Department of Health and Human Services; 2018.
- [20] C. M. Apovian, "Obesity: definition, comorbidities, causes, and burden," *Am. J. Manag. Care*, vol.22, no.7 Suppl, pp. s176-185, 2016.
- [21] R. Gafoor, H. P. Booth, and M. C. Gulliford, "Antidepressant utilization and incidence of weight gain during 10 years' follow-up: population based cohort study," *BMJ*, vol.361, pp. 1951, 2018.
- [22] Obezite, Lipid Metabolizması, Hipertansiyon Çalışma Grubu: Obezite Tanı ve Tedavi Kılavuzu. Ankara: Türkiye Endokrinoloji ve Metabolizma Derneği, 2019.
- [23] J. L. Miner, "The adipocyte as an endocrine cell," *J. Anim. Sci.*, vol.82, no. 3, pp. 935-941, 2004.
- [24] C. Iacobini, G. Pugliese, C. Blasetti Fantauzzi, M. Federici, and S. Menini, "Metabolically healthy versus metabolically unhealthy obesity," *Metabolism*, vol.92, pp.51-60, 2019.
- [25] S. R. Daniels, "Complications of obesity in children and adolescents," *Int. J. Obes. (Lond.)*, vol.33, no. Suppl 1, pp.S60-65, 2009.
- [26] M. E. Estensen, A. Hognestad, U. Syversen, I. Squire, L. Ng, J. Kjekshus, K. Dickstein, and T. Omland, "Prognostic value of plasma chromogranin A levels in patients with complicated myocardial infarction," *Am. Heart J.*, vol.152, no.5, pp.e1-6, Nov. 2006.
- [27] A. M. Jansson, H. Røsjø, T. Omland, T. Karlsson, M. Hartford, A. Flyvbjerg, and K. Caidahl, "Prognostic value of circulating chromogranin A levels in acute coronary syndromes," *Eur. Heart J.*, vol.30, no.1, p.25-32, Jan. 2009.
- [28] E. Ferrero, S. Scabini, E. Magni, C. Foglieni, D. Belloni, B. Colombo, F. Curnis, A. Villa, M. E. Ferrero, and A. Corti, "Chromogranin A protects vessels against tumor necrosis factor alpha-induced vascular leakage," *FASEB J.*, vol.18, pp.554-555, 2004.
- [29] B. Tota, T. Angelone, and M. C. Cerra, "The surging role of Chromogranin A in cardiovascular homeostasis," *Front. Chem.*, vol.2, pp.64, 2014.
- [30] A. Corti, and B. Tota, "CgA in heart diseases: more than meets the eye," *Lancet Diabetes Endocrinol.*, vol.1, no.2, pp. 90, 2013.
- [31] L. Crippa, M. Bianco, B. Colombo, A. M. Gasparri, E. Ferrero, Y. P. Loh, F. Curnis, and A. Corti, "A new chromogranin A-dependent angiogenic switch activated by thrombin," *Blood*, vol.121, no.2, pp.392-402, 2013.
- [32] K. B. Helle, M. H. Metz-Boutigues, M. C. Cerra, and T. Angelone, "Chromogranins: from discovery to current times," *Pflugers Arch.-Eur. J. Physiol.*, vol.470, pp.143-154, 2018.
- [33] S. K. Mahata, and A. Corti, "Chromogranin A and its fragments in cardiovascular, immunometabolic and cancer regulation," *Annals NYAS*, vol.1455, no.1, pp.34-58, 2019.
- [34] Y. Cao, "Angiogenesis modulates adipogenesis and obesity," *J. Clin. Invest.*, vol.117, no.9, pp.2362-2368, 2007.
- [35] P. Collinson, "The role of cardiac biomarkers in cardiovascular disease risk assessment," *Curr. Opin. Cardiol.*, vol.29, no.4, pp.366-371, 2014.
- [36] P. Pervanidou, A. Akalestos, D. Bastaki, F. Apostolakou, I. Papassotiropou, and G. Chrousos, "Increased circulating High-Sensitivity Troponin T concentrations in children and adolescents with obesity and the metabolic syndrome: a marker for early cardiac damage?," *Metabolism*, vol.62, no.4, pp.527-531, 2013.
- [37] M. N. Lyngbakken, P. L. Myhre, H. Røsjø, and T. Omland, "Novel biomarkers of cardiovascular disease: Applications in clinical practice," *Crit. Rev. Clin. Lab. Sci.*, vol.56, no.1, pp.33-60, 2019.
- [38] T. E. Kaier, B. Alaour, and M. Marber, "Cardiac myosin-binding protein C-From bench to improved diagnosis of acute myocardial infarction," *Cardiovasc. Drugs Ther.*, vol.33, no.2, pp.221-230, 2019.
- [39] S. Govindan, A. McElligott, S. Muthusamy, N. Nair, D. Barefield, J. L. Martin, E. Gongora, K. D. Greis, P. K. Luther, S. Winegrad, K. K. Henderson, and S. Sadayappan, "Cardiac myosin binding protein-C is a potential diagnostic biomarker for myocardial infarction," *J. Mol. Cell. Cardiol.*, vol.52, no.1, pp.154-164, Jan. 2012.
- [40] D. El Amrousy, H. Hodeib, G. Suliman, N. Hablas, E. R. Salama, and A. Esam, "Diagnostic and prognostic value of plasma levels of cardiac myosin binding protein-C as a novel biomarker in heart failure," *Pediatr. Cardiol.*, vol.38, no.2, pp.418-424, 2017.
- [41] Y. Sato, H. Fujiwara, and Y. Takatsu, "Biochemical markers in heart failure," *J. Cardiol.*, vol.59, no.1, pp.1-7, 2012.
- [42] C. W. Tong, G. F. Dusio, S. Govindan, D. W. Johnson, D. T. Kidwell, L. M. De La Rosa, P. C. Rosas, Y. Liu, E. Ebert, M. K. Newell-Rogers, J. B. Michel, J. P. Trzeciakowski, and S. Sadayappan, "Usefulness of released cardiac myosin binding protein-C as a predictor of cardiovascular events," *Am. J. Cardiol.*, vol.120, no.9, pp.1501-1507, Jul. 2017.
- [43] J. O. Baker, R. Tyther, C. Liebetrau, J. Clark, R. Howarth, T. Patterson, H. Möllmann, H. Nef, P. Sicard, B. Kailey, R. Devaraj, S. R. Redwood, G. Kunst, E. Weber, and M. S. Marber, "Cardiac myosin binding protein C: a potential early biomarker of myocardial injury," *Basic Res. Cardiol.*, vol.110, no.3, pp.23, May 2015.
- [44] X-J.Chen, W. Zhang, Z-P.Bian, Z-M.Wang, J. Zhang, H-F.Wu, Y-F Shao, J-N Zhang, and S. Zhao, "Cardiac myosin-binding protein C release profile after cardiac surgery in intensive care unit," *Ann. Thorac. Surg.*, vol.108, pp.1195-1201, 2019.
- [45] Loyola Medicine. New Blood Test Could Detect Heart Attacks More Quickly. Ultra-early marker could lead to faster treatments. 2014 Feb. Access: <http://www.newswise.com/articles/new-blood-test-could-detect-heart-attacks-more-quickly>

- [46] S. Govindan, D. W. Kuster, B. Lin, D. J. Kahn, W. P. Jeske, J. M. Walenga, F. Leya, D. Hoppensteadt, J. Fareed, and S. Sadayappan, "Increase in cardiac myosin binding protein-C plasma levels is a sensitive and cardiac-specific biomarker of myocardial infarction," *Am. J. Cardiovasc. Dis.*, vol.3, no.2, pp.60-70, 2013.
- [47] D. W. Kuster, A. Cardenas-Ospina, L. Miller, C. Liebetrau, C. Troidl, H. M. Nef, H. Möllmann, C. W. Hamm, K. S. Pieper, K. W. Mahaffey, N. S. Kleiman, B. D. Stuyvers, A. J. Marian, and S. Sadayappan, "Release kinetics of circulating cardiac myosin binding protein-C following cardiac injury," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 306, no. 4, pp.H547-556, 2014.