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Research Article

Total Serum Protein as an Independent Predictor of Heart Failure with Preserved Ejection Fraction in Obese Pediatric Population

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Abstract

Objectives: We aimed to clarify the prognostic role of Total Serum Protein (TSP) in obese children with HFpEF and its using as an effective and non-invasive approach for screening of target population.

Methods: In total, 587 patients who enrolled in our unique program aimed for children's obesity treatment were referred. Among these patients, we identified and retrospectively studied 64 patients who met our criteria and compared them with 24 lean healthy subjects. Baseline examination, routine blood testing and transthoracic echocardiography were obtained.

Results: We revealed that obese patients had higher TSP levels compared to them with normal weight. They also had worse echocardiographic results including a lower Left Ventricular Ejection Fraction (LVEF) and E/A ratio. Positive correlations between TSP and Pulmonary Artery Systolic Pressure (PASP), Left Atrial Volume Index (LAVI), and Interventricular Septal Systolic/Diastolic Dimension (IVSs, IVSd) and negative TSP correlations with LVEF and E/A ratio were found, too. Compared to the commonly used Albumin-to-Globulin Ratio (AGR), the TSP was a better metabolic predictor. There were more significant correlations in obese subgroup with HFpEF than to those without HFpEF.

Conclusions: We first indicated that higher TSP levels are positively associated with obesity and HFpEF in children and could be used as a more easily available biomarker which provides a more-accurate HFpEF risk evaluation of obese pediatric population group than other objective indices, possibly allowing early implementation of appropriate intervention in daily practice and leads to better outcomes and early prevention in patients with higher HF risk.

Keywords: Heart failure; Preserved ejection fraction; Obesity; Total serum protein

Introduction

Reduced Ejection Fraction (EF) has traditionally been used to represent Heart Failure (HF) syndromes, but it is now widely acknowledged that nearly Half of HF patients have Preserved EF (HFpEF). The diagnosis of HFpEF requires the following conditions to be satisfied: (1) signs or symptoms of HF; (2) normal or mildly abnormal systolic Left Ventricular (LV) function; (3) evidence of diastolic LV dysfunction [1]. Epidemiological studies have suggested a high prevalence of HFpEF in adults (1.1 - 5.5%), incidence has increased over the past decades [2,3]. Numerous studies have enhanced understanding of HFpEF [4-10]. However, all of them have been conducted in adults, and there are very limited informations regarding HFpEF in children [11].

Childhood obesity has reached epidemic proportions worldwide [12]. It has long been described as a major comorbidity in HFpEF patients [13-15]. Obesity has been proposed as a major driver of systemic inflammation, ultimately predisposing to myocyte remodeling and the development of HFpEF [16-19]. Obesity and HFpEF is substantiated by prior community-based studies, demonstrating an association of obesity with future HFpEF [20-22]. Obesity and related cardio-metabolic traits are also more strongly associated with the risk of future HFpEF rather than HFrEF, suggesting that obesity-associated HFpEF represents a distinct clinical phenotype within the broad spectrum of HFpEF [23,24].

However, in epidemiological studies mild to moderate overweight or obesity status was reported to have a protective effect in patients with HF [25,26]. This phenomenon was termed "the obesity paradox" and initially observed in small population studies [27,28] and confirmed in large observational studies in both HFrEF and HFpEF patients [29-31]. But other studies have not shown that the obesity paradox exists in HFpEF [32-34], and thus, the causal link between this scientific observation and its clinical implications are limited and remain hotly debated. Several hypotheses are proposed to explain the presence or absence of the obesity paradox [35, 36], and have been extensively reviewed [37-39].

Because of the potential cardiovascular consequences associated with obesity, it is vital to identify children at risk of HFpEF-identification of promising prognostic factors could improve their

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long-term survival. Numerous prognostic markers associated with HF have been identified, but their clinical applicability is limited and precise risk stratification remains challenging [40-42]. There is a lack of consensus on how we define HFpEF. This lack of uniformity in disease definition stems in part from an incomplete understanding of disease pathobiology, phenotypic heterogeneity, and natural history. Although most criteria rely on the presence of clinical symptoms and preserved ejection fraction, there is substantial variation regarding the use of biomarkers, abnormal cardiac structure and function ascertained by echocardiography, and previous hospitalizations to define HFpEF [43-45]. Unlike other diseases within cardiovascular medicine (atrial fibrillation, hypertension, etc.) where definitions are centered around a specific diagnostic test, HFPEF is a clinical syndrome for which we rely on a constellation of symptoms, signs, and other manifestations. Thus, simple but effective prognostic biomarker models are needed to improve the management of the HF epidemic.

In recent studies, the correlation between Serum Albumin (sALB) and Globulins (sGLB), commonly used in clinical practice as Albumin-Globulin Ratio (AGR), has been confirmed to be associated with impaired survival in patients with HF [46]. However, the TSP, including not only sALB and sGLB, but also other inflammatory proteins, as a cumulative and effective prognostic biomarker in early diagnosis of HF has not been studied previously [47]. What is more, TSP measurement is often not included in the routine battery of blood tests of cardiac patients, presumably because interpretations may be uncertain in a clinical setting. There is an ongoing debate whether TSP can be used as a causal risk factor, or merely a nonspecific marker of disease.

The hypothesis underlying the current study was that HFpEF status can be accessed via total protein level in serum that assess multiple pathways of disease as a low-risk, non-invasive approach for screening of obese children.

Methods

Patients

All medical records of patients were referred between August 20, 2017 and December 15, 2019 to the School of Obesity-a unique interdisciplinary outpatient program aimed for children's obesity treatment supervised by the Department of Metabolic Disease of the Pediatric Clinic at the Children's Faculty Hospital, Kosice, since 2017 as the only one of its type in Slovakia. The programme has been performing according to the Declaration of Helsinki, and the hospital ethics review board approved the protocol. Only patients whose parents signed the approved voluntary informed consent document for this study have been including.

The data were analyzed retrospectively to identify pediatric patients (<18 years old) with HFpEF. HFpEF was defined as (1) HF signs or symptoms with LVEF >50% and (2) objective evidence of diastolic dysfunction obtained by echocardiography [48]. Clinical signs and symptoms of HF were based on a modification of the previously described criteria in adults: history of acute pulmonary edema, or the presence of at least 2 of the following clinical features with no other identifiable cause and improvement following diuresis: dyspnea, bilateral edema of the lower extremities, or hepatomegaly.

64 subjects who were enrolled in our School of Obesity program were included with following inclusion criteria: under the age of 18 years, diagnosis of overweight or obesity, absence of comorbidities and were compared with 24 lean healthy subjects. Exclusion criteria were as follows: (1) unavailable data of baseline TSP levels, (2) previously diagnosed at least one of any following diseases: significant hematological disorders, thyroid dysfunction, liver or renal insufficiency, infectious or systemic inflammatory diseases and malignant tumors, (3) history of surgical correction of cardiovascular lesions, (4) hypertrophic/restrictive cardiomyopathies, (5) chromosomal abnormalities or (6) interrupted cooperation during follow-up. Lean controls were healthy children matched for age and sex, in whom lipid and glucose metabolism disorders or obesity were not presented.

All patients had to be followed up every 3 months for the 12 months by the trained nurses or cardiologists who were blinded to the aim of this study. The same analysis was performed in the control group and the results were compared.

Data extraction and baseline examination

Data regarding patient demographics, echocardiographic examination, and laboratory measurements including TSP levels were extracted from medical records.

Height and weight were measured, and Body Mass Index (BMI) (kg/m^2) was calculated as Weight (kg) divided by the square of height (m^2). BMI percentiles and Waist Circumference (WC) were measured according to World Health Organization's recommendations [49,50]. Overweight was defined as a BMI at or above the 85th percentile and below the 95th percentile for children and teens of the same age and sex. Obesity was defined as a BMI at or above the 95th percentile for children and teens of the same age and sex [51].

Blood Pressure (BP) was measured with a standard oscillometric sphygmomanometer, and stethoscope placed over the brachial artery pulse, proximal and medial 2 cm above the cubital fossa. The cuff used was appropriate for the size of the child's upper right arm. Systolic and diastolic BP were measured three times after 10 min rest in the supine position, according to the recent recommendations and the average of the three measurements was calculated [52]. Hypertension was defined as BP ≥95th percentile to <95th percentile + 12 mmHg or 130/80 to 139/89 mmHg (for children aged 1-12 years) or as BP> 130-139/80-89 mmHg (for children aged 13 years and older) [53].

Biochemical measurements

Serum was isolated from blood samples collected after overnight fasting. Venous blood (5 ml) was drawn into a red top vacutainer serum tube and placed upright 30 to 60 minutes until clot formation. The tubes were centrifuged in a swinging bucket rotor (1.300 g x 20 min) and the serum was pipetted into 1.5 ml vials. Plasma glucose, serum Triglycerides (TAG), Total Cholesterol (TC) and High-Density Lipoprotein (HDL) cholesterol concentrations were measured with standard laboratory techniques on colorimetric enzymatic assay systems (Siemens ADVIA 1800, Siemens Healthineers, Erlangen, Germany). Low Density Lipoprotein (LDL) cholesterol was calculated according to Fridewald's formula [54]. Fasting serum insulin was measured by a sandwich ECLA method (Roche MODULAR E170, Hoffmann-La Roche AG, Basel, Switzerland).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by photometric kinetic methods (Siemens ADVIA 1800). The levels of albumin (sAlb), globulin (sGlb) and TSP were quantitatively measured by the method based on the biuret reaction, in which an alkaline copper solution reacts with peptide linkages to form a complex that absorbs light at wavelength 540 nm. The sensitivity of reaction was increased in accordance with Lowry method - by the addition of phosphotungstomolybdic acid (Folin-Ciocalteu / phenol reagent).

DM was defined as a fasting plasma glucose ≥126 mg/dl (7.0 mmol/l) in multiple determinations [55]. Dyslipidemia was considered to be present in children if they had fasting total cholesterol ≥200 mg/dl (5.0 mmol/l) or triglyceride ≥150 mg/dl (3.75 mmol/l) [56]. Presence of Metabolic Syndrome (MS) was determined according to IDF 2007 criteria [57].

Echocardiography

Two-dimensional transthoracic echocardiographic examinations were obtained in all subjects in calm state and in the left lateral decubitus position using a Siemens Acuson SC 2000 Prime ultrasound system, with a 2.5 MHz transducer (Siemens Healthineers, Erlangen, Germany), with a frame rate ≥50 frame/sec. All measurements were performed according to the recommendations of the American Society of Echocardiography [58]. All images were digitally stored with at least three cardiac cycles for offline analysis. The conventional recorded parameters included the Left Ventricular Ejection Fraction (LVEF), E/A ratio, Left Atrial Volume Index (LAVI), Pulmonary Artery Systolic Pressure (PASP), and Interventricular Septal Diastolic and Systolic Dimensions (IVSd, IVSs). LVEF was assessed by the biplane Simpson's method, E/A ratio was assessed by color M-mode Doppler. PASP was calculated as 4* (peak TR velocity)^2. As a cut-off value to identify HFpEF was considered EF ≥50% by the end of follow-up. Cut-off values for all recorded parameters were considered according to recent studies [59]. The imaging procedures were conducted by the same professional echocardiographer, masked to the cohort data.

Statistical analysis

Data were processed using methods of descriptive and inductive statistics, depending on the type and number of monitored variables. For the purpose of inductive statistics, we assumed that our data represent a random sample of the relevant population. The first step was a one-dimensional analysis - the tabulation of all monitored variables using frequency tables. The second step was a two-dimensional analysis - the assessment of pairs of monitored variables. To compare numerical and categorical variables (e.g. obesity level), analysis of variance was used to determine the statistical significance of differences, if the distribution of variables was normal. The last step was a multidimensional analysis-a multiple linear regression analysis, where the relationship between several numerical variables was examined simultaneously. Therefore, EF is presented as a dependent (outcome) variable, the baseline and biochemical parameters including TSP as independent (explanatory) variables. Statistical analysis was conducted using Prism 8 (GraphPad Software Inc, San Diego, CA). All of the statistical tests were considered statistically significant if $p < 0.05$. Data were summarized as means ± SD.

Results

In total, 587 patients were referred to our School of Obesity programme from August 2017 through December 2019. Among these patients, we identified 64 patients who met inclusion/exclusion criteria, including 4 with HFpEF (6.25%). The demographic data of these patients are summarized in (Table 1). Compared with the patients without obesity, the ones who were obese had higher levels of TSP (76.5 ± 4.5 *vs.* 71.5 ± 3.5, $p < 0.05$). We also found that body weight, BMI, BMI percentile, WC and systolic and diastolic BP were significantly higher in obese subjects compared to lean controls. Obese (and overweight) subjects had higher levels of ALT, TAG, total and LDL cholesterol compared to control group, while HDL cholesterol was lower.

We found significant differences in the distribution of TSP compared to them with normal weight, as well as sAlb and AGR (Table 2). In this pilot study, simple linear regression analysis showed a positive correlation of TSP with TC. Positive correlations were also found between TSP and sAlb, AGR, respectively. Multiple regression analysis revealed that after adjusting for BW, BMI, BMI percentile, WC and systolic and diastolic BP, sAlb ($r = 0.36$, $p < 0.05$) and AGR ($r = 0.57$, $p < 0.05$) were the only two markers correlating with TSP (Table 3).

Table 1: Baseline characteristics.

Parameter	Obese/Overweight (Ow) Group (n = 64)	Control Group (n = 24)
HFpEF confirmed	4	1
Age (years)	13.2 ± 5.3**	12.8 ± 6.2
Body weight (kg)	76 ± 15.9**	59.8 ± 13.4
Height (cm)	157.1 ± 15.6**	150.4 ± 12.6
BMI (kg/m^2)	29.4 ± 4.4**	25.8 ± 4.1
BMI percentile	95.1 ± 3.5**	37.1 ± 2.9
BMI Z-score	-0.41 ± 1.03**	2.06 ± 0.54
Waist circumference (cm)	93.6 ± 12.9**	70.5 ± 9.6
Systolic BP (mmHg)	126.6 ± 16.3*	120.8 ± 16.6
Diastolic BP (mmHg)	73.5 ± 10.4**	69.9 ± 6.4
Fasting glucose (mmol/l)	4.7 ± 0.4	4.6 ± 1.4
Fasting insulin (IUI/l)	18.2 ± 6.9*	7.5 ± 4.6
Total cholesterol (mmol/l)	4.5 ± 1.0*	4.4 ± 0.6
Triglyceride (mmol/l)	1.29 ± 0.8**	0.9 ± 0.4
HDL cholesterol (mmol/l)	1 ± 0.2*	1.5 ± 0.2
LDL cholesterol (mmol/l)	3.3 ± 1.2*	2.7 ± 0.6
AST (U/l)	31 ± 13	25 ± 11
ALT (U/l)	40 ± 29*	19 ± 7
sAlb (g/l)	45.4 ± 4.2*	40.2 ± 3.7
AGR	1.8 ± 0.4***	1.5 ± 0.4
TSP (g/l)	76.5 ± 4.5*	71.5 ± 3.5

Data are mean as ± SD. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.
AGR: Albumin-To-Globulin Ratio; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: Body Mass Index; BP: Blood Pressure; HDL: High- Density Lipoprotein; LDL: Low-Density Lipoprotein; sAlb: Serum Albumin; TSP: Total Serum Protein.

Table 2: Correlation between total serum protein (TSP) and baseline characteristics.

Parameter	TSP in Obese/Ow Group (N = 64)	TSP in Control Group (N = 24)
	r	r
Body weight (kg)	0.08	0.27
Height (cm)	0.02	0.29
BMI (kg/m^2)	0.16	0.18
BMI percentile	0.24	0.27
Waist circumference (cm)	0.14	0.12
Systolic BP (mmHg)	-0.01	0.04
Diastolic BP (mmHg)	-0.22	-0.19
Fasting glucose (mmol/l)	-0.23	-0.01
Fasting insulin (IUI/l)	-0.02	< 0.05
Total cholesterol (mmol/l)	0.47*	0.42*
Triglyceride (mmol/l)	0.31	0.08
HDL cholesterol (mmol/l)	0.23	0.1
LDL cholesterol (mmol/l)	-0.04	0.19
AST (U/l)	0.16	0.07
ALT (U/l)	0.03	< 0.05
sAlb (g/l)	0.68*	0.59**
AGR	0.65*	0.63**

Data are mean as ± SD. *p<0.05; **p<0.01.
AGR: Albumin-To-Globulin Ratio; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: Body Mass Index; BP: Blood Pressure, HDL: High Density Lipoprotein; LDL: Low-Density Lipoprotein; R: Correlation Coefficient; sAlb: Serum Albumin; TSP: Total Serum Protein.

Table 3: Multiple regression analysis of some metabolic parameters (pooled groups).

Parameter	TSP		
	r	tc	ß coefficient
Total cholesterol (mmol/l)	0.65	0.47	0.91
sAlb (g/l)	0.36*	2.85	-0.08
AGR	0.57*	1.12	-0.11

*p<0.05.
AGR: Albumin-To-Globulin Ratio; r: Correlation Coefficient, sAlb: Serum Albumin; tc: Thigh Circumference; TSP: Total Serum Protein.

Table 4 compared echocardiographic characteristics between obese (and overweight) and lean subjects. For this entire cohort, the participants had a worse echocardiographic results including a lower LVEF (60.8 ± 10.6 *vs.* 63.5 ± 10.1, p <0.001) and E/A ratio (1.67 ± 0.43 vs. 2.05 ± 0.56, p <0.05) than the lean group.

Simple linear regression analysis showed positive correlations of TSP with PASP, LAVI, IVSs and IVSd. Negative correlations of TSP with LVEF and E/A ratio (Table 5). All findings were confirmed using multiple regression analysis, too (Table 6).

As shown in (Table 7), TSP was a better metabolic predictor than AGR not only in relationship with LVEF, but also with other metabolic and echocardiographic parameters, but AGR showed a similar predictive value as TSP (Table 8). shows more significant correlations in obese subgroup with HFpEF than to those without HFpEF.

Table 4: Echocardiographic characteristics.

Parameter	Obese/Ow Group (N = 64)	Control Group (N = 24)
LVEF (%)	60.8 ± 10.6***	63.5 ± 10.1
LV diastolic dimension (mm)	47.5 ± 3.3*	43.4 ± 4.4
Relative wall thickness	0.36 ± 0.04*	0.32 ± 0.03
LV mass index (g/m^2)	38.5 ± 9.5*	34.2 ± 8.9
E/A ratio	1.67 ± 0.43*	2.05 ± 0.56
LAVI (ml/m^2)	28.5 ± 8.1***	27.2 ± 8.3
PASP (mmHg)	31.7 ± 5.2***	31.3 ± 4.9
IVSs (mm)	11.6 ± 6.1***	12.5 ± 7.0
IVSd (mm)	11.9 ± 6.0***	11.6 ± 2.0

Data are mean as ± SD. *p<0.05; **p<0.01; ***p<0.001.
IVSd: Interventicular Septal Diastolic Dimension; IVSs: Interventricular Septal Systolic Dimension; LAVI: Left Atrial Volume Index; LVEF: Left Ventricular Ejection Fraction; PASP: Pulmonary Artery Systolic Pressure; TAPSE: Tricuspid Annular Plane Systolic Excursion.

Table 5: Correlation between total serum protein (TSP) and echocardiographic characteristics.

Parameter	TSP in Obese/Ow Group (N = 64)	TSP in Control Group (N = 24)
LVEF (%)	-0.84**	-0.72
LV diastolic dimension (mm)	-0.61*	-0.58
Relative wall thickness	0.57*	0.53
LV mass index (g/m^2)	0.63*	0.59
E/A ratio	-0.57*	-0.54
LAVI (ml/m^2)	0.65**	0.63
PASP (mmHg)	0.71**	0.66
IVSs (mm)	0.54*	0.5
IVSd (mm)	0.58*	0.59

*p<0.05; **p<0.001.
IVSd: Interventicular Septal Diastolic Dimension; IVSs: Interventricular Septal Systolic Dimension; LAVI: Left Atrial Volume Index; LVEF: Left Ventricular Ejection Fraction; PASP: Pulmonary Artery Systolic Pressure; TAPSE: Tricuspid Annular Plane Systolic Excursion.

Table 6: Multiple regression analysis of some echocardiographic parameters (pooled groups).

Parameter	TSP		
	r	tc	ß coefficient
LVEF (%)	-0.39*	0.41	0.37
LV diastolic dimension (mm)	-0.48**	0.59	0.22
Relative wall thickness	0.52**	0.24	0.19
LV mass index (g/m^2)	0.49**	0.37	0.08
E/A ratio	-0.38**	0.31	0.17
LAVI (ml/m^2)	0.52*	0.43	0.16
PASP (mmHg)	0.49**	0.95	0.25
IVSs (mm)	0.37*	0.44	0.19
IVSd (mm)	0.51*	0.13	0.05

*p<0.05; **p<0.001.
IVSd: Interventicular Septal Diastolic Dimension; IVSs: Interventricular Septal Systolic Dimension; LAVI: Left Atrial Volume Index; LVEF: Left Ventricular Ejection Fraction; PASP: Pulmonary Artery Systolic Pressure; r: Correlation Coefficient; TAPSE: Tricuspid Annular Plane Systolic Excursion; tc: Thigh Circumference; TSP: Total Serum Protein.

Table 7: Correlation of TSP and AGR with chosen metabolic and echocardiographic parameters.

Parameter	TSP in Obese/Ow Group (n = 64)	TSP in Control Group (n = 24)	AGR in Obese/Ow Group (n = 64)	AGR in Control Group (n = 24)
	r	r	r	r
Total cholesterol (mmol/l)	0.47*	0.42	0.25**	0.21
Albumin (g/l)	0.68*	0.59	0.57*	0.55
LVEF (%)	-0.84**	-0.72	-0.10*	-0.1
LV diastolic dimension (mm)	-0.61*	-0.58	0.52*	0.47
Relative wall thickness	0.57*	0.53	0.48*	0.43
LV mass index (g/m^2)	0.63*	0.59	0.55*	0.52
E/A ratio	-0.57*	-0.54	-0.23*	-0.22
LAVI (ml/m^2)	0.65**	0.63	0.58**	0.56
PASP (mmHg)	0.71**	0.66	0.29*	0.28
IVSs (mm)	0.54*	0.5	0.13*	0.1
IVSd (mm)	0.58*	0.59	0.11*	0.11

*p<0.05; **p<0.001.
IVSd: Interventicular Septal Diastolic Dimension; IVSs: Interventricular Septal Systolic Dimension; LAVI: Left Atrial Volume Index; LVEF: Left Ventricular Ejection Fraction; PASP: Pulmonary Artery Systolic Pressure; r: Correlation Coefficient; TAPSE: Tricuspid Annular Plane Systolic Excursion; TSP: Total Serum Protein.

Table 8: Correlation of TSP with chosen metabolic and echocardiographic parameters in the individual subgroups with and without HFpEF.

Parameter	TSP in Obese/Ow Group With HFpEF (n = 4)	TSP in Obese/Ow Group Without HFpEF (n = 60)	TSP in Control Group Without HFpEF (n = 23)
	r	r	r
Total cholesterol (mmol/l)	0.49*	0.45*	0.41
Albumin (g/l)	0.66*	0.63*	0.57
LVEF (%)	-0.84**	-0.82**	-0.7
LV diastolic dimension (mm)	-0.61*	-0.62*	-0.58
Relative wall thickness	0.58*	0.54*	0.53
LV mass index (g/m^2)	0.67*	0.59*	0.58
E/A ratio	-0.55*	-0.55*	-0.51
LAVI (ml/m^2)	0.61**	0.61**	0.66
PASP (mmHg)	0.73**	0.69**	0.63
IVSs (mm)	0.55*	0.50*	0.51
IVSd (mm)	0.56*	0.57*	0.54

*p<0.05; **p<0.001.
IVSd: Interventicular Septal Diastolic Dimension; IVSs: Interventricular Septal Systolic Dimension; LAVI: Left Atrial Volume Index; LVEF: Left Ventricular Ejection Fraction; PASP: Pulmonary Artery Systolic Pressure; r: Correlation Coefficient; TAPSE: Tricuspid Annular Plane Systolic Excursion; TSP: Total Serum Protein.

Discussion

Main finding

In the present study, we report for the first time a strong positive correlation between circulating TSP and the occurrence of HFpEF. More importantly, these results are demonstrated among obese pediatric population. In the literature, there are limited data available about the prognostic significance of TSP in the field of cardiovascular disease, not to mention in children. This fact is amplified by low prevalence of HFpEF in children (0.5%) [11]. our findings also indicated that obesity is may be considered as one of the possible markers of a higher risk of HFpEF development, as similar as TSP, which level was higher in obese children compared to group with normal weight.

Although the study might be limited by follow-up duration, the present data provided novel and important information with regard to the key issue whether the baseline TSP concentration can be a marker for predicting the possible clinical outcomes of obese children's HFpEF as a consequence of the obesity. In addition, these results should provide a basis for better understanding the pathological influence of obesity in HFpEF development in children.

Possible mechanisms

First of all, obesity is characterized by the accumulation of adipose tissue, associated with chronic low-grade inflammatory process [60]. It has already been demonstrated that most of the inflammatory obesity-related proteins are directly produced by the adipose tissue [61]. The question raises whether the increased serum levels of TSP in obesity depend on an altered metabolic status affecting TSP release by the liver or on a direct production from the adipose tissue [62]. Here, we speculate that TSP could constitute an important link between obesity and its comorbidities (HFpEF) by mediating some of the inflammatory effects associated with obesity status. Recently, the contribution of chronic inflammation to HFpEF has been described [63].

Previously, the correlation between sAlb and sGlb with HF has been emphasised. sAlb and sGlb, the two major components of serum proteins, have been confirmed to be involved in the systemic inflammatory process. sAlb indicates nutritional status and relates to chronic inflammation in HF [64,65]. Moreover, increased levels of sGlb could serve as a marker of chronic inflammation response and reflect a cumulative exposure of various proinflammatory cytokines [46]. sAlb is a negative-phase reactant and its synthesis is decreased in both acute and chronic inflammation. Chronic inflammation is also known because acute-phase proteins increase. It is a critical contributor to HF occurrence, development and survival, and is also related to the risk of recurrence among patients with HF [66]. Thus, TSP considered as a marker of immune status may be also a marker of HF risk.

Another alternative explanation is that comorbidities associated with development of HF are also associated with worsening TSP profile. These observations suggest its interesting role as a strong surrogate marker for incident HF among the children, a marker that possibly integrates known and unexplored pathways. *Regan et al*. investigated more than 500 different serum proteins in patients with HFpEF [67]. They have found that biomarkers of angiogenesis, fibrosis, fatty acid metabolism and inflammation are associated with HFpEF and improve discriminative capabilities on top of clinical factors and NT-proBNP. These findings highlight the importance of these pathways in HFpEF and identify potential novel circulating diagnostic biomarkers. Further insight may impact future therapeutic interventions.

In general, the mechanisms underlying TSP changes in obesity, HF and other diseases are not clearly understood. There are many pathological conditions that may influence it. Increase in TSP is associated with dehydration (sAlb likely to be also elevated, too), chronic infection or inflammation (e.g. AIDS, hepatitis, osteomyelitis, endocarditis), paraproteinemia (e.g. myeloma and other causes) or autoimmune disorders (e.g. rheumatoid disease, systemic lupus erythematosus) except 'organ specific' autoimmune diseases without

Autoimmune hepatitis. Low levels only occur as a result of conditions causing low values of the major components, i.e. albumin and the immunoglobulins - particularly IgG (e.g. glomerulonephritis). A low TSP but normal albumin may be the first indication that a patient has humoral immunodeficiency [68].

What is more, *Castleberry et al*. found that obesity itself was not a risk factor for the pediatric cardiomyopathy population as a whole, including symptomatic and asymptomatic individuals [69]. High circulating lipoprotein levels in obese patients may bind and detoxify lipopolysaccharides that play a role in stimulating the release of inflammatory cytokines, all of which may serve to protect them [70,71].

TSP is commonly measured (1) to diagnose nutrition-related chronic deficiencies [72], (2) as a liver function test to support the diagnosis of liver or kidneys disorders which can stimulate the increase or inhibition of its production (meanwhile, the TSP levels are usually decreased in conditions that are commonly associated with liver or kidneys dysfunction), (3) to diagnose chronic inflammations or infections, haematological and (auto) immunodeficiency disorders [73]. However, though TSP has been considered as a biomarker in many other aspects, its role in human cardiovascular physiology remains unknown in the pediatric population nor adults compared to its individual fractions.

Overweight and obese children and adults have elevated serum levels of C-reactive protein, interleukin-6, tumor necrosis factor-α, and leptin [74]. In addition, the concentrations of fibrinogen, orosomucoid, alpha1-antitrypsin, haptoglobin, ceruloplasmin, alpha 1-acid glycoprotein, growth hormone binding protein, ferritin and retinol-binding protein 4, increase with increasing weight [75-77]. It has also been demonstrated that obesity is associated with an increased TSP profile, suggesting the role of body's regulatory mechanism to maintain the adequate protein levels in the serum [78,79].

It has been shown that AGR, the most frequently used biomarker from this group, is associated with subsequent stroke, myocardial infarction or vascular death [80,81]. On the other hand, the relation between AGR and survival has not been well described in patients with chronic HF. At least, *Verma et al*. noted the relation between AGR and outcomes in HF patients - low AGR was associated with high 6- and 12-month mortality [82]. Moreover, patients with low AGR were at higher risk of readmission due to HF [83].

Previous studies have also demonstrated that hypoalbuminemia was associated with impaired survival in patients with HF [84-92]. However, no study investigated the cumulative effect of TSP on patients with HF nor HFpEF. It is in agreement with our findings as we described below.

It needs to be emphasized that all mentioned studies were realized with adult patients and not with children. It shows a huge potential for further investigations. In conclusion, our research presents a first step in this extensive sphere.

Clinical implications

Data reported here allow us to add TSP to the growing list of circulating protein raised in human obesity and somehow involved not only in inflammation and/or in immune response (TNF-, IL-6, CRP, and leptin), but also in HFpEF (CRP, NT-proBNP, NT-proANP, neuropilin, osteopontin) [93-96].

The results of the present study indicate that TSP may provide a clinically useful tool in combination with other standardized clinical, laboratory and imaging predictors of HFpEF in obese children. The prediction of HFpEF risk is a cornerstone of its future management - accurately provided risk evaluation can be of benefit to patients. Patients with worse prognosis might prosper more from an aggressive treatment and a closer follow-up [97]. There exist previous risk models for patients with HF, which adopted a systems biology approach [98,99]. Incorporating information from demographic, biomarker, genomic, proteomic and the initial response to therapy might create a more effective prediction model and hopefully aid in understanding HFpEF prognosis. Hence,

Designing a simple survival model based on routine blood biochemical indexes for clinicians is helpful for better identification of patients at high HFpEF risk.

As a clinical prognostic factor, simplicity is indispensable for daily use. Thus, TSP is a more easily available biomarker which possibly provides a more-accurate HFpEF risk evaluation of obese pediatric

populational group than other objective indices, possibly allowing early implementation of appropriate intervention in daily practice and leads to better outcomes in patients with higher HF risk.

Further validation of the diagnostic accuracy of this approach will require extensive testing in greater numbers of patients at multiple locations as well as a prognostic cohort. It is possible that inclusion of TSP with other predictors of HFpEF will enhance both the fidelity and the efficacy of this approach for diagnostic purposes.

Our recommendations

HFpEF remains challenging to diagnose despite advances in cardiac biomarkers, non-invasive imaging modalities, and provocative testing. Fundamentally, it is important to recognize that part of the problem is that HFpEF is a clinical syndrome with a multitude of contributing risk factors, causes, and phenotypic manifestations. Hence, we propose the following focus areas for future research: (1) determination of TSP for HFpEF progression and clinical trajectories, (2) the potential role of TSP to distinguish HFpEF from non-cardiac causes of dyspnea or other comorbidities, (3) prospective validation of TSP as a potential component of some proposed diagnostic algorithm. Of these focus areas, we would like to highlight that something as fundamental as the relationship between chosen biomarker (including TSP) clinical evaluation and prognosis of HFpEF in pediatric (not only obese) population remains largely understudied. How does TSP level relate to different population groups with HFpEF, and are there potential therapeutic implications? In sum, academic institutions with the capacity for advanced diagnostic testing, should prioritize research into development of novel diagnostic tools for HFpEF in (obese) children.

Study Limitations

Several limitations of this study should be acknowledged. First, we could not assess this causal relationship because of the retrospective study design. Follow-up studies are merited to investigate the TSP levels as a prospective risk factor of obesity and/or HFpEF. Second, there was no detailed information about nutritional or dietary status recorded which may influence TSP concentration, especially state of hydration - meaningful interpretation of results requires that a patient's hydration state is normal. Third, we measured only TSP and AGR from a group of serum proteins. Furthermore, TSP was measured once, on the day of the admission. Assessment of additional measurements, as well as markers including prothrombotic proteins, the fibrinogen, prothrombin, and other inflammatory proteins would improve the reliability of our results, as well as no laboratory screening toward other comorbidities including immune disease, plasma cell tumors or parasitic disease was done. Fourth, detailed echocardiographic assessments were not performed in the present study because of its retrospective design. Advanced echocardiography may help to clarify the relationship not only between TSP and previously mentioned parameters including LVEF, but also with others. Additionally, all examined parameters were derived from adults due to limited data known in the pediatric population. Finally, our analysis was based on a relatively small cohort from one rural area and limited to population from the School of obesity programme. The findings of our study may not be generalizable to the entire population. Despite these limitations, our work represents the first study that focuses on the predictive value of TSP in diagnostic of HFpEF in obese children.

Conclusions

In conclusion, our findings suggest that higher TSP levels are positively associated with obesity and HFpEF in children and could be used as a biomarker that not only serve as a screening and/or prediction tool of development of new onset hepatic, renal or nutritional insufficiency, but also to identify individuals with HFpEF. The novel observation of these results may provide a clue to further elucidating the pathophysiology and appropriate diagnostics of HFpEF. We believe that the present study provides the basis for future studies on this disease.

Although our report provides novel and useful clinical informations of pediatric (obese) patients with HFpEF, some important clinical problems remain unsolved. As discussed, the patient's nutritional state may be highly related to the occurrence of HFpEF, as well as the importance of TSP in this problem. A more precise nutritional evaluation could provide the answer to this question. Another important question is whether monitoring of TSP levels may be effective in diagnostics and potential treatment of HFpEF. In other words, the reason for elevation of TSP levels in (obese) pediatric patients with HFpEF is still not precisely known. Furthermore, it is also unclear whether TSP elevation will be reversible when HFpEF spontaneously improves. In order to provide a diagnostic and therapeutic strategy for HFpEF, a physiological or biochemical approach and consideration of these questions are essential. Future prospective studies would provide the answer to these questions.

The clinical recognition and understanding of HFpEF in children have just started. Thus, correct diagnosis and careful observation of (obese) pediatric patients with this condition will provide more precise clinical information about this type of HF.

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