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Spexin and Fetuin A in Morbid Obese Children

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***Abstract*—**Spexin, expressed in the central nervous system, has attracted much interest in feeding behavior, obesity, diabetes, energy metabolism and cardiovascular functions. Fetuin A is known as the negative acute phase reactant synthesized in the liver. Eosinophils are early indicators of cardiometabolic complications. Patients with elevated platelet count, associated with hypercoagulable state in the body, are also more liable to cardiovascular diseases (CVDs). In this study, the aim is to examine the profiles of spexin and fetuin A concomitant with the course of variations detected in eosinophil as well as platelet counts in morbid obese children. 34 children with normal-body mass index (N-BMI) and 51 morbid obese (MO) children participated in the study. Written-informed consent forms were obtained prior to the study. Institutional ethics committee approved the study protocol. Age- and sex-adjusted BMI percentile tables prepared by World Health Organization were used to classify healthy and obese children. Mean age ± SEM of the children were 9.3 ± 0.6 years and 10.7 ± 0.5 years in N-BMI and MO groups, respectively. Anthropometric measurements of the children were taken. BMI values were calculated from weight and height values. Blood samples were obtained after an overnight fasting. Routine hematologic and biochemical tests were performed. Within this context, fasting blood glucose (FBG), insulin (INS), triglycerides (TRG), high density lipoprotein-cholesterol (HDL-C) concentrations were measured. Homeostatic model assessment for insulin resistance (HOMA-IR) values were calculated. Spexin and fetuin A levels were determined by enzyme-linked immunosorbent assay. Data were evaluated from the statistical point of view. Statistically significant differences were found between groups in terms of BMI, fat mass index, INS, HOMA-IR and HDL-C. In MO group, all parameters increased as HDL-C decreased. Elevated concentrations in MO group were detected in eosinophils ($p < 0.05$) and platelets ($p > 0.05$). Fetuin A levels decreased in MO group ($p > 0.05$). However, decrease was statistically significant in spexin levels for this group ($p < 0.05$). In conclusion, these results have suggested that increases in eosinophils and platelets exhibit behavior as cardiovascular risk factors. Decreased fetuin A behaved as a risk factor suitable to increased risk for cardiovascular problems associated with the severity of obesity. Along with increased eosinophils, increased platelets and decreased fetuin A, decreased spexin was the parameter, which reflects best its possible participation in the early development of CVD risk in MO children.

***Keywords*—**Cardiovascular diseases, eosinophils, fetuin A, pediatric morbid obesity, platelets, spexin.

I. Introduction

OBESITY is one of the contributing factors to many clinical problems such as diabetes mellitus, metabolic syndrome (MetS), cancer and CVDs. Childhood obesity is even more important because it may lead to such chronic diseases during adulthood. Morbid obesity in children is drawing attention in recent years due to the increases in its prevalence. In its advanced stage, MetS may develop. HDL-C is one of the MetS components for diagnosis [1], [2]. It has been reported that HDL-C concentration is inversely correlated with the risk of coronary heart disease and is accepted as a key component of predicting cardiovascular risk [3].

Recently, in some clinical and experimental studies, possible associations between cardiovascular risk and spexin as well as fetuin A have been investigated [4]-[9].

Serum fetuin A, an anti-inflammatory mediator and therefore, negative acute phase reactant, has been found to be associated with an increased risk of CVDs. It was decreased in chronic heart failure (CHF). As an anti-inflammatory mediator and a major inhibitor of calcification, fetuin A may play role in the pathogenesis of CHF [10], [11]. Fetuin A is associated with disease severity and exacerbation frequency in patients with chronic obstructive pulmonary disease [12]. Fetuin A increased in diabetics [13]. In older adults, the association of low fetuin A with increased risk for CVD mortality in patients without diabetes was reported [6].

The participation of spexin is being discussed in the early development of CVD risk [14]. A study reported an inverse correlation between spexin and leptin in adolescents. Therefore, a potential role for spexin in the regulation of satiety and certain cardiovascular risk factors was suggested in obese children [7]. In experimental studies, it was pointed out that spexin inhibited food intake in mice [15]. In rats, spexin treatment ameliorated diabetes induced deleterious cardiometabolic disturbances [16]. In another study performed on rats, it was reported that increased arterial pressure values and decreased heart rate observed with spexin injection were suggested as the indicators of the participation of this parameter in the modulation of cardiovascular activities [8], [9].

The matter of cell blood counts including leukocytes and platelets has been a great concern due to their associations with cardiometabolic risk [17]. Particularly, eosinophil and platelet counts have drawn interest in obesity and MetS [18]-[23]. Individuals with elevated eosinophils as well as elevated platelets are known to be more subjected to CVDs.

The aim of this study was to determine eosinophil and platelet counts as well as spexin and fetuin A concentrations in pediatric population, particularly their profiles among MO children. The possible associations of these parameters with cardiovascular risk factors related to blood pressure, glucose homeostasis and lipid metabolism were also investigated.

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II. Patients and Methods

A. Patients

34 and 51 children were included in Group 1 and Group 2, respectively. The first group comprises children, whose age and sex dependent BMI percentile values were between 85 and 15. The second group consists of children, whose age and sex dependent BMI percentile values were above 99. The study protocol was approved by the Ethical Committee of Tekirdag Namik Kemal University, Faculty of Medicine. Informed consent forms were obtained from the participants.

B. Measurements

Anthropometric measurements including weight and height as well as waist, hip, head and neck circumference values were recorded.

C. Definition of Morbid Obesity

World Health Organization percentile tables were used for composing healthy control and MO groups [24]. Children, whose age and sex dependent BMI percentile values were between 85-15 and above 99 were defined as those with N-BMI and morbid obesity, respectively.

D. Calculation of Indices

BMI [body weight/(body height)^2] as well as fat mass index (FMI) [body fat/(body height)^2] values were calculated. FBG and INS were used to calculate HOMA-IR values.

E. Hematologic and Biochemical Parameters

Complete blood cell (CBC) analysis was performed using automated CBC count analyzer. Systolic and diastolic blood pressure values were recorded. Routine biochemical parameters including FBG, INS, TRG, HDL-C were determined. Concentrations of spexin and fetuin A were measured by enzyme-linked immunosorbent assay.

F. Statistical Analysis

Results were presented either as mean ± SEM or median as appropriate. Data were evaluated using SPSSx Version 16 statistical package programme. Unpaired t and the Mann–Whitney U-tests were used depending on the type of the distribution. Pearson's and Spearman's rank correlation tests were performed to evaluate the relationships among the parameters. Scatter plots with linear regression lines were drawn. $p < 0.05$ was considered as statistically significant.

III. Results

A total of 85 children were included into the scope of the study. Of them, 34 were with N-BMI and 51 with morbid obesity. Parameters, which may be associated with the severity of obesity and introduced as cardiovascular risk factors, were listed in Table I. The results were expressed as mean ± standard error of mean (SEM) for BMI, TRG, HDL-C, FBG, eosinophils and platelets. For INS, HOMA-IR, spexin and fetuin A, median values were given.

Statistically significant increases were found in MO children for BMI, FMI, INS, HOMA-IR and eosinophils. Spexin and HDL-C concentrations were significantly reduced in the same group.

Eosinophil and platelet counts were shown in Figs. 1 and 2, respectively. Figs. 3 and 4 show box plots drawn for spexin and fetuin A concentrations determined in N-BMI and MO groups.

TABLE I
Some Hematological and Biochemical Parameters in Groups

Groups	N-BMI (x ± SEM)	MO (x ± SEM)	P
BMI (kg/m^2)	16.6 ± 0.4	28.6 ± 0.8	0.001
FMI (kg/m^2)	3.0 ± 0.3	10.4 ± 0.7	0.001
TRG (mg/dL)	92.4 ± 8.5	103.3 ± 5.8	NS
HDL-C (mg/dL)	56.0 ± 2.0	49.5 ± 1.7	0.05
FBG	90.0 ± 1.5	91.0 ± 1.0	NS
INS ^{m} (μIU/mL)	7.7	17.9	0.001
HOMA-IR ^{m}	1.7	4.0	0.001
Eosinophils	0.226 ± 0.026	0.300 ± 0.044	0.05
Platelets	316 ± 15	347 ± 14	NS
Fetuin-A ^{m} (mg/L)	445	366	NS
Spexin ^{m} (ng/L)	652	426	0.05

^{m} median

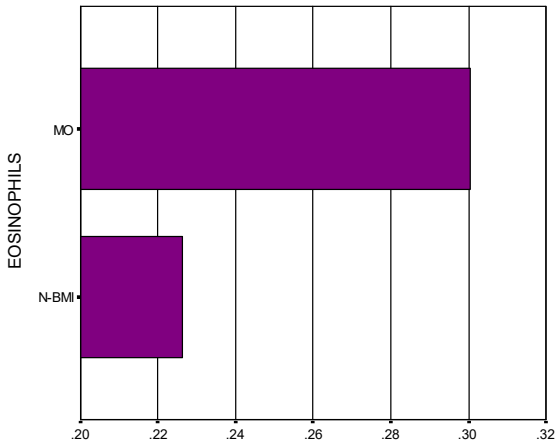

Fig. 1 Eosinophil counts in N-BMI and MO children

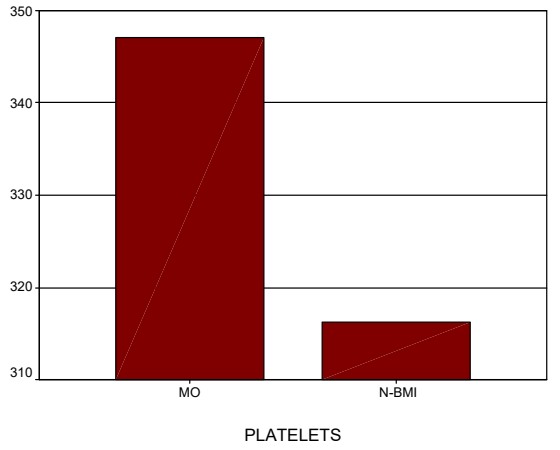

Fig. 2 Platelet counts in N-BMI and MO children

Increased eosinophil ($p < 0.05$) and platelet ($p > 0.05$)

counts were observed in MO children compared to values obtained in children with N-BMI.

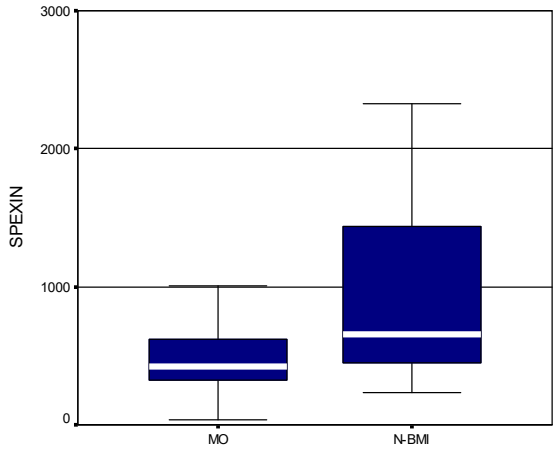

Fig. 3 Spexin levels in N-BMI and MO children. Box plots display the distribution of the data. The rectangles span the interquartile range. The segments inside the rectangles show the medians

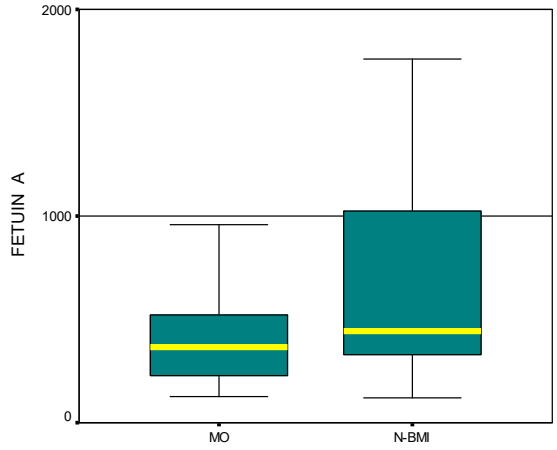

Fig. 4 Fetuin A levels in N-BMI and MO children. Box plots display the distribution of the data. The rectangles span the interquartile range. The segments inside the rectangles show the medians

Significantly lower concentrations were measured for spexin ($p < 0.05$) and fetuin A ($p > 0.05$) in MO group in comparison with concentrations detected in N-BMI group.

In MO group, a statistically significant partial correlation was found ($r = 0.388$; $p = 0.023$) between spexin and HDL-C when adjusted for FMI (Fig. 5). This association was confined to this group. Such a correlation was not detected in children with N-BMI.

IV. Discussion

Spexin and fetuin A are two of the new generation obesity markers [25]. There are few reports investigating the involvement of these parameters in cardiovascular risk [6], [7]. In our study, eosinophil as well as platelet counts and concentrations of fetuin A and spexin were evaluated in children with N-BMI and morbid obesity. Increased eosinophil and platelet counts were detected in the presence of decreased fetuin A and spexin concentrations in MO children.

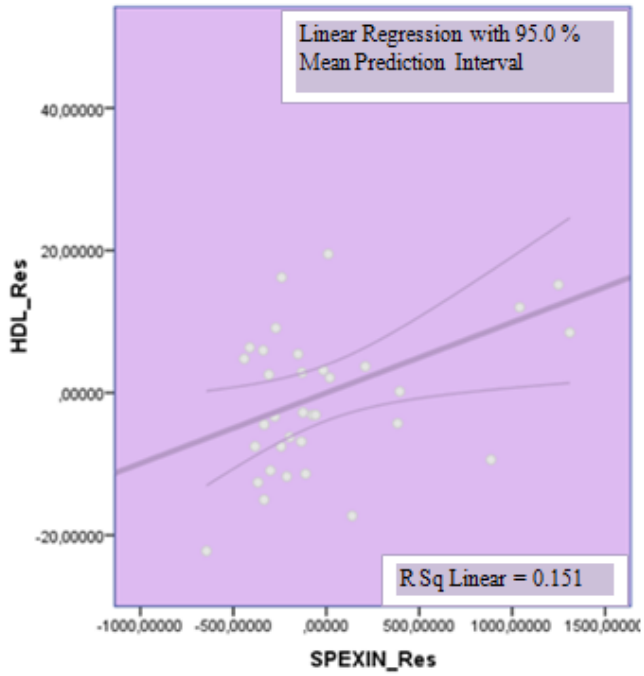

Fig. 5 Partial correlation between spexin and HDL-C controlling for FMI in the group of MO children

Low fetuin A levels are associated with greater CVD risk [4]-[6], [26]. An inverse association was detected between fetuin A and vascular calcification [27]-[29]. Decreased fetuin A levels observed in our study may be introduced as the indicator of down-regulated anti-inflammatory activity of this parameter in MO children.

In a very recent report, the association of spexin levels with MetS was introduced in children [30]. Also, significantly decreased spexin levels were found in obese children [30], [31]. We have also found significantly decreased concentrations for spexin in MO children. All of these studies suggested the potential role of spexin in childhood obesity.

In a previously reported study, spexin was found to be positively correlated with HDL-C in polycystic ovary syndrome [32].

HDL-C is one of the cardiovascular risk factors. It is also considered as one of the MetS components.

In our study, HDL-C as a cardiovascular risk factor was found to be correlated with spexin adjusted for FMI in MO children. To the best of our knowledge this finding was the first in the literature, giving spexin an important role during the evaluation of pediatric morbid obesity from the cardiovascular a well as MetS risks point of view. Within this context, spexin may be a novel, promising, therapeutic target for further CVD as well as MetS research and drug design. The role of spexin as a biomarker possibly associated with cardiovascular and MetS risk leads to further investigations.

References

[1] S. Xu and Y. Xue, “Pediatric obesity: Causes, symptoms, prevention and

treatment," *Exp. Ther. Med.,* vol. 11, pp. 15-20, Jan. 2016.

[2] A. Ladla, P. Tongkrajai, S. Srisaenpang, P. Siviroj, S. Yutthakasemsunt, S. Tiamkao, V. Chotmongkol and K. Sawanyawisuth, "Which diagnostic criteria of metabolic syndrome are predictors of cardiovascular diseases in elderly populations?," *J. Clin. Transl. Endocrinol.,* vol. 23, no. 100248, Mar. 2021.

[3] D. J. Rader and G. K. Hovingh, "HDL and cardiovascular disease," *Lancet.*, vol. 384, no. 9943, pp. 618-625, Aug. 2014.

[4] X. Chen, Y. Zhang, Q. Chen, Q. Li, Y. Li and W. Ling, "Lower plasma fetuin-A levels are associated with a higher mortality risk in patients with coronary artery disease," *Arterioscler. Thromb. Vasc. Biol.,* vol. 37, no. 11, pp. 2213-2219, Nov. 2017.

[5] M. K. Jensen, T. M. Bartz, K. J. Mukamal, L. Djoussé, J. R. Kizer, R. P. Tracy, S. J. Zieman, E. B. Rimm, D. S. Siscovick, M. Shlipak and J. H. Ix, "Fetuin-A, type 2 diabetes, and risk of cardiovascular disease in older adults: the cardiovascular health study," *Diabetes Care.,* vol. 36, no. 5, pp. 1222-1228, May 2013.

[6] G. A. Laughlin, K. M. Cummins, C. L. Wassel, L. B. Daniels and J. H. Ix, "The association of fetuin-A with cardiovascular disease mortality in older community-dwelling adults: the Rancho Bernardo study," *J. Am. Coll. Cardiol.,* vol. 59, no. 19, pp. 1688-1696, May 2012.

[7] S. Kumar, M. J. Hossain, A. Javed, I. J. Kullo and P. B. Balagopal, "Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity," *Pediatr. Obes.,* vol. 13, no. 6, pp. 374-380, Jun. 2018.

[8] S-Y. Lv, Y-C. Zhou, X-M. Zhang, W-D. Chen and Y-D. Wang, "Emerging roles of NPQ/Spexin in physiology and pathology," *Front. Pharmacol.,* vol. 10, pp. 457, May 2019.

[9] L. Toll, T. V. Khroyan, K. Sonmez, A. Ozawa, I. Lindberg, J. P. McLaughlin, S. O. Eans, A. A. Shahien and D. R. Kapusta, "Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception," *FASEB J.,* vol. 26, no. 2, pp. 947–954, Feb. 2012.

[10] M. Keçebaş, S. Güllülü, S. Sağ, F. Beşli, E. Açikgöz, E. Sarandöl and A. Aydinlar, "Serum fetuin-A levels in patients with systolic heart failure," *Acta Cardiol.,* vol. 69, no. 4, pp. 399-405, Aug. 2014.

[11] A. Akyüz, "Association of Fetuin-A with Carotid Intima-Media Thickness and Vascular Diseases", In: Patel V., Preedy V. (eds) *Biomarkers in Cardiovascular Disease. Biomarkers in Disease: Methods, Discoveries and Applications,* Springer, Dordrecht., 2016.

[12] M. Minas, P. Mystridou, P. Georgoulias, S. Pournaras, K. Kostikas and K. I. Gourgoulianis, "Fetuin-A is associated with disease severity and exacerbation frequency in patients with COPD," *COPD.,* vol. 10, no. 1, pp. 28-34, Feb. 2013.

[13] M. Keskin, C. Culha, N. E. Gulcelik, E. Ademoglu, A. Keskin, and Y. Aral, "Fetuin-A levels determine cardiovascular risk in young diabetic patients," *Biomed. Res. India.*, vol. 28, no. 15, pp. 6767-6772, 2017.

[14] A. Khadir, S. Kavalakatt, D. Madhu, S. Devarajan, J. Abubaker, F. Al-Mulla and A. Tiss, "Spexin as an indicator of beneficial effects of exercise in human obesity and diabetes," *Sci. Rep.,* vol. 10, no. 1, pp. 10635, Jun. 2020.

[15] S. Lv, Y. Zhou, Y. Feng, X. Zhang, X. Wang, Y. Yang and X Wang, "Peripheral spexin inhibited food intake in mice," *Int. J. Endocrinol.,* vol. 2020, no. 4913785, Aug 2020.

[16] K. Abd El-Fattah Abul-Fadle, N. El-Huda A. Mohammed, R. M. Al-Sayed, M. M. Abdul-Rahman and A. I. Farag, "Effect of spexin treatment on cardiometabolic changes in obese type 2 diabetic rats," *Al-Azhar Med. J.*, vol. 49, no. 2, pp.735-758, 2020.

[17] R. Kelishadi, M. Hashemipour, P. Ashtijou, P. Mirmoghtadaee, P. Poursafa, N. Khavarian, and S. Ghatrehsamani, "Association of cell blood counts and cardiometabolic risk factors among young obese children," *Saudi Med. J.,* vol. 31, no. 4, pp. 406-412, Apr. 2010.

[18] K. Sakai, S. Inoue, T. Matsuyama, M. Takei, H. Ota, T. Katagiri, and Y. Koboyashi, "Eosinophils may be involved in thrombus growth in acute coronary syndrome," *Int. Heart J.,* vol. 50, no. 3, pp. 267-277, May 2009.

[19] A. V. Finn, "Eosinophils: an overlooked player in acute myocardial infarction. Editorial," *Coron. Artery Dis.,* vol. 26, pp. 99-100, March 2015.

[20] F. L. Roufosse, "Eosinophils: How they contribute to endothelial damage and dysfunction," *Presse Med.,* vol. 42, pp. 503-507, Apr. 2013.

[21] A. Furman-Niedziejko, P. Rostoff, R. Rychlak, K. Golinska-Grzybala, M. Wilczynska-Golonka, M. Golonka, and J. Nessler, "Relationship between abdominal obesity, platelet blood count and mean platelet volume in patients with metabolic syndrome," *Folia Med. Cracov,* vol. 54, no.2, pp. 55-64, 2014.

[22] O. Donma and M. Donma, "Eosinophils and platelets: Players of the game in morbid obese boys with metabolic syndrome," *Int. J. Med. Health Sci.,* vol. 11, no. 5, pp. 257 – 260, May 2017.

[23] O. Donma and M. Donma, "The potential involvement of platelet indices in insulin resistance in morbid obese children," *Int. J. Med. Health Sci.,* vol. 14, no. 3, pp. 85 – 88, Mar. 2020.

[24] World Health Organization (WHO). The WHO Child Growth Standards. Available at: http://www.who.int/childgrowth/en/ Accessed on June 10, 2016.

[25] M. M. Donma, S. D. Erselcan, A. Yilmaz, S. Guzel and O. Donma, "The evaluation of new generation inflammatory markers in children with morbid obesity and metabolic syndrome," *Nam. Kem. Med. J.,* vol. 8, no. 3, pp.479-488, Dec. 2020.

[26] S. A. Aroner, D. E. St-Jules, K. J. Mukamal, R. Katz, M. G. Shlipak, M. H. Criqui, B. Kestenbaum, D. S. Siscovick, I. H. de Boer, N. S. Jenny, M. J. Budoff, J. H. Ix and MK Jensen "Fetuin-A, glycemic status, and risk of cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis," *Atherosclerosis.,* vol. 248, pp. 224-229, May 2016.

[27] L. E. Laugsand, J. H. Ix, T. M. Bartz, L. Djousse, J. R. Kizer, R. P. Tracy, A. Dehghan, K. Rexrode, O. L. Lopez, E. B. Rimm, D. S. Siscovick, C. J. O'Donnell, A. Newman, K. J. Mukamal and M. K. Jensen, "Fetuin-A and risk of coronary heart disease: A Mendelian randomization analysis and a pooled analysis of AHSG genetic variants in 7 prospective studies," *Atherosclerosis.,* vol. 243, no. 1, pp. 44-52, Nov. 2015.

[28] K. Türkmen, H. Kayıkçıoğlu, O. Özbek, A. GaIpov, F. H. Yerlikaya, A. Toker and H. Z. Tonbul, "Relationship between fetuin-A, inflammation, coronary artery calcification in hemodialysis and peritoneal dialysis patients," *Turk. Neph. Dial. Transpl.,* vol. 21, no. 2, pp. 111-117, 2012.

[29] K. Mori, Y. Ikari, S. Jono, M. Emoto, A. Shioi, H. Koyama, T. Shoji, E. Ishimura, M. Inaba, K. Hara and Y. Nishizawa, "Fetuin-A is associated with calcified coronary artery disease," *Coron. Artery Dis.,* vol. 21, no. 5, pp. 281-285, Aug. 2010.

[30] M. Behrooz, E. Vaghef-Mehrabany and A. Ostadrahimi, "Different spexin level in obese vs normal weight children and its relationship with obesity related risk factors," *Nutr. Metab. Cardiovasc. Dis.,* vol. 30, no. 4, pp. 674-682, Apr. 2020.

[31] T. Chen, F. Wang, Z. Chu, L. Sun, H. Lv, W. Zhou, J. Shen, L. Chen and M. Hou, "Circulating spexin decreased and negatively correlated with systemic insulin sensitivity and pancreatic β cell function in obese children," *Ann. Nutr. Metab.,* vol. 74, no. 2, pp. 125-131, 2019.

[32] G. A. Ilhan, and B. Yildizhan, "Spexin as a new metabolic biomarker in women with polycystic ovary syndrome," *Fert. Ster.,* vol. 110, no. 4, e118, Sept. 2018.