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ASSOCIATION OF SERUM VITAMIN D-BINDING PROTEIN WITH COMPONENTS OF METABOLIC SYNDROME IN TYPE 2 DIABETIC PATIENTS IN GORGAN

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The metabolic syndrome (MetS) is an important problem in the development of several disorders. Variations in Vitamin D binding protein (VDBP) may affect insulin secretion and glucose metabolism. This study was conducted to assess serum VDBP between MetS subjects with Type 2 diabetes mellitus (T2DM), and subjects without MetS and its association with MetS components, in Gorgan. 243 patients of T2DM were included in this study. 81 patients with MetS and vitamin D deficiency (group1), 81 patients with MetS and normal vitamin D level (group2) and 81 subjects without MetS and normal vitamin D level (group3). Adult Treatment Panel-III (ATP-III) guidelines are used to define the MetS subjects. Serum VDBP levels were significantly lower in the MetS subjects than the subjects without MetS. The triglyceride (TG) and fasting blood glucose (FBG) levels were significantly lower and higher in subjects with MetS and normal vitamin D when compared to subjects with MetS, and vitamin D deficiency. Serum VDBP levels were significantly lower in MetS subjects and vitamin D deficiency compared to subjects without MetS and normal vitamin D. There were positive and negative correlations between VDBP and FBG and TG in groups 1 and 2. It could be concluded that controlling of VDBP is an effective way for controlling MetS components in subjects with MetS. It suggests that the estimation of VDBP level in different populations appears to play an important role in evaluating and considering the relationship between VDBP and MetS components in subjects with MetS

Key words: *VDBP, Metabolic syndrome, components, Type 2 diabetes mellitus, Gorgan*

INTRODUCTION

Metabolic syndrome (MetS) includes several disorders whose coexistence increases the risk of development of cardiovascular diseases and type 2 diabetes mellitus (T2DM)[1]. MetS shows a cluster of cardiovascular disease (CVD) risk factors associated with high mortality rates, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance, and high blood glucose[2]. People with MetS have a five-time higher risk of type 2 diabetes (T2D), and are two to three times more at risk of CVDs than those without MetS[3]. In today's advanced industrial societies, MetS is a major problem in the development of chronic diseases, and understanding the factors that affect MetS is thus a key research challenge[4]. The MetS indicates a different prevalence in some other populations. Many studies have shown that MetS changes in different ethnic groups and gender[5-7].The global epidemic proportions of MetS were estimated to be around 20–25%. Comparing regions, it was estimated that 12-37% of the Asian population had MetS, whereas

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approximately 12-26% of the European population had MetS[8]. The extent of MetS prevalence varies globally, especially in Asian countries because of differences in lifestyles and ethnicities[9]. One of the substances responsible for the transport of vitamin D is a serum monomeric glycoprotein, also called vitamin D binding protein (VDBP) with a low-molecular weight 52–59 kDa, which is the major carrier protein of vitamin D, and is essential for the intracellular metabolism of vitamin D. VDBP binds almost 90% of circulating vitamin D. Unlike vitamin D, VDBP is probably not subject to seasonal variations [10-11]. VDBP may have additional metabolic roles beyond vitamin D transport. Variations in VDBPs are postulated to influence the amount and activity of vitamin D, which may affect insulin secretion and glucose metabolism[12]. Studies based on knocking out the gene coding for VDBP in mice have shown that this protein has a significant impact on vitamin D activity. The relation between the level of VDBP and the vitamin D level in the blood has also been demonstrated in humans[13-14]. Some studies have shown that there is an association of glycemic control with variations in serum and urine levels of VDBP in diabetic subjects. Yuan et al. indicated that the serum level of VDBP and the urine ratio of VDBP to creatinine in T2DM were significantly higher than control subjects[15]. Another study revealed a significant increase in the urinary excretion of VDBP in subjects with T1DM[16]. Studies on the relationship of VDBP with insulin resistance indices showed that insulin resistance and elevated insulin level are associated with lower levels of VDBP. This may indicate a risk factor for glucose dysregulation[13]. Most of the studies revealed controversial results that it may be an association between the level of VDBP and metabolic disorders. There are arguable research findings among the different studies. However, there is no exact evaluation of VDBP and its association with MetS components in type 2 diabetic patients. The aim of the study was to clarify and evaluate serum VDBP between MetS subjects with Type 2 diabetes mellitus (T2DM), and subjects without MetS and its association with MetS components, in Gorgan.

MATERIALS AND METHODS

Patients were addressed to the non-governmental laboratory in Gorgan. 5 ml blood samples were obtained from studied groups after 12 hours fasting during the period of time from October 2022 to January 2023. Serum samples were prepared and stored at -20°C until measurement. An oral and written consent was obtained from participants after explanation of the purpose and procedures of the study. The study was approved by ethical committee guidelines in the Faculty of Medicine; Golestan University with the ethical code of IR.GOUMS.REC.1401.279.This study was conducted on 243 subjects in three groups. 81 patients with metabolic syndrome and vitamin D deficiency (group1, 45 females and 36 males), 81 patients with metabolic syndrome and normal level of vitamin D (group2, 38 females and 50 males) and 81 subjects without metabolic syndrome and normal level of vitamin D (group3, 40 females and 41 males). The age range for all subjects was 45-55 years. The type2 diabetes mellitus subjects with diabetic ketosis, no diabetic nephropathy or retinopathy complications, and no administration of insulin or Vitamin D and calcium considered as the exclusion criterion. The criteria of the National Cholesterol Education Programme, Adult Treatment Panel III (NCEP, ATP III) was used for inclusion of metabolic syndrome [17]. Metabolic syndrome determined if any of the study subjects had any three or more of the following criteria: Waist circumference (WC): >102 cm (male), >88 cm (female); triglyceride (TG) levels: >150 mg/dl; high density lipoprotein cholesterol (HDL-C) levels: <40 mg/dl (male), <50 mg/dl (female); blood pressure: >110//85 mmHg; and fasting blood glucose (FBG) levels: >110 mg/dl. The biochemical parameter (FBG, TG, HDL-C) were determined by using commercial kits (PARS AZMON, Iran) and spectrophotometer method. The VDBP and vitamin D levels (Cat. No E1402Hu BT lab., and Cat. No ab213966 25 (OH)D, China) were measured by The Enzyme-Linked Immunosorbent Assay (ELISA) kits (Deficiency and normal levels of vitamin D defined if 25(OH) vitamin D level is below 20 ng/ml and higher than 30 ng/ml, not shown). A tape in centimeters was used to assess waist circumference (WC). Systolic and

diastolic blood pressures were measured using a digital blood pressure monitor and BMI was calculated by using the formula weight (in kilograms, kg) divided by square body height (in meters, m).

Statistical analysis: The data were analyzed statistically using the SPSS-18 software. All values reported as mean ± standard deviation (SD). Comparison between study groups were assessed by the Mann-Whitney U test. the Shapiro-Wilk test was used to determine the normal distribution of the data.Correlation of VDBP with metabolic syndrome components in different study groups were determined by Spearman's rho, and Pearson's correlation coefficient test.

RESULTS AND DISCUSSION

Results

Table 1 shows the comparison of biochemical parameters in groups with and without MetS, and normal vitamin D levels. Based on the results, there are significant differences in the mean values of WC, SBP, DBP, FBG, TG, HDL-C and VDBP (P<0.05). WC, SBP, DBP, FBG and TG levels were significantly higher and HDL-C and VDBP were significantly lower in the subjects with MetS than the subjects without MetS.

Table 2 shows the comparison of biochemical parameters in groups with MetS, and with normal vitamin D and vitamin D deficiency. There are significant differences in mean values of TG and FBG levels (P<0.05). TG level was significantly lower and FBG was significantly higher in subjects with MetS and normal vitamin D when compared to subjects with MetS, and vitamin D deficiency.

Table 3 shows the comparison of parameters in groups with MetS and vitamin D deficiency, and without MetS with normal vitamin D levels. There are significant differences in mean values of WC, SBP, DBP, FBG, TG, HDL-C and VDBP levels (P<0.05).WC, SBP, DBP, FBG and TG levels were significantly higher and HDL-C and VDBP levels were significantly lower in group 1 compared to group 3.

Table 4 shows the correlation of VDBP with MetS components of all groups. Based on the results, there were positive and negative correlations between VDBP and FBG (r=0.295, **P=0.017**) and TG (r=-0.328, **P=0.003**) in groups 1 and 2, respectively.

Table 1: Demographic and biochemical characteristics of subjects with and without MetS; and with normal Vitamin D level.

Parameters	Subjects with MetS and normal Vitamin D (n= 81	Subjects without MetS and normal Vitamin D (n=81)	P-value
Age (Year)	54.68 ±10.56	50.52 ±14.35	0.165
BMI (Kg/m2)	26.80 ± 3.03	26.12 ± 5.17	0.295
WC (cm)	106.36 ± 10.26	97.76 ± 13.44	**<0.001**
SBP (mmHg)	132.62 ± 20.55	118.43 ± 18,42	**<0.001**
DBP(mmHg)	84.37 ± 11.65	75.55± 7.42	0.142
FBG (mg/dl)	158.36 ± 45.02	118.31 ± 58.46	**<0.001**
TG (mg/dl)	170.68 ± 67.01	120.02 ± 50.86	**<0.001**
HDL-C (mg/dl)	43.07± 10.03	49.02 ± 11.73	**<0.01**
Vit DBP (ng/ml)	1.64±1.30	2.24 ± 1.81	**0.017**

P-value < 0.05 was significant. MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBg: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, Vit.DBP: Vitamin D binding protein.

Table 2: Demographic and biochemical characteristics of subjects with MetS; and with normal and deficiency of Vitamin D level.

Parameters	Subjects with MetS and normal Vitamin D (n= 81	Subjects with MetS and Vitamin D deficiency (n=81)	P-value
Age (Year)	54.68 ±10.56	50.06 ±10.79	0.175
BMI (Kg/m2)	26.80 ± 3.03	26.70 ± 3.11	0.728
WC (cm)	106.36 ± 10.26	106.30± 9.85	0.888
SBP (mmHg)	132.62 ± 20.55	128.63± 22.484	0.214
DBP(mmHg)	84.37 ± 11.65	82.19± 11.47	0.222
FBG (mg/dl)	158.36 ± 45.02	180.76± 62.93	**0.042**
TG (mg/dl)	170.68 ± 67.01	200.31± 81.47	**0.030**
HDL-C (mg/dl)	43.07± 10.03	41.6± 8.78	0.494
Vit DBP (ng/ml)	1.64±1.30	1.58 ± 0.63	0.137

P-value < 0.05 was significant. MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBg: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, Vit.DBP: Vitamin D binding protein.

Table 3: Demographic and biochemical characteristics of subjects without and with MetS; and with normal and deficiency of Vitamin D level.

Parameters	Subjects without MetS and normal Vitamin D (n=81)	Subjects with MetS and Vitamin D deficiency (n=81)	P-value
Age (Year)	50.52 ±14.35	50.06 ±10.79	0.645
BMI (Kg/m2)	26.12 ± 5.17	26.70 ± 3.11	0.875
WC (cm)	97.76 ± 13.44	106.30± 9.85	0.142
SBP (mmHg)	118.43 ± 18,42	128.63± 22.484	**<0.01**
DBP(mmHg)	75.55 ± 7.43	82.19± 11.47	**<0.01**
FBG (mg/dl)	118.31 ± 58.46	180.76± 62.93	**<0.001**
TG (mg/dl)	120.02 ± 50.86	200.31± 81.47	**<0.001**
HDL-C (mg/dl)	49.02 ± 11.73	41.6± 8.78	**<0.01**
Vit DBP (ng/ml)	2.24 ± 1.81	1.58 ± 0.63	**<0.001**

P-value < 0.05 was significant. MetS: Metabolic syndrome, BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, Vit.DBP: Vitamin D binding protein.

Table 4: Correlation of vitamin D binding protein with demographic characteristics and metabolic syndrome components in three study groups.

	Subjects with metabolic syndrome and vitamin D deficiency		**Subjects with metabolic syndrome and normal vitamin D**		**Subjects without metabolic syndrome and normal vitamin D**	
Parameters	r	P-value	r	P-value	r	P-value
Age (Year)	-0.170	0.122	-0.158	0.152	-0.183	0.096
BMI (Kg/m2)	-0.025	0.824	-0.115	0.312	-0.148	0.184
WC (cm)	0.019	0.862	-0.041	0.715	-0.025	0.824
SBP (mmHg)	-0.206	0.062	-0.190	0.093	0.118	0.286
DBP(mmHg)	-.0185	0.095	-0.178	0.121	0.088	0.377
FBG (mg/dl)	0.295	**0.017**	0.019	0.868	-0.008	0.941
TG (mg/dl)	0.142	0.192	-0.328	**0.003**	-0.090	0.417
HDL-C (mg/dl)	-0.152	0.161	0.044	0.700	-0.020	0.858

P-value < 0.05 was significant. BMI: body mass index, WC: Waist circumference, SBP: Systolic blood pressure,

DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol.

Discussion

Based on the results, VDBP was significantly lower in subjects with MetS and normal deficiency of vitamin D compared to subjects without MetS and normal vitamin D levels. VDBP was significantly higher in subjects with MetS and normal vitamin D compared to subjects with MetS and vitamin D deficiency. Some studies have shown that VDBP did not be different significantly according to the presence or absence of metabolic syndrome, which is not in accordance with our findings[18]. The correlation of VDBP with MetS components in subjects with MetS and vitamin D deficiency shows that there was a negative correlation between VDBP and FBG levels. There was also a positive correlation between VDBP and TG in subjects with Mets and normal vitamin D. A study on subjects with MetS showed that VDBP level negatively correlated with body mass index and levels of diastolic pressure. Other findings have revealed that VDBP levels were significantly lower in MetS subjects than in control subjects. There were also significant negative correlations of VDBP levels with systolic blood pressure, glucose and age, which are not in agreement with our results except with FBG[19-20]. Some studies indicated that serum levels of VDBP were significantly lower in T2DM patients than control subjects, which is in agreement with our results that type 2 diabetic patients with MetS showed lower level of VDBP[21-22]. It has been reported that there were no association between VDBP and HDL-C, SBP, DBP and WC which is in agreement with our results [23]. In an in contrast study conducted that VDBP level was found to be elevated in the presence of MetS which is in accordance with our findings[24]. Some studies have indicated the associations between vitamin D metabolism, lipolysis and MetS, and some other studies have shown the association of MetS with alterations in vitamin D levels [25-27]. However, it is not exactly clear whether the VDBP polymorphism affects the MetS components through alterations in vitamin D levels. Although, a number of environmental and genetic factors have been found related to metabolic disorders[28-29]. Some findings indicate the association of VDBP and vitamin D3 with insulin in overweight and obese females. Their

results showed that females with higher VDBP revealed lower insulin resistance. They reported that there was no significant association between VDBP and vitamin D, FBG levels, BMI or insulin resistance. They deduced that higher VDBP levels may be associated with lower levels of insulin. Therefore, the evaluation of VDBP in different populations appears to be a significant clinical estimation of diabetes mellitus prevalence increases and glucose intolerance [30]. Parveen et al., investigated the association between serum 25(OH) D and VDBP in T2DM patients. They revealed that T2DM patients had significantly lower serum VDBP levels than controls [31]. Some other studies showed the effect of obesity on VDBP and 25 (OH) D levels in African American and white females. They showed that 25 (OH) D levels were lower in African American females than in whites. However, the VDBP level was similar in African Americans and white females. They showed that 25 (OH) D levels were lower in African American women than in whites. However, the VDBP level was similar in African Americans and white women. There also was not a relation to BMI in both racial groups [32]. Some studies revealed that insulin resistance and increased levels of insulin were associated with lower serum levels of VDBP. This may be an important factor for glucose dysregulation. It has also indicated that VDBP level seems to be affected by age, race, and fasting insulin and bioavailable vitamin D levels [13]. It looks like that the VDBP may regulate the amount of insulin secretion in the pancreatic β-cells. Thus, it may affect the prevalence of insulin resistance and type 2 diabetes mellitus [13]. Some studies have shown that in subjects with MetS, VDBP level negatively correlates with hip circumference, BMI and diastolic blood pressure[19]. Findings from other studies showed the correlation between VDBP and anthropometric parameters is different. VDBP correlates negatively[33-34] and positively [35] with body weight and BMI, while other studies indicated no correlation[32, 14]. Our results are in agreement that there was no correlation between VDBP and BMI and wait circumference[32, 14]. A study revealed negative correlations between the VDBP level and triglyceridemia[36], which is in accordance with our findings. It seems that VDBP may affect insulin secretion by controlling the amount of vitamin D in pancreatic β-cells. This may influence the prevalence of insulin resistance and type-2 diabetes mellitus [13]. The results of this study may showed that the association between the VDBP levels and some MetS components related to the increase of prevalence of metabolic disorders in MetS subjects in our study area. Our study groups consisted of different levels of vitamin D deficiency and different degrees of MetS and its components in our study subjects. Some subjects had three, four or five MetS components according to the MetS definition. This may affect the level of VDBP in subjects with MetS. Our study subjects were non-obese. We considered that VDBP level changes with MetS in non-obese subjects. This study did not focus on obese type 2 diabetic patients with MetS.

Conclusions

There were statistically significant correlations observed between the levels of VDBP and some components of MetS (FBG and TG). Therefore, it suggest that it could be concluded that controlling of VDBP level is an effective way for controlling MetS components in subjects with MetS. It suggests that the estimation of VDBP in different populations appears to be an important clinical value for evaluating and considering the relationship between VDBP and MetS components in subjects with MetS and T2DM patients.

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نشرة العلوم الصيدلية
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إرتباط فيتامين د مع البروتين في البلازما مع مكونات المتلازمة الأيضية لدى مرضى السكري من النوع الثاني في ولاية جرجان

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تعد متلازمة التمثيل الغذائي (MetS) مشكلة مهمة في تطور العديد من الاضطرابات و قد تؤثر الاختلافات في إرتباط فيتامين د مع البروتين (VDBP) على إفراز الأنسولين واستقلاب (عملية الأيض) للجلوكوز . وتم في هذه الدراسة تقييم (VDBP) في الدم بين الأشخاص الذين يعانون من (MetS) المصابين بمرض السكري من النوع الثاني(T2DM) ، والتي لا يعانون من (MetS) وارتباطها بمكونات (MetS) في مدينة جرجان .

وإشترك في هذه الدراسة ٢٤٣ مريضا من المصابين بمرض السكرى النوع الثانى (T2DM) ٨١ مريضاً يعانون من (MetS) و كذلك نقص فيتامين د (المجموعة ١)، ٨١ مريضاً يعانون من (MetS) وفيتامين د عندهم في المستوى المعتاد (المجموعة ٢)، ٨١ مريضاً بدون (MetS) وفيتامين د في المستوى المعتاد (المجموعة ٣).

وتم إستخدام إرشادات Panel-III (aTP-III) لتحديد الأشخاص الذين يعانون من .(MetS) وكانت مستويات (VDBP) في الدم أقل بشكل ملحوظ في حالات (MetS) مقارنة بالأشخاص الذين ليس لديهم (MetS)). وكانت مستويات الدهون الثلاثية (TG) أقل ومستويات الجلوكوز في الدم الصائم (FBG) أعلى بشكل ملحوظ في الأشخاص الذين يعانون من (MetS) وفيتامين D الطبيعي بالمقارنة مع الأشخاص الذين يعانون من (MetS) ونقص فيتامين.D وكانت مستويات (VDBP) في الدم أقل بشكل ملحوظ في الأشخاص الذين يعانون من (MetS) ونقص فيتامين د مقارنة باللأشخاص الذين لا يعانون من (MetS) وفيتامين د الطبيعي. كانت هناك ارتباطات إيجابية وسلبية بين VDBP و FBG و TG في المجموعتين ١ و ٢ .

وتوصلت الدراسة إلى أن التحكم في مستوى (VDBP) يعد وسيلة فعالة للتحكم في مكونات (MetS) في الأشخاص الذين يعانون من (MetS) . و أن تقدير مستوى (VDBP) في مجموعات مختلفة من المرضى يلعب دورًا مهمًا في تقييم ودراسة العلاقة بين مكونات (VDBP و MetS) في الأشخاص الذين يعانون من (MetS).