Original article

Assessment of Prolactin level in Vitamin D DeficiencyType-2 Diabetes Mellitus Patients

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A R T I C L E I N F O

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Abstract

The study aims are to evaluate prolactin (PRL) level in vitamin D (VitD) deficiency type-2 diabetes mellitus. A cross-sectional study was conducted in120 type-2 diabetic patients, aged between 25-80 years, classified based on vitamin D results into two groups, case (<30 ng/ml) and control (>30ng/ml) groups, anthropometrics variables (age, gender, and BMI) and diabetes duration were examined. Blood glucose, vit D and PRL were measured by spectrophotometry and ELISA techniques. Type-2 DM and overweight were more frequent in females (63.0%, 77.6%) than males respectively. VitD deficiency was common in females compared to males 75.0% vs 45.5%. This study showed no correlation between vitD and PRL (P= 0.588), also there was no correlation between serum PRL and duration of diabetes (P = 0.360), PRL significantly correlated to blood glucose level. Study concludes that, vit D deficiency is more common in female, and overweight male is more vulnerable to vitD deficiency, prolactin level correlate positively with blood glucose level.

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Introduction

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both, including two categories, type1 (IDDM) and type 2 (NIDDM) .Type 1 primarily due to pancreatic islet B-Cell destruction causing absolute deficiency of insulin secretion while type 2 resulting from insulin resistance results in a relative insulin deficiency (Bishop, 2000). A number of factors can increase the risk of developing type 2 diabetes, being overweight or obese, having a waist size of 31.5 inches or more (women) or more than 37 inches (men), eating an unhealthy diet, Physical inactivity, having a first-degree relative with type 2 diabetes and having high blood pressure or raised cholesterol levels.

Vitamin D plays vital role in controlling the growth of normal as well as cancerous cells. It acts through several mechanisms on glucose metabolism, directly on insulin producing cells in the pancreas to produce more insulin, on the muscle and fat cells to improve insulin action by reducing insulin resistance, indirectly improves insulin production and its action by improving the level of calcium inside the cells, therefore can help in the prevention as well as treatments of type 2 diabetes (Zaidi, 2013).

Some studies showed that the prevalence of vitamin D insufficiency was significantly higher among diabetic patients. Also it has been approved that vitamin D deficiency has a role in increasing the rate of cardio-vascular diseases in diabetic patients (Al-Timimi and Ali, 2013 and Mansouritorghabe *et al.,* 2013).

Prolactin is a highly versatile hormone whose functions are related to reproduction, growth and development, immune regulation, brain function, and angiogenesis. Also takes a crucial

part in regulating whole-body insulin sensitivity and glucose metabolism by increasing β-cell proliferation, promoting cumulative insulin secretion (Wang *et al.*, 2013). Besides its lactogenic properties PRL exerts various physiological actions in humans and rodents via its binding to a membrane receptor, accumulating evidence suggests that PRL has a crucial impact on energy balance by acting on pancreas and adipose tissues, that is appears to play an important role in obesity and diabetes (Carre and Binart, 2014).

2. Some studies showed that high circulating prolactin was associated with lower prevalence of diabetes, impaired glucose regulation (IGR) and protection against diabetic retinopathy. Furthermore a Cross-sectional study showed an inverse association between low PRL concentrations and prevalent T2DM risk in men and women after multivariable-adjusted (Mansouritorghabe et al. 2013, Haug *et al.,* 1985, and Balbach *et al.*, 2013).

   Recent animal studies and tissue-culture studies have led to the suggestion that prolactin (PRL) and growth hormone (GH) are involved in the regulation of 25-hydroxyvitamin D-1α-hydroxylase activity (Haug *et al.*, 1985).

   The incidence and prevalence of type 2DM is increasing in adult Sudanese population with wide differences among the different areas, besides that, there is increasing evidence on association between vitamin D and diabetes. For these reasons this study aimed to determine whether the variation of prolactin concentration associated with fasting glucose, duration of the disease, and BMI (body mass index), also to improve nutritional state of diabetic patients, through evaluating vitamin D in order to minimizing diabetic complications.

Materials and Methods

Materials

A cross-sectional study was carried out at Almotakamil Primary Healthcare Center, Khartoum state (from March to June 2014). 120 type2 diabetic patients were enrolled in this study. Patients age ranged between 25-80 years and were classified to two groups based on vitamin D levels: <30ng/ml patients are considered as a case group while >30ng/ml as control group.

Samples collection

Five veni-puncture blood samples were collected after overnight fasting and serum was obtained by centrifugation at 3000 rpm and kept at −20°C till used, then utilized for estimation of fasting blood glucose, vitamin D and prolactin.

Ethical consideration

Ethical approval was obtained from Al Neelain University college of Medical Laboratory Sciences, verbal informed consent was taken, and all participants were informed about the aim of the study.

Methods

Calculation of BMI

Weight and height of the two groups were measured then body mass index (BMI) was calculated using the formula: weight in kilograms (kg) divided by height in squared meters (m^2).

Estimation of vitamin D

Quantitative solid phase competitive inhibition enzyme immunoassay was used for estimation of vit D (lot E 140116AE, Euroimmune AG, Germany). 200 µl of sample was diluted with biotin in a micro plate well which is coated with monoclonal anti vitamin D antibodies, during incubation an unknown amount of 25-OH vitamin D were competed for the antibody sites, while unbound 25-OH vitamin D was removal by washing. 100 µl of streptavidin-peroxidase was added to detect bound biotin labelled 25-OH vitamin D, 100 µl of peroxidase substrate tetra-methyl benzidine (TMB) was added to promote colour reaction, and the colour intensity was inversely proportional to the 25-OH vitamin D concentration in the samples. Results were calculated by using standard curve (Sunrise-Tecan) [Hollis, 2004 and Snellman *et al.,* 2004).

Estimation of glucose

Glucose oxidase catalyzed glucose to gluconic acid and hydrogen peroxide, then peroxidase and achromogenic oxygen acceptor (4-aminoantipyrine+phenol) were added to from a coloured compound (Quinoneimine), which absorbed at 520 nm by Biosystem BTS-310 spectrophotoometer-Spain (Burtis 2005).

Prolactin estimation

Quantitative two sites immunoenzyomertic assay was performed for prolactin measurement (PRL kit Cat.NO.0025255) by using TOSOH-AIA full Automated Immunoassay Analyzer. Sample (30µl) was added to immobilize monoclonal antibody on magnetic solid phase and enzyme-labelled monoclonal antibody in the AIA pack test cups. The magnetic beads were washed to

remove unbound enzyme –labelled monoclonal antibody. Then incubated with afluorogen-substrate, 4-methylumbelliferyl phosphate (4MUP), the amount of bound enzyme–labelled antibody was directly proportional to the prolactin concentration (Fahie-Wilson, 2000 and Beltran *et al*., 2008).

Statistical consideration

All data entered and analyzed using statistical analysis software SPSS version 12 Pearson's correlations (*r*, *sig*) were used to assess the relationship between PRL and vit D. Statistically significant follow up differences or effects were determined using multiple comparison tests; level of significance was set at P-value ≤ 0.05.

Results

Gender percentage

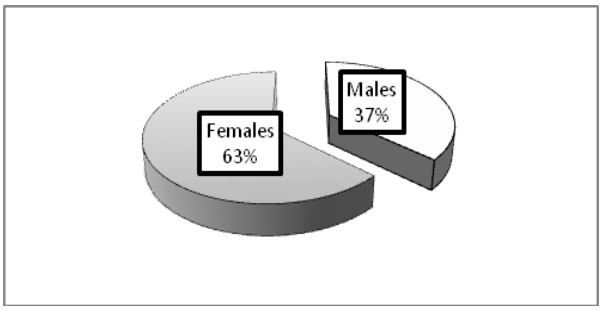

Fig.1 Percentage of DM among males and females (n=120)

BMI and Vit D status in male and female

Table (1) Percentages of BMI (<26.5 and >26.5) and VitD (<30 ng/ml and >30 ng/ml) among gender.

Groups classification	BMI		VitD levels	
	< 26.5	>26.5	<30 ng/ml	>30 ng/ml
Male	34.10 %	65.90 %	45.45 %	54.55 %
Female	22.40 %	77.60 %	75.00 %	25.00 %

Table (2) Correlation of BMI and Gender with VitD status (Normal and deficient)

Variables	Male BMI <26.5	Male BMI >26.5	Female BMI <26.5	Female BMI >26.5
Normal Vit D	66.70%	48.28%	17.65%	22.04%
Deficient t Vit D	33.30%	51.72%	82.35%	77.96%
Total %	100.0%	100.0%	100.0%	100.0%

Results expressed as percentage (%) in (n120).

Prolactin level in patients and control

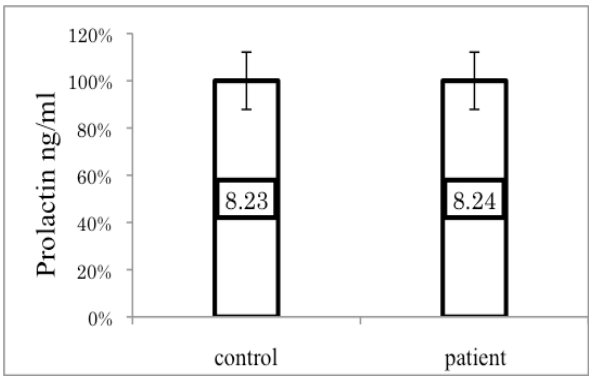

Fig.3.2 Shows mean concentration of PRLin control (normal vitamin) and patients (vitamin deficient), results expressed as Mean ± SD and significant considered as *p*-value ≤0.05.

Table (3) Person's correlation of vitamin D versus prolactin concentration

Parameter	R	Sig.
Prolactin	-0.053	0.588

Table 3. R= (-) negative correlation (+) positive correlation, Sig= strength of correlation

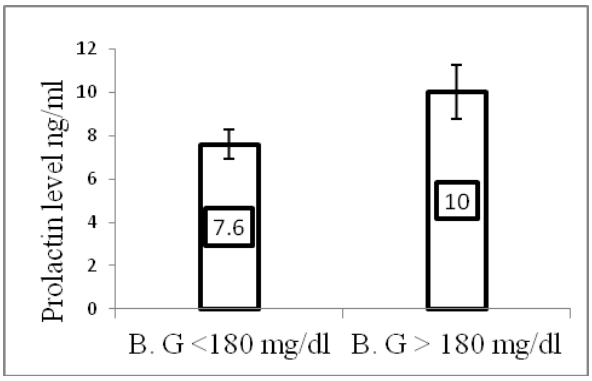

Fig. 2. Mean prolactin level in diabetes patients with blood glucose <180 and >180 mg/dl.

Table (4) Prolactin level and duration of the disease

Duration of D.M	Mean of PRL ± SD	*p*-value
< 10 years	9.7±7.2	0.360
> 10 years	9.0±8.6	

Discussion

DM is a major health problem through the world that has been increased in the last decade due to changes in life style. Most of recent studies focusing on the relationship between vitD and DM show that, low level of vit D is high risk for the development of insulin resistance and Type 2 diabetes. In addition prolactin receptor is required for normal glucose homeostasis and modulation of beta cell mass. The present study aims to determine whether the variation of prolactin level associated with fasting glucose and duration of the disease, also to find out if there is relationship between vit D and prolactin.

The present study showed that, the prevalence of type 2 DM and obesity are more frequent in females (63.0% and 77.6%) than in males (37% and 65.9%) respectively.

This finding agreed with previous study done by Hilawe *et al.,* (2013) who reported that, in many countries of sub-Saharan Africa, women are more likely to be obese or overweight than men and accepted to have higher prevalence of diabetes mellitus compared to men (Hilawe *et al.*, 2013).

This study revealed that, vitD deficiency is more common in type 2 DM females versus males (75% females vs. 45.45% males), our finding confirmed by Tahir and Ismail**, (**2015) who stated that, type 2 DM female subjects were more prone for VitD deficiency as compared to male (71.09% vs 61.09%, P <0.03) (Majumder *et al*., 2014). As current study showed there was no significant difference in the mean prolactin level in those had normal vitD compared with deficient vitD (8.2±6.8, 8.2±4.1) respectively.

Researchers in previous study concluded that, prolactin involved in the regulation of 25-hydroxyvitamin D-1α-hydroxylase activity suggest feedback loop between renal tubular cells and the anterior pituitary, which observed by finding of present study that prolactin inversely correlate with vitD, indicate that increased prolactin level inhibits 25-hydroxase enzyme and thus active vitD synthesis.

However, Balbach reported an inverse association between low PRL concentration and prevalentT2DM risk in men and women (Balbach et al. 2013). Arshag performed a case control study and showed that, PRL level although in most cases stayed in normal, was significantly higher in diabetic compared to normal group. According to observations (figure 5), those who had blood glucose >180mg/dl had significantly higher mean PRL level (10.0ng/ml ± 9.0) than those had blood glucose <180 mg/dl (7.6 ng/ml ± 0.6). In addition, table (2) showed, there was no correlation between serum PRL and duration of diabetes (p-value = 0.36), this finding agreed with previous report done by (Arshag et al. 1985).

Conclusions

* vit D deficiency is more common in females, and overweight males are more vulnerable to vit D deficiency.

* Prolactin levels correlate positively with blood glucose levels.

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