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Evaluation of Zinc Level among Vitamin D Deficient Type2 Diabetes Mellitus Patients

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ABSTRACT

Diabetes is a major and growing health problem worldwide and in the Sudan, zinc and vit D play a functional role on insulin function and glucose tolerance. Study aim to evaluate zinc level in type2 DM patients with vit D deficiency. Cross-sectional study was conducted on 120 type2 DM patients aged between 25-80 years old, classified based on vit D results into two groups, <30 ng/ml considered as cases and >30ng/ml as control. Vit D, zinc and glucose were determined in fasting blood samples, using competitive ELISA and atomic spectroscopy. The percentage of DM was higher among females (63%), and vit D deficiency was common among females (75%), results of BMI showed females more obese than males (77.6% females have BMI > 26.5 compared with 65.9% for males), in males .vit D inversely correlate with BMI, and there was no correlation in females, vit D result in week negative correlation with zinc (r= -0.0195), and no association between zinc and blood glucose (P-value = 0.46). Zinc and vit D are essential nutrients in DM patients, thus deficiency lead to early complications in type 2 DM patients, which need regular monitoring

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INTRODUCTION

The number of people with diabetes and pre-diabetes are exponentially increasing worldwide due to population growth, aging, urbanization, unhealthy eating habits, increasing prevalence of obesity and physical inactivity[1]. Diabetes mellitus is a leading cause of morbidity and mortality worldwide, with an estimated 346 million adults being affected in year 2011 [2]. The prevalence is expected to double between years 2005-2030, with the greatest increases expected in low- and middle-income developing countries of the African, Asian, and South American regions [2,3]. At present, 80% of the world's population with diabetes lives in low- and middle-income countries [2]. Ninety percent of those with diabetes have type-2 diabetes[4].

Vitamin D is a hormone related to skeletal integrity [5]. Recently, the extra skeletal effects of vitamin D have raised considerable interest [6]. Vitamin D deficiency appears to be related to the development of diabetes mellitus type 2 [7, 8,9,10]. Mild to moderate vitamin D insufficiency has been proposed as a risk factor for type 2 diabetes [11]. Higher plasma vitamin D has been shown to be related with a lower risk for the development of diabetes mellitus in high risk patients [7]. Vitamin D deficiency has been described in the metabolic syndrome [12], specific vitamin D receptor gene polymorphisms having been found to be related to components of the metabolic syndrome [13]. Moreover, vitamin D seems to affect glucose homeostasis.

Several studies have indicated a relationship between vitamin D status and the risk of diabetes or glucose intolerance. Vitamin D has been proposed to play an important role and to be a risk factor in the development of insulin resistance and the pathogenesis of type 2 DM by affecting either insulin sensitivity or β-cell function, or both [14,15,16].

1,25-dihydroxyvitamin D plays an important role in glucose homeostasis via different mechanisms. It not only improves insulin sensitivity of the target cells (liver, skeletal muscle, and adipose tissue) but also enhances and improves β-cell function. In addition, 1,25-dihydroxyvitamin D protects β-cells from detrimental immune attacks, directly by its action on β-cells, but also indirectly by acting on different immune cells[17].

Insulin is stored as a hexamer containing two Zinc ions in -cells of the pancreas and released into the portal venous system at the time of -cells de-granulation [18]. The Zn(II) ions which are co-secreted with insulin suppress inherent amyloidogenic properties of monomeric insulin [19]. High concentrations of glucose and other secretagogues decrease the islet cell labile Zinc.

Zinc is important in insulin action and carbohydrate metabolism [20]. Oxidative stress plays an important role in the pathogenesis of diabetes and its complications. Zinc is a structural part of key anti-oxidant enzymes such as superoxide dismutase, and Zinc deficiency impairs their synthesis, leading to increased oxidative stress [21]. Studies have shown that diabetes

is accompanied by hypozincemia[22], and hyperzincuria[23]. In addition Zinc deficiency is more common in developing countries [24], where diabetes is also showing an exponential increase in prevalence.

Animal studies have shown that Zinc supplementation improves fasting insulin level and fasting glucose in mice [25]. Human studies have also shown the beneficial effects of Zinc supplementation in both type-1 [26, 27] and type-2 diabetes [28,29]. Zn2+ is also involved in the modulation of vit D gene activation[30].

MATERIAL AND METHOD

Cross-sectional study was conducted at primary health care center (Almotakamil) at Khartoum state,during April 2014 to May2014, 120 diabetic patients type2 (aged between 25-80 years) were classified in to two groups based on vitamin D level(<30ng/ml deficient,>30ng/ml control)

Target group was diabetes mellitus type 2 vitamin D deficiencies, after an overnight fasting, 6 ml of peripheral blood was taken(2ml in fluoride oxalate container and4ml in plain container). The blood samples were centrifuged at 3000 rpm for 10 min and serum stored at −20°C. Utilized for different metabolic parameter (vitamin D, zinc), and plasma for Fasting blood glucose.

Ethical approval was obtained from Alneelain university collage of medical laboratory, informed consent was taken from each participant after the full explanations about the study.

Estimation of vitamin D

Quantitative of vit D level a solid phase competitive inhibition enzyme immunoassay was used to determine vitD (in use the vit D ElISA kit (lot E 140116AE)(EuroIMMUN AG) Germany according to the manufactured protocol, 200 µL of sample diluted with biotin Microplate well which coated with monoclonal anti vit D antibodies, during incubation antigen antibodies reaction occurred .then unbounded 25-OH vit D was removal by washing, 100 µL of streptavidin-peroxidase will added to detect bound biotin labeled 25-OH vit D.100 µL tetramethylbenzidine promotes a color reaction, the color intensity is inversely proportional 25-OH vitD concentration in the sample calculated by using curve (sunrise-TECAN)(31,32).

Estimation of Glucose

Glucose oxidases catalyze the oxidation of beta D- glucose present in the plasma or serum to glucono- 1, 5 lactone with formation of hydrogen peroxide andlactone, which are hydrolyzed to D gluconic acid. By peroxidase enzyme the hydrogen peroxide broken down to water and oxygen. Oxygen reacts with oxygen acceptor such as ortho toludine which

converted to colored compound measured spectrophotometer(33).10 µL of plasma added to 1ml of reagent incubated for 10 minute then read at 520nm.

Estimation of zinc the measurement of zinc by atomic absorption

Principle: The electron of the atom promoted to higher orbital's (excited state)for a short period of time by absorbing a defined quantity of energy .The amount of energy (wave length)is specific to a particular electron transition in a particular element. The radiation measured by using detector and the absorbance is converted to analyze concentration or mass using Bear Lamber low (Perkin-Elmer.1994).For determination of zinc 1ml of serum diluted with 4ml of distil water(D.W), at wave length 213.9 nm

Measurement of BMI

Weight and height were measured and BMI was calculated by dividing weigh in (Kg) by squire of height in (m).

Statistical analysis

Data from all patients were presented as percentage and (mean ± SD), differences between means of patients and control groups were considered statistically significant with p-value threshold <0.05 using independent T-test. Significant correlation (r) was calculated using linear correlation test.

RESULTS AND DISCUSSION

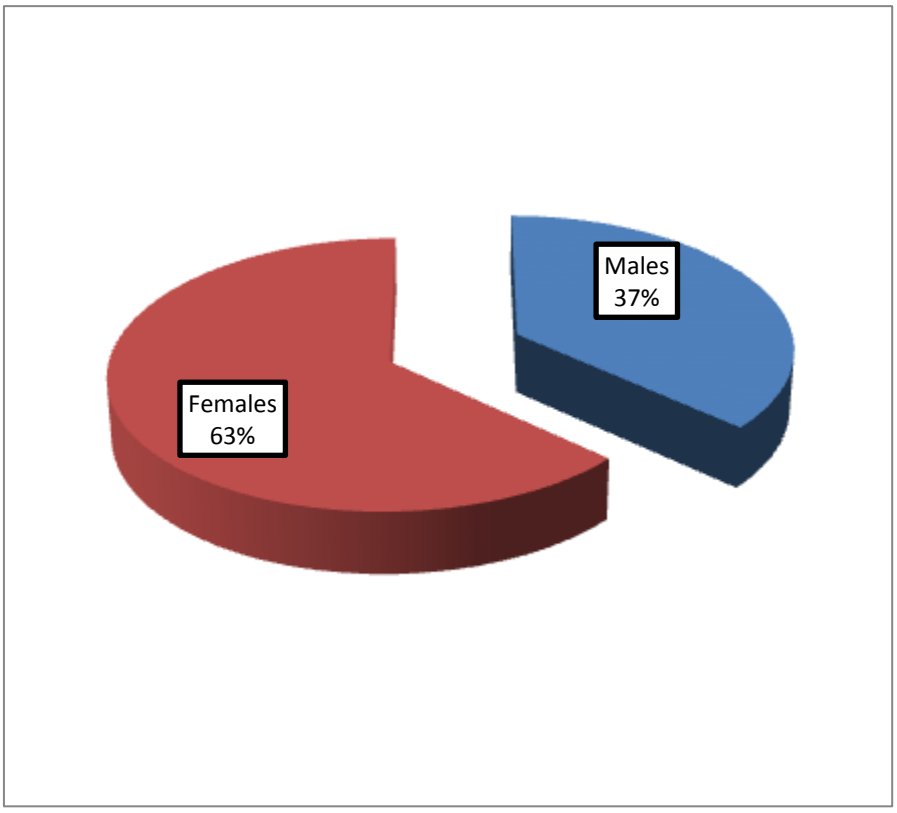

Figure 1:Shows percent of male and female among type2 DM (n=120)

Table 1: Presenting the percentages of BMI (<26.5 and >26.5) and Vit D<30 ng/ml and >30 ng/ml) level among gender. Results expressed as %.

	BMI		Vit D levels	
Group classification	< 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: Presenting association between vit D, BMI and gender among type2 DM patients, results expressed as percentage (%) in (n=120).

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 Vit D	33.30%	51.72%	82.35%	77.96%
Total %	100.0%	100.0%	100.0%	100.0%

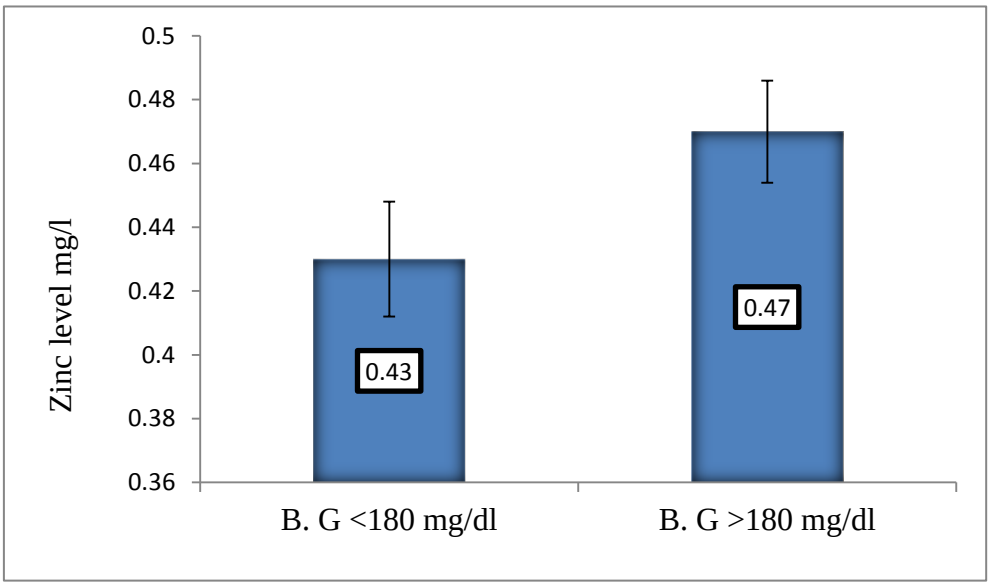

Figure 2: Mean of Zinc Level Compare With Blood Glucose

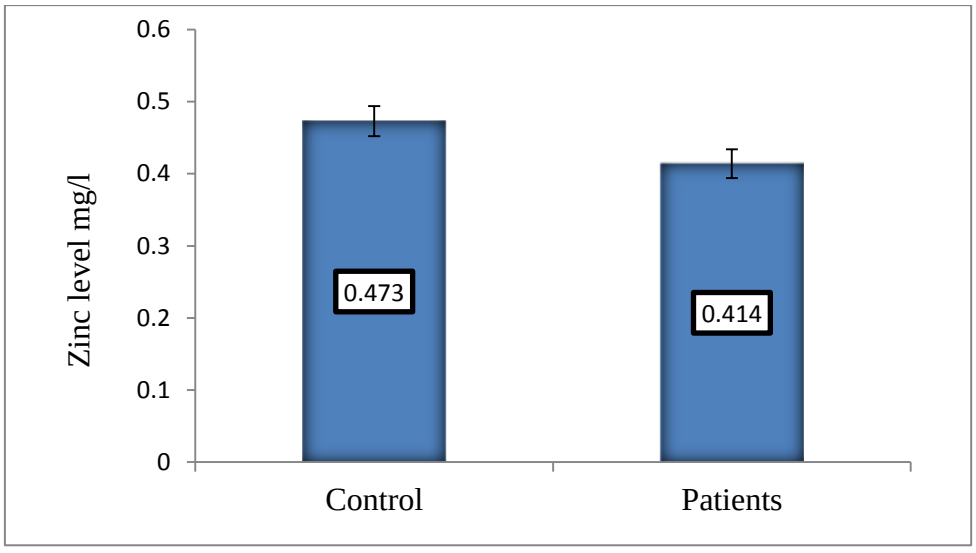

Figure 3: Mean of Zinc Level in Study Groups

Table 3: Showed correlation between vitamin D and Zinc

Parameters	r	Sig
Zn	-0.019	0.843

The number of people with diabetes and pre-diabetes are exponentially increasing. Studies on humans have shown the beneficial effects of Zinc supplementation in patients with diabetes. The present study aims to evaluate zinc in Diabetes Mellitus type 2 with vitamin D deficiency. A key regulator of the phosphorylation state of the insulin receptor is known to be a target of Zinc ions. Studies have shown that Zinc may play a role in improving peripheral insulin sensitivity, as it can potentiate insulin-stimulated glucose transport.

The present studies showed that the percentage of diabetes is higher among female (63%) than male (37%) this finding confirm with study done in Mediterranean Island and not confirm with study done in china by Wenying et al.(34) which estimate that the prevalence of diabetes is higher in male than female.

In addition our study observed that the percentage of vitamin D deficiency is common among female (75%) than male (45.45%), that agree with study done by Giancarlo Isaia et al.(35)reported that High Prevalence of Hypo vitaminosis D in Female Type 2 Diabetic Population. Vitamin D deficiency progressively reduces insulin secretion, and this reduction soon becomes irreversible (36). It was also shown that insulin deficiency may be associated with lower vitamin D–binding protein and 1,25(OH)2D3 serum levels . These decreases are somewhat dependent on androgen concentration, but they are counteracted by estrogens (37).

The result of frequency showed that the percent of BMI >26.5 in female higher (77.60%) than male (65.90%) which agree with study done byButheinah A. Al-Sharafi and Abdallah A. Gunaid in Yemen (38) report The prevalence of obesity in patients with type 2 diabetes mellitus in Yemen is high with respect to the Yemeni population, especially in females. According to observation serum vitamin D in female with BMI <26.5 is higher deficient (82.35%) than female with BMI>26.5 (77.96%) but in male with BMI>26.5 is higher deficient (51.72%) than male with BMI <26.5(33.30%)Which agree with study done by Konradsen et al.(39) reported that there is an inverse association between BMI and the serum level of 25-OH- vitD and1,25-vit D. The result of present studies shown there is no significant difference between mean of zinc levels of patients(0.414+ 0.020) when compared with control group(0.473+0.021) (p-value 0.024). The present study has shown there is no significant difference in mean of zinc level in patient with glucose less than 180mg/dl (0.43+-0.018) when compare with zinc level in patient with blood glucose more than 180mg/dl (0.47+-0.016) with p-value(0.046) this finding agree with study done by Glaucia et al.(40)which report from accumulating data in clinical studies suggest that zinc is

independently associated with alterations in glucose metabolism. Deficiencies may increase risk of the development of insulin resistance and T2DM. However, there is inadequate evidence based data available to inform public health strategies. In addition to that analysis of frequency vitamin D concentration is inversely correlated with zinc level(r-0.0195)with p-value (0.842).

CONCLUSION

Zinc is essential for glucose metabolism in trace amount thus fluctuations lead to disturbance in glucose and lipid metabolism specially in type 2 DM patients with vit D deficiency, both deficient of zinc and vit D lead to appearance of early complications in type 2 DM patients, which need regular monitoring for design supplementation protocols. Further studies are required to understand underline mechanism.

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