Evaluation of Cupper Levels among Vitamin D Deficient Type 2 Diabetes Mellitus Patients Cross-sectional study

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Abstract:
Background: Diabetes is an epidemic disease in most countries and in the Sudan. Vit D may play a functional role on glucose tolerance through its effects on insulin secretion and insulin sensitivity and copper the role metabolism of glucose, and acting as antioxidants for preventing tissue peroxidation - Study aim to evaluate copper level in type2 DM patients with vitD deficiency.

Materials and Methods: Cross-sectional patients study was conducted on 120 type2 DM patients aged between 25-80 years old, classified based on vitD results into two groups, <30 ng/ml considered as cases and >30ng/ml as control. VitD, copper and glucose were determined in fasting blood samples, using competitive ELISA and atomic spectroscopy.

Results: DM is more common in females (63%) than in males (37%). 75% of DM females and 45.45% of DM males suffering from

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vitD deficient, results of BMI>26.5 showed females were more obese than males (77.6%), (65.9%) respectively. In males vitD inversely correlate with BMI, and there was no correlation in female, vitD result in week negative correlation with copper(r= -0.2644), and no association between copper and blood glucose (P-value = 0.10).

**Conclusion:** The study conclude that, copper increase in DM patient with vitamin D deficient, which both may lead to cells damage in type 2 DM patients.

**Key words:** DM diabetes mellitus, vitD vitamin D, Cu Copper

**Introduction**

Diabetes is an epidemic disease in most countries. Worldwide, an estimated 150 million people are affected by diabetes, and this number is likely to reach 300 million by the year 2025 if successful strategies are not implemented for its prevention and control (1) The incidence of type 2 Diabetes Mellitus (t2DM) is increasing at an alarming rate both nationally and worldwide with more than 1 million new cases per year diagnosed in the US alone (2) Type 2 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion (3). The Patients with diabetes mellitus are at increased risk for cardiovascular diseases, thus cardiovascular complications are the leading cause of diabetes-related morbidity and mortality (4).

Vitamin D is one of fat soluble vitamins, refer to a group of related metabolites, it stimulates intestinal absorption of calcium and phosphorus for bone growth and metabolism (5). In addition, Vitamin D is important for normal glucose metabolism. It acts through several mechanisms on glucose metabolism, directly acts 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 (6).

Some studies have shown that vitamin D may play a functional role on glucose tolerance through its effects on insulin secretion and insulin sensitivity (7). role of vitamin D in insulin secretion and function includes the presence of vitamin D receptors in human pancreatic β-cells, the detection of 1α-hydroxylase activity, and insulin gene transcription responsiveness to vitamin D in pancreatic β-cells (8). Therefore, vitamin D could play a role in the pathogenesis of type 2 diabetes, by affecting either insulin sensitivity or β cell function, or both. However, the interaction of vitamin D with insulin sensitivity and β cell function has not been examined in a group of well-defined subjects, because abnormal glucose tolerance could adversely affect insulin sensitivity and β cell function (9).

Copper is the third most abundant mineral in the human body. Copper is present in the body combined with various enzymes to form metallo-enzymes such as ceruloplasmin, SOD. These enzymes play a major role in redox reactions, such as superoxide dismutase which plays key role in antioxidant defense. Copper is associated with altered glucose metabolism through the stimulation of glycation and release of copper ion enhancing the oxidative damage (10). It is known that copper plays an important role in the development and maintenance of immune system function (11). Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites, serving as cofactors or components for enzyme systems which are involved in glucose metabolism, increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation (12). Trace elements participate in production of reactive oxygen species (ROS), which contribute to oxidative stress. Oxidative stress contributes to the pathogenesis of many diseases including DM. Previous studies have shown that copper causes oxidative
stress. Copper acts as a prooxidant and may participate in metal catalysed formation of free radicals. The increased production of free radicals is likely to be associated with development of type 2 (13), it is also manifested that some reactive oxygen species (ROS) are produced during diabetes due to imbalance of essential metals. This oxidative stress might decrease the insulin gene promoter activity and mRNA expression in pancreatic islet cells due to hyperglycemic condition (14).

Materials and Methods

Cross-sectional study was conducted at primary heath care center (Almotakamil) in Khartoum state during the period from May to July. 120 patients with DM type2 aged between 25-80 years classified based on vitD results into two groups, group one <30 ng/ml considered as cases and >30ng/ml as control.

Samples collection

Overnight fasting blood specimen was taken, centrifuged at 3000 rpm for 10 min and serum stored at −20°C until utilized. Ethical approval was obtained from Alneelain university faculty of medical laboratory, informed consent was taken from each participant after the full explanations about the study.

Ethical consideration

The study has been approved by the local ethics committee of Al-Neelain University. The study participants give their written informed consent. Sample and clinical information were used anonymously.

Measurement of BMI

Weight and height were measured and BMI was calculated by dividing weigh in (Kg) by squire of height in (m).
Estimation of Glucose
Glucose oxidase catalyzes the oxidation of beta D-glucose present in the plasma or serum to glicono- 1, 5- lactose with the formation of hydrogen peroxide, which react with 4-aminoantipyrine and phenol formed quinoneimine color, then absorbed at 546 nm using spectrophotometer BTS-310 Biosystem, the concentration obtained by calculation of O. D of test against O. D of standard, 10 μl of serum added to 1ml of reagent incubated for 10 min (Trinder, 1969).

Estimation of vitamin D
Quantitative of vitD level solid phase competitive inhibition enzyme immunoassay was used to determine vitD (in use the vitD Elisa kit (lot E 140116AE) (EuroIMMUN AG) Germen according to the manufacture protocols. 200 µL of sample diluted with biotin Micro plate well which coated with monoclonal anti vitD antibodies, during incubation antigen antibodies reaction occurred, then unbounded 25-OH vitD was removal by washing. 100μL of streptavidin-peroxidase will added to detect bond biotin labeled 25-OH vitD. 100μL tetramethylbenzidine promotes a color reaction .the color intensity is inversely proportional 25-OH vitD concentration in the sample, then calculated by using standard curve (Sunrise-TECAN) (Holick, Chen, 2008; Hollis, 2004).

Estimation of cupper by atomatic 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 analyte concentration or mass using Bear Lamber low (Perkin-Elmer.1994). For determination of cupper 1ml of serum diluted with 1ml of distal water wave length 324.8 nm.
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

Fig (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 VitD <30 ng/ml and >30 ng/ml) level among gender. Results expressed as %.

<table>
<thead>
<tr>
<th>Group classification</th>
<th>BMI</th>
<th>VitD levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 26.5</td>
<td>&gt;26.5</td>
</tr>
<tr>
<td>Male</td>
<td>34.10 %</td>
<td>65.90 %</td>
</tr>
<tr>
<td>Female</td>
<td>22.40 %</td>
<td>77.60 %</td>
</tr>
</tbody>
</table>

Table.2. Presenting association between vitD, BMI and gender among type2 DM patients, results expressed as percentage (%) in (n120).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male BMI</th>
<th>Female BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male BMI</td>
<td>Male BMI</td>
</tr>
<tr>
<td></td>
<td>&lt;26.5</td>
<td>&gt;26.5</td>
</tr>
<tr>
<td>Normal ViD</td>
<td>66.70%</td>
<td>48.28%</td>
</tr>
<tr>
<td>Deficient ViD</td>
<td>33.30%</td>
<td>51.72%</td>
</tr>
<tr>
<td>Total %</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Mohamed ELM, Amar M I, Hutheiefa Ibrahim Younis Mohammed. **Evaluation of Cupper Levels among Vitamin D Deficient Type 2 Diabetes Mellitus Patients**

Cross-sectional study

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![Graph showing mean Cupper levels in control and patient groups](image)

**Fig.5.** Shows mean Cupper level in study groups (control and patients), results presented as (Mean±SD) and (P-value <0.05)

**Table (1) Showed correlation between vitamin D and Cupper**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>-0.264</td>
<td>0.006</td>
</tr>
</tbody>
</table>

r= -0.264 negative correlation Sig=strength of correlation

![Graph showing Cupper levels in different glucose groups](image)

**Fig.6.** Shows mean Cupper level in group one (Blood glucose < 180 mg/dl) group tow (Blood glucose > 180 mg/dl), result expressed as (Mean±SD) and (P-value <0.05).

**Discussions**

Diabetes mellitus is an endocrine disorders and health problem affecting millions of individuals worldwide and it is a major cause of morbidity in developed countries (15), which result from defective insulin synthesis and secretion. Previous studies evaluate the association between vitamin D and copper in DM patients, accordingly the present study aims to evaluate copper levels in type2 DM patients with vitamin D deficient.
The results of frequency showed DM is higher among females (63.0%) than males (37.0%) which agreed with Marina in India who reported the prevalence of diagnosed DM was higher among female than male(16). In addition our study observed that, vitD deficiency is common among female (75.0%) than male (45.5%) which agree with Mansour study who stated that, majority of female patients 73.6% were vitamin D deficient while 46.9% of male patients. This result could be attributed to less sun exposure in female patients relative to male patients in our community (17). Butheinah and Abdallah observed that, overweight are more frequent in females than in male, moreover, the mean BMI, as a continuous variable associated with morbidity and mortality, was significantly higher in females than in males (18), our results revealed that (77.6%) female have BMI > 26.5 compared with (65.9%) male. The majority of the male subjects have normal BMI (<26.5) showed low percentage of vitD deficiency (33.3%) compared with BMI (>26.5) (51.7%), which indicated that, increase BMI correlate with VitD deficiency. In contrast female subjects showed no association between BMI and vitD deficiency, subsequently our finding agreed with Ehsaneh who stated that there is negative relationship between vitD and BMI adjusted for gender (19).

The results of present study provide experimental evidence that, there was insignificant difference between mean copper levels of patients in comparison with control group with \( P\text{-value} 0.123 \), which reinforced by person’s regression of week negative correlation between vitD and copper level \( r =0.264 \), this indicate that copper increased in patients with vitamin D deficiency, David stated that, excessive intake of vitamin D can contribute to or exacerbate an existing copper deficiency. The opposite may also occur: excess copper intake or retention may produce a deficiency of vitamin D or increase their requirement.

Synergistic vitamins, those whose requirements are increased by copper deficiency, include vitamin D (20).
On the other hand, it appears that increased oxidative stress in presence of hyperglycemia may lead to increased availability of transition metals like copper released from its storage site. Copper in its free form is a potent cytotoxic element because of its redox chemistry it readily participates in Fenton and Heiber-Weiss reactions to generate reactive oxygen species (ROS) (21), thus both increase in copper and vitamin D deficient synergistically causes the damage of the cells in vitamin D deficient DM patients.

The present study observed that, patients have high blood glucose have low copper level, which may be due to hyperosmolar caused by high blood glucose that lead to haemodilution and thus decrease in copper levels.

**Conclusion**

The study concludes that, copper increase in DM patient with vitamin D deficient, which both may lead to cells damage in type 2 DM patients. Female is more vulnerable vitamin D deficient than male and copper correlate negatively with blood level of glucose.

**REFERENCES**

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