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Vitamin D3 Deficiency is a risk Factor for the Low Ferritin in Iraqi Alzheimer's type-2- Diabetic Patients

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ABSTRACT

Alzheimer's disease and Type-2- Diabetes Mellitus are prevalent chronic diseases linked by likely a bi-directional relationship, this relationship is partially understood. the aim of the present study is highlighting the biochemical relationship between the two complicated diseases in the term of biochemical parameters vitamin D3, Calcium, Ferritin and iron. Thirty (30) patients suffers from both Alzheimer's and type-2-diabetes participated in the present study, the study design involved two groups : the patients group included Alzheimer's type-2-diabetic patients with the age ranged between (48-55) years and termed as (G) , this group compared with healthy subjects as a control group (C) which composed of thirty subjects matched in age with the patients. The biochemical parameters: HbA_{1c}, FBS, vitamin D3, Calcium, Ferritin and Iron were determined in sera of the patients and control groups. It has been reported that the levels of HbA_{1c}, FBS were highly significant increased while the levels of vitamin D3, calcium, ferritin and iron were highly significant decreased in sera of Alzheimer's type 2- diabetic patients compared with the healthy subjects. The present study is the first in Iraq reporting that vitamin D_3 deficiency is regarded as a risk factor to the low level of ferritin in Iraqi Alzheimer's type-2- diabetic patients. Additionally, the biochemical parameters (vitamin D3, Calcium, Ferritin and iron) collectively submit a supportive finding to the bi-directional relationship between Alzheimer's disease and type -2- diabetes mellitus.

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1. INTRODUCTION

Alzheimer's disease (AD) is regarded as the most prevalent neurodegenerative disease worldwide characterized by deposition of β-amyloid plaque (AB) and hyper phosphorylated tan in addition to deregulated metabolism in the brain [1]. Diabetes mellitus (DM) is a life style related pandemic disease strongly linked with the metabolism of all the body [2] , diabetes complication are more prevalent is older adults compared with young people [3]. Clinically, type-2- diabetes mellitus constitutes about 90% of diabetic cases and resulted from a biochemical interaction between genetic and environmental factor such as nutritional imbalance, lifestyle, stress and environmental pollutants such as dioxins and air pollution. Interestingly ,comorbidities related to the central nervous system including neurodegenerative disease (in particular Alzheimer's disease) may associated with diabetes ,thus the biochemical relationship between type-2- diabetes mellitus and Alzheimer's disease is likely bi-directional [4]. Since diabetes mellitus is strongly linked with metabolism [2] , a number of specific biochemical parameters must be reviewed , vitamin D is an endogenous fat-soluble metabolic is biosynthesized in the skin via exposure to ultraviolet B radiation , it modulates calcium signaling by promoting the expression of the calcium sensing receptor and also regulated the activity of calcium binding proteins and increasing their expression [5]. Vitamin D as a dietary precursor of 1,25,(OH)2 D3 is regarded as a key nutrient for maintaining healthy for preventing several diseases. Additionally 1,25,(OH)2 D3 plays a reactive role in the regulation of cellular calcium signaling [2]. In the context of Alzheimer's disease ,calcium is a second messenger that plays a key role in neurotransmission and memory formation [5]. In this regard , calcium is a second Messenger that plays a key role in neurotransmission and memory Formation [5]. Vitamin D3 as a supplement has a helpful importance in diabetic cases particularly regarding complication prevention , this is prevalent in the Arabic Countries [6].

Indeed , vitamin D3 can be Obtained through the Diet or from the sun exposure [5]. Ferritin is a key protein strongly reflects the iron balance and homeostasis caused by its level is used as a biochemical marker for Iron storage. Remarkably , either the elevation or the lowering of ferritin levels may be linked with the complications of Diabetes Mellitus [3]. Iron is the key abundant metal In the human brain , thus abnormal iron levels are recorded in different regions of the human brains of Alzheimer's disease [1]. Since the biochemical relationship between Alzheimer's disease and Type-2- Diabetes Mellitus is not fully understood ,the aim of the present study is to highlight the complicated biochemical relationship between the two diseases in the term of biochemical parameters linked With the metabolism : vitamin D3 ,calcium ,ferritin and iron.

2. METHOD

2.1. Study design and patients selection

The current study involved thirty (30) Iraqi Alzheimer's disease coupled with type-2 diabetes patients (males and females) with the age ranged between (48-55) years , patients group was termed as (G) , this group was compared with healthy persons as a control group (C) which composed of (30) subjects or cases (males and females) who were approximately in the same age as the patients. Remarkably , the study protocol involved an exclusion criteria that excluded all the cases suffered from other chronic diseases than Alzheimer's and type-2-diabetes mellitus. Patients suffered from type-2- diabetes mellitus for the last 6 months to 2 years and recently diagnosed as Alzheimer's cases by C.T scan and MRI after attending the nervous and psychological division of either Al-Yarmook teaching hospital or Ibn Rushud training hospital in Baghdad. Also , the medical history of these patients involves laboratory tests indicating they suffer from type-2-diabetes mellitus.

2.2. Blood sample collection

Five (5) ml of venous blood were sampled and put in gel tube from each person participated in the present study , blood sera were separated from the whole blood by the centrifuge for 5-7 min at 3000 r.p m. the resulted sera were divided into four portions (for each subject) ,each division put within eppendorf) and kept frozen at (-20 o C) until the beginning with the biochemical laboratory tests .

2.3. The biochemical determination

Fasting blood sugar (FBS) was determined by enzymological method. Glycated hemoglobin (HbA1c) was determined by high performance liquid chromatography (HPLC). Vitamin D level was determined by a procedure based on double antibody type of enzyme linked immuno sorbent assay (ELISA) , It is termed as a sandwich method because the tested antigen are sandwiched between two layers of antibodies. (capture and detection antibodies) , the pre-coated antibody is human Anti-25-OH vitamin D3 monoclonal antibody and the adjusting antibody is human polyclonal antibody with biotinylated Anti-25-OH vitamin D3 labled. Tris – buffered or phosphate-buffered saline was used as a wash buffer and 2N of HCL was used as a stop solution. Calcium ,iron and Ferritin were determined by mindary BS 800 analyzer , this novel device combines innovation and high performance into an integrated solution , this developed device used an indirection electrode method to determine a wide range if chemicals. With this scalable platform , complete lines of clinical biochemistry reagents in addition to controls and calibrators or any solution used in the laboratory tests are made as the test needs . A buffer solution was used to dilute samples before being disposed to the electrodes flow cell The laboratory tests were conducted the international center for research and development (ICRD) in Baghdad .

2.4 Statistical Analysis

The data were quantified and documented by student T test in the term of probability (P) by comparing the difference between the values resulting from each group . when $p \leq 0.05$ the , the difference is regarded as significant while when $p \leq 0.001$ the difference is regarded as highly significant. On the other hand when $p>0.05$ the different is regarded as as non-significant.

3. RESULTS AND DISCUSSION

Table 1. The levels of glycated hemoglobin (HbA1c) and fasting blood sugar (FBS) (Mean±S.D) in sera of Alzheimer's type 2 diabetic patients (G) compared the with the control group (C).

HbA_{1c} (%)	Control group (C)	4.3±0.7	p G/C = 9.04 E $^{-5}$ (H.S)
	Patients group (G)	6.8±1.8	
FBS (mg/dL)	Control group (C)	91.08±9.23	p G/C = 2.88 E $^{-6}$(H.S)
	Patients group (G)	137.54±14.97	

H.S : high significant ($p \leq 0.001$).
S.D : standard deviation.

Table 2. The levels of vitamin D3 and calcium (Mean±S.D) in sera of Alzheimer's type 2 diabetic patients (G) compared the with control group (C).

Vitamin D_3 ng/ml	Control group (C)	35.02 ± 3.77	p G/C = 10.44 E $^{-10}$ (H.S)
	Patients group (G)	17.80 ± 8.32	
Ca^{2+} ng/ml	Control group (C)	9.76 ± 1.12	p G/C = 1.53 E $^{-5}$ (H.S)
	Patients group (G)	8.01 ± 0.52	

Table 3. The levels of ferritin and iron (Mean±S.D) in sera of Alzheimer's type 2 diabetic patients (G) compared the with control group (C).

Ferritin ng/ml	Control group (C)	98.00 ± 45.92	p G/C = 6.13 E $^{-13}$(H.S)
	Patients group (G)	18.7 ± 6.68	
Fe^{+2} µg/ml	Control group (C)	119.47 ± 33.46	p G/C = 6.03 E $^{-12}$ (H.S)
	Patients group (G)	62.93 ± 11.54	

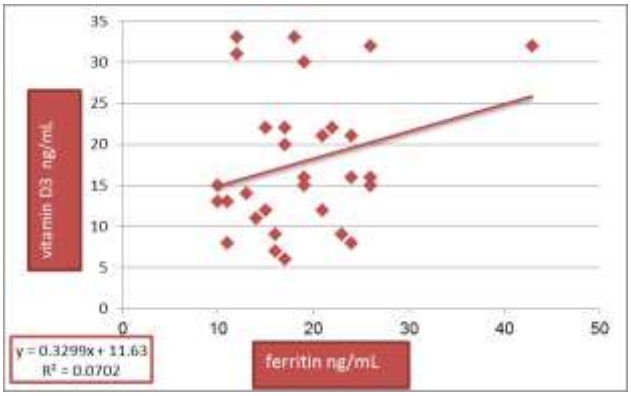

Figure 1. The direct biochemical relationship between vitamin D3 and ferritin levels in Iraqi Alzheimer's Type -2- Diabetic Patients (r=+0.265).

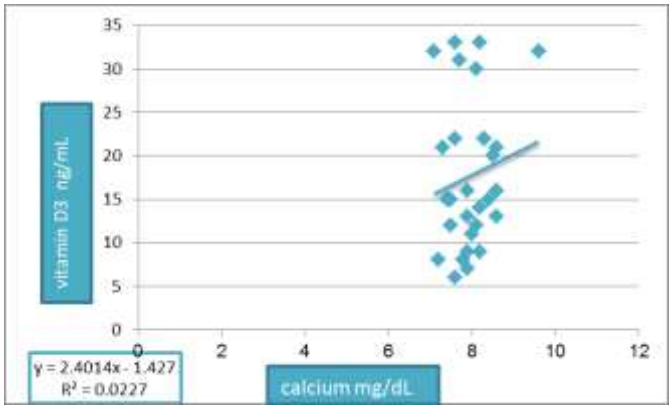

Figure 2. The direct biochemical relationship between vitamin D3 and calcium levels in Iraqi Alzheimer's Type -2- Diabetic Patients (r= +0.150696).

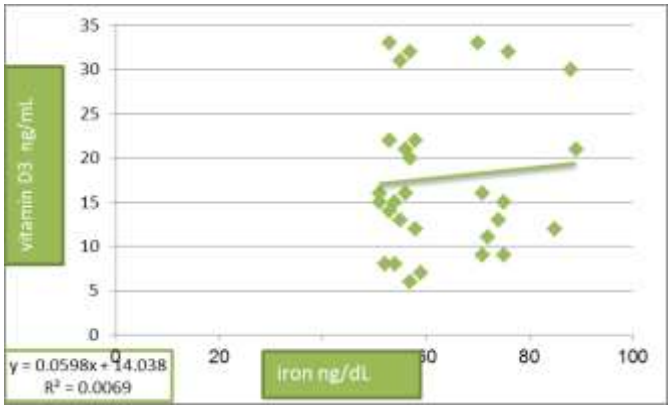

Figure 3. The direct biochemical relationship between vitamin D3 and iron levels in Iraqi Alzheimer's Type -2- Diabetic Patients (r= +0.082868).

The Results of the present study have reported that HbA1c level was highly significant increased (9.04 E -5) in sera of Alzheimer's type-2- diabetic patients (G) (6.8±1.8) compared with the control group (4.3±0.7) , table 1. Similarly , FBS level was highly significant increased (2.88 E -6) in sera of G (137.54±14.97) compared with C (91.08±9.23) , table 1. Regarding table 2 , Vitamin D3 level was highly significant decreased (p=10.44 E -10) in sera of Alzheimer's type-2- diabetic patients (G) (17.80±8.32) ng/ml compared with the

control group (C) (35.02±3.77) ng/ml. In the same context, Ca+2 level (ng/ml) was highly significant decreased (p= 1.53 E -5) in sera of G (8.01±0.52) ng/ml compared with C (9.76±1.12) ng/ml as seen in table.2.

Similarly to table 2, the level of ferritin was highly significant decreased (p=6.13 E -13) in G (18.7.6.68) ng/ml compared with C (98.00±45.92) ng/ml as seen in table 3. Interestingly , the level of Fe+2 was highly significant decreased (p=6.03 E -12) in sera of G (62.93 ± 11.54) µg/ml compared with C (119.47 ± 33.46) µg/ml . . As reported by figure 1 , a direct biochemical relationship was suggested between vitamin D3 and ferritin levels in Iraqi Alzheimer's Type -2- Diabetic Patients with correlation coefficient r=+0.265. Similarly , a direct biochemical relationship was reported between vitamin D3 and calcium levels in the same patients with r= +0.150696 , as seen in figure 2. Moreover , a third direct biochemical relationship was suggested between vitamin D3 and iron levels in those patients with r= +0.082868 as shown by figure 3.

Fasting blood sugar (FBS) and Glycated hemoglobin (HbA1c) were determined as routine diagnostic tests for diabetes mellitus. A recent study has reported a remarkable association between type-2- diabetes mellitus and the risk factor of dementia. Interestingly, both of these diseases are degenerative and progressive and reflect definitive risk factors [4]. On the other hand , a previous study has confirmed that the abnormalities linked with the homeostasis of vitamin D3 and calcium are prevalent in diabetes mellitus and may be associated with elevated morbidity and mortality [2]. Regarding diabetes mellitus , a recent study has revealed that vitamin D3 promotes glucose transport by binding with its receptors on beta cells in the pancreas and the result is increasing insulin gene transcription and glucose transport across the target cells [6].

Moreover , Alzheimer's disease is regarded as a central metabolic disease because of glucose hypo-metabolism associated with impaired insulin signaling pathway in the brain and subsequently play a key role in memory disorder [4]. The Status of Vitamin D are affected by insulin resistance in type-2- diabetes mellitus. Interestingly , the quickly increase in the intracellular calcium triggers the release of insulin from the β cells of pancreas. Consequently , vitamin D3 and calcium supplementation may relieve the symptoms of diabetes mellitus [2]. Collectively , the present Study highlights the bi-directional relationship between Alzheimer's disease and type -2- diabetes mellitus in Iraqi patients in the term of vitamin D3 and calcium. Regarding iron , a recent study has revealed that iron dys-regulation play a key role in the pathogenesis of the neurodegerative diseases. Nevertheless , the mechanisms that regulate iron level in the brain is partially understood [7]. Iron is the most abundant metal in the brain [8]. At this point , serum ferritin is regarded as the iron storage and the A cute Phase Protein Which Can be Increased in the Inflammatory status [9]. Although several studies have revealed abnormal iron accumulation in the different regions of Alzheimer's patients' brain , the status of iron in the sera of Alzheimer's patients was not highlighted. On the Other Hand , It has recently confirmed that the too much iron storage raises the risk of insulin resistance in diabetic patients . Anyway , the serum ferritin level is directly correlated with the iron stores in the body and can be used as a good indicator for iron status in human body [3].Definitely, ferritin is the iron storage acute phase protein that play a key role in human iron homeostasis caused by serum ferritin levels are in dynamic balance with the body's iron stores. Consequently , ferritin reflects the iron status and regarded as an indicator for the inflammatory diseases[10].

Interestingly , both iron overload and iron deficiency may trigger neuro-degeneration [7]. The higher levels of ferritin are remarkable in cases of transferrin saturation , liver function panel and complete blood count while the lower levels of ferritin reflects iron deficiency in pathological cases may affected by age , gender and conditional specific thresholds but the details is still partially understood [10]. Anyway , the distribution of iron homeostasis may affected by the mitochondrial functions and consequently triggers the progression of neurodegenerative mechanisms [7]. Although ferritin and iron levels are increased in approximately all the inflammatory diseases [10]. The present study has reported a lower level of ferritin and iron (regardless what happen in both Alzheimer's and type-2 diabetes) is characterized by a wide range of inflammatory conditions. At this point , the lower level of vitamin D3 is associated with lower level of ferritin in anemia cases [11]. Collectively , the present study is the first in Iraq reporting that the deficiency of vitamin D3 may regarded as a risk factor for the lower level of ferritin in Iraqi Alzheimer's type -2- Diabetic Patients. However, the mechanism that explain the biochemical relationship between the lower levels of both Vitamin D3 and Ferritin is partially unknown and need a lot of research to be fully understood . One Possible mechanism has shown that vitamin D3 modulates the level of of cytokines that in turn inhibit the inflammatory Milieu which subsequently lead to Anemia With lower levels of Ferritin and Iron , While the other mechanism supposed that Vitamin D3 directly stimulates Erythroid Precursors [12].

In the same context , a previous study has revealed that vitamin D3 has a biochemical relationship with the hematologic system by modulating the hematological parameters and cell migration into Peritoneal and pulmonary cavities in Alloxan –Diabetic Mice [13]. A recent study has reported that Vitamin D3 Play a key role in erythropoiesis [14], while another recent study has confirmed that vitamin D3 deficiency promotes the severity of platelet morphology deterioration in patients with anemia who have both iron and B12 deficiency [15]. Regarding the immune system it has been recently reported that the microbiota , peripheral immune activation and non microglia brain cells like astrocytes and infiltrating immune cells have a remarkable impact on the neuroinflammation [16]. At this point , another recent study has revealed that the adaptive immune system has the more impact on Alzheimer's disease and highlighted the need to novel strategies to modulate it for the target therapeutic purposes [17]. The suggested mechanism for lower levels vitamin D3 as a risk factor to lower levels of ferritin in Iraqi Alzheimer's Type -2- Diabetic Patients is abbreviated in Figure 4.

The positive correlation coefficient between Vitamin D3 and the other biochemical parameters (as reported by figures 1,2 and 3) promote the role of vitamin D3 in the immune system for Iraqi Alzheimer's Type -2- Diabetic Patients. Generally , the level of vitamin D3 must be controlled within the normal values , a recent study highlighted the role of laboratory tests to determine its levels and the optimal vitamin D3 intake to control its level and avoid its deficiency [18]. It has been recommended that (20 ng/mL) is essential for bone health to be protective [19] , Vitamin D deficiency is regarded as below 20 ng/ml
and vitamin D insufficiency as [21-29] ng/ml , hence the supplements are the first option to treat the deficiency [20]. According to supplements of vitamin D3 , the moderate dose (4000 IU/day) is recommended and favorable [21].

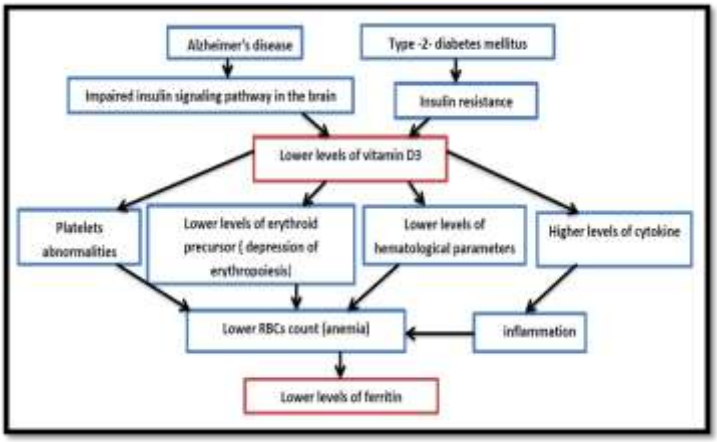

Figure 4. The suggested mechanism for lower levels vitamin D3 as a risk factor to lower levels of ferritin in Iraqi Alzheimer's Type -2- Diabetic Patients.

4. CONCLUSION

The present study is the first reporting that the relationship between vitamin D3 and ferritin levels in Iraqi Alzheimer's type -2- Diabetic Patients is definitely direct. Since vitamin D3 become deficient , its regarded as a risk factor for the lower levels of ferritin. Additionally , the four measured biochemical parameters (vitamin D3 ,Calcium , Ferritin and iron) are collectively regarded as an indicator for the bi-directional relationship between Alzheimer's disease and type -2- diabetes mellitus. Determination of the levels of vitamin D3 ,Calcium , Ferritin and Iron in Alzheimer's Type -2- Diabetic Patients' sera may promote the diagnostic power of Alzheimer's type -2- Diabetic cases and open the way to examine the role of other biochemical parameters to explain the biochemical relationship between the two chronic diseases.

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