Study the Effects of Vitamin D as Immune-Modulatory Agent in Type II Diabetes Mellitus Patients

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Received in :12 May 2013 , Accepted in : 24 July 2013

Abstract

This study was designed to show the roles of vitamin D as immune-modulatory agent in serum type II Diabetes Mellitus Patients collected from type II Diabetes Mellitus and controls. They have been classified into two groups as the following:

1) Patients of type II DM group includes (20) individuals from both sexes with age range (35–65) years.
2) Control group: includes (20) healthy individuals from both sexes, with age range (30 – 45) years and no previous disease which may interfere with the parameters analyzed in this research. All the blood samples were analyzed for vitamin D_3, albumin, C- reactive protein (CRP), erythrocyte sedimentation rate (ESR), immunoglobulins (IgG, IgM, IgA), α_1-antitrypsin and total protein (TP).

Keywords: vitamin D, type II diabetes mellitus, inflammation, Acute - phase proteins.

Introduction

Diabetes Mellitus (DM) is a heterogeneous, metabolic disease which is characterized by hyperglycemia and long term complications [1].

Type II DM is caused by combination of insulin resistance (impaired sensitivity of tissues to insulin action) and relative insulin deficiency with increased hepatic glucose production [2].

Cholecalcifrol is a prohormone that is synthesized in the skin by photochemical of 7-dehydrocholestrol, it is subsequently hydroxylated to 25-hydroxy-cholecalciferol [25(OH) D_3] in the liver and finally to the active metabolite, 1,25-dihydroxy – cholecalciferol [1,25$(OH)_2D_3$] in the kidney [3].

Opinions differ on how to define vitamin D deficiency. A recent statement by the institutes of medicine agreed upon level of 10 ng/ml (25nmol/L) to be clear that vitamin D deficiency is determined as the level where parathyroid hormones (PTH) start to rise [4].

The vitamin D hormone, 1,25 $(OH)_2$ D exerts its effects mainly by activating the nuclear vitamin D receptor (VDR), a member of the nuclear transcription super family of ligand-activated transcription factors, and when bound to this vitamin D an attractive molecule to investigate in the context of diabetes treatment [5,6].

Indeed, the activated form of vitamin D, 1, 25$(OH)_2$ D_3 influence insulin secretion and is an important immune modulator [7,8].

Vitamin D deficiency is more common in type II DM than in type I DM independently of age, sex or insulin therapy [9].

The initial observations linking vitamin D to type II DM in human came from studies showing that both healthy and diabetic subjects had a seasonal variation of glycemic control currently, there is evidence supporting that vitamin D status is important to regulate some pathways related to type II DM development since the activation of inflammatory pathway interferes with normal metabolism and disrupt proper insulin signaling, it is hypothesized that vitamin D could influence glucose homeostasis by modulating inflammatory response [10].

The aim of this study is to show the roles of vitamin D include immune-modulation effect and anti-inflammatory agent in type II DM patients compared with control

Experimental

Sampling is classified in two groups:

1) Patients of type II Diabetes mellitus group: include (20) patients from both sexes, with age range (35–65) years.

2) Control group: includes (20) healthy individual from both sexes, with age years (30–45) years and no previous disease which may interfere with the parameters analyzed in this study.

Specimen collection and preparation

Ten milliliters (ml) disposable plastic syringes of 21 G needles were used to draw eight ml of venous blood from each patient and control groups after 12 hours fasting. The blood samples were divided into two tubes:

1. Two ml of blood samples were transferred into plastic tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) and left for 20 - 30 minutes at 37Cº. The blood was later used for the determination of (ESR).

2. The second part of blood samples were transferred into plastic plane tubes no anticoagulant: blood was left to clot for 20 - 30 minutes at 37Cº. Serum was obtained by centrifugation for 10 minutes at 3000 rpm and was divided into small epindrof tubes capacity 1.5 ml and kept at – 20Cº until time of analysis. The separated serum was later used for the determination of the levels of vitamin D_3, C-reactive protein (CRP), Albumin, $\propto_1$-antitrypsin, immunoglobulins (IgM, IgG, IgA) and total protein.

Determination of vitamin D_3:

Vitamin D_3 was measured using high performance liquid chromate graphy (HPLC) technique according to (AL- Dulaimy, W. Y- M. and Al-sarrag, N.F.Y, 2013 method) [11].

Determination of Albumin:

Albumin level was determined in serum sample of all studied groups according to (Doumas, etal method) [12].

Determination of C - reactive protein (CRP):

CRP was measured in serum samples of all studied groups according to (Young, D.S. method) [13].

Determination of Erythrocyte Sedimentation Rate (ESR):

Erythrocyte Sedimentation Rate (ESR) was determined in whole samples of all studied groups according to (Bick, R. L method) [14].

Determination of Serum Immunoglobulins (IgG , IgM, IgA):

Immunoglobulins (IgG, IgM, IgA) have been determined in serum samples of all studied groups by a ready kit purchased from (parsazmum company), Iran. The method depends on immune turbidometric test which the immunoglobulins form a complex with antibodies in solution which the absorbance is read by spectrophotometry [15].

Determination of $\propto_1$ - antitrypsin:

$\propto_1$– antitrypsin was measured in serum samples of all studied according to the method depends on immune turbidometric test [15].

Determination of total protein (TP):

Total protein was determined in serum of all studied group according to Biuret method [16].

Statistical Analysis:

Data presented in table (1) were the means and standard deviation student's t-test was used to compare the significance of the difference in the mean values of any two groups, P value less than 0.05 was considered statistically significant.

Results and discussion

This study evaluate the biochemical parameters levels of [vitamin D_3, Albumin, CRP, ESR, IgG, IgM, IgA, $\propto_1$– antitrypsin and total protein TP] in sera of type II diabetes mellitus patient group compared with control group. Data in table (1) shows a significant decrease in vitamin D_3, albumin and IgM in sera patients of type II DM compared with control group ($P \leq 0.05$). While table (1) shows that a significant increase in the level of c-reactive protein (CRP) , ESR, IgG, IgA and $\propto_1$– antitrypsin in sera of type II Diabetes mellitus patient group compared with control group ($P \leq 0.05$). Also table (1) shows no significant difference in total protein (TP) in sera of type II Diabetic Mellitus Patient group compared with control group ($P > 0.05$).

The result of a significant decrease in level of vitamin D_3 in sera of type II diabetic patient group compared with control group agrees with the study of (Palomer, X. et al 2008) [17], which suggested that vitamin D_3 has a role in abnormal glucose metabolism as well as in type II diabetes serum albumin level has linked clinical practice to several diseases. Low albumin levels can suggest inflammation, this is the study of (Sesmilo, G. et al 2004) [18].

This study is compatible with our result in table (1) showing a significant decrease in albumin level in sera of patients of type II DM compared with control group ($P \leq 0.05$).
A significant increase in the level of C- reactive protein (CRP) in sera of type II DM patient group compared with control group ($P \leq 0.05$). Our result agrees with the study of (Badawi, A. et al 2010) [19].

That suggests C-reactive protein is an acute-phase reactant inflammation biomarker and that elevated synthesis of Pro-inflammatory cytokines and acute-phase proteins characterize the pre-clinical stages of type II DM and exhibit a graded increase with disease progression. Also a recent study estimated that one-third of type II DM cases can be associated with elevated serum CRP [20].
It is known that adipose tissue can synthesis and release the main pro-inflammatory cytokine alfa-tumor necrosis factor (TNF-alpha), interleukin-1 (IL–1) and interleukin-6 (IL–6) and that the pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways which are relevant to insulin resistance [21].

A significant increase in the level of ESR in sera of patients with type II DM compared with control ($P \leq 0.05$). Our result agrees with study of (Donth, et al 2003) [22].
Donth study suggests that inflammations play a role in the pathogensis of type II DM most probably by determinal effects of inflammation on beta cells function.

Also in diabetes mellitus disease, the levels of variety of plasma proteins increase as a result of increased red cell agglomeration leading to accelerated sedimentation [14].

Table (1) shows that both immunoglobulins IgG and IgA were elevated while IgM was decreased in sera of type II patient group compared with control group. These findings are compatible with the results by (Michael, L. Bishop et al., 2005) [23] suggested that infections and inflammation diseases indicating in flammatory condition seen when there is an increase in both IgG, IgA immuogobulins, ∝- antitrypsin , CRP levels while there is a decrease in IgM and albumin level that stimulate the immune system .

Table (1) shows a significant increase in the ∝- antitrypsin level in sera of type II DM patient group compared with control group. This result agrees with the previous study of (Michael, L. Bishop et al., 2005) [23]. While table (1) shows no significant difference in total protein (TP) level in sera of type II patient group compared with control group.

Vitamin D has long been known to play important role in immune function. All cells of the immune system express the vitamin D receptor (VDR) including monocytes, macrophages, dendritic cell, neutrophils, T-Lymphocytes as well as B-Lymphocytes (Veldman et al 2000) [24].
Vitamin D modulates the adapative immune system as well, through direct effects on T-cell activation and on the phenotype and function of antigen-presenting (Kamen, DL et al 2010) [25] and (Von Essen, Mr. et al 2010) [26].

Type II diabetes development involves impaired pancreatic B cell function, insulin resistance and inflammation. It has been suggested that both environmental and genetic factors seem to be involved in type II diabetes development [27]; also, human and experimental data support the role of vitamin D on these pathways.

Due to the presence of both 1-∝- hydroxylase and VDR in pancreatic Beta cells, vitamin D is important for insulin synthesis and release [28].
This study demonstrated that type II DM an inflammatory diseases through the determination of biochemical parameters (Albumin, CRP, ESR, IgG, IgM, IgA, ∝ - antitrypsin and total protein (TP) and the deficiency in vitamin D play a crucial role in progression of type II diabetes mellitus.

References

1. Ionecu, T.C. (1998). "Proposed for a new classification of diabetes mellitus". Rom. J. inter. Med, 36 (1-2): P121-34.
2. American Diabectes Association, Inc: (2007). "Diabetes magnitude and mechanism", clinical Journal 25: 25-28.
3. Holick, M. (2002). "Vitamin D: the underappereciated D-lightful hormone that is important for skeletal and cellular health "CurropinEndocrinol Diabetes; 9:87-98.
4. Ross, A. C; Munson, J.E; Abrams, S.A; Aloia, J.F.; Brannon, P.M; Clinton, S.K.; Durazo-Arvizu, R.A; Gallagher, J.C; Gallo, R.I; Jones, G; Kovacs, C. S; and Mayne S. T. "The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine what clinicians need to know" J. Clin. Pract 64:1084-1089.
5. Christakoss, S; Barletta F; Huening, M; Dhawan P; Liu, Y; and Porta, A. et al (2003). "Vitamin D target proteins: function and regulation". J. Cell Biochem 88 :238–44.
6. Vidal, M; Ramana C.V; and Dusso, A.S. (2000). "Stat 1-vitamin D receptor interactions antagonize 1, 25-dihydroxy vitamin D transcriptional activity and enhance stat 1 mediated transcription", Mol. Cell. Biol. 22:2777–87.
7. Mathieu, C; and Adorinol (2002). "The coming of age of 1, 25-dihydroxy vitamin D_3 analogs as immunomodulatory agents", Trends Mol. Med. 8:174–9.
8. Matheiu, C; Gysemans, C; Giulietti, A; and Bouillon, R. (2005). "Vitamin D and diabetes", Diabetologia, 48:1247–57.
9. Dicesa, R.D.j; ploutz-synder, R; Weinstock, R.S; and Moses, A.M. (2006). "Vitamin D deficiency is more common in type II DM than in type I DM". Diabetes care, 29 :174.
10. Chagas, C.E.A; Borges, M.C; Martini, L.A; and Rogero, M.M. (2012). "Focuson Vitamin D, inflammation and type IIDiabetes", Nutrients, 4:52–67.
11. Al-Dulaimy, W.Y.M; and Al-Sarrag, N.F.Y. (2013). "study of some non-traditional roles a scribed to Vitamin D include immunemodulating effects and anti – inflammatory insera of Iraqi myocardial infarction patients" ,word Family medicine Journal. 11::issue 4.
12. Doumas, B. T; Watson, W. A; and Biffs, H.G. (1977). Clin. Acta (31):87–96.
13. Young, D. S. (1995), "Effect of drugs on clinical laboratory tests". 4th ed. AACC press.
14. Bick, R.L. (1993). "Heamatology clinical Laboratory practice" 1st ed, P: 253, Baltimore publishing company.
15. Shivanada, Nayak B. (2008). Manpal Manual of clinical Biochemistry, (For laboratory and MSC. students): (2008) 3rd. ed, p: 104-106 , medical publishers (p) Ltd , New Delhi , india
16. Tietz, N.W. (1987). "Fundamentals of clinical chemistry" p: 940, W.B. Sannders Co. Philadelphia, P. A.
17. Palomer, X; Gonzalz-Clemente, J.M; Blanco Vaca, F; and Mauricio, D. (2008). "Role of vitamin D in the pathogensis of type II diabetes mellitus". Diabetes Obes. Metab. 10: 185–197.
18. Sesmilo, G. et al. (2004). Effect of vitamin D on glucose metabolism. Ann Intern Med, 133(2):111–122.
19. Badawi, A.; Klip, A; Haddad, P. Cole; E.C; David; Bailo, B.G; El-Sohemy, A; and Karmali, M. (2010). "Type II diabetes mellitus and inflammation": prospects for biomarkers of risk and nutritional intervention. Diabetes Metab. Synd Obes. 3:173–186.
20. Dehghan, A; Kardys, I; de Maat, M.P., et al (2007). "Genetic variation, C-reactive protein levels, and incidence of diabetes". Diabetes, 56:872–878.
21. Mahajan, V.V.; APTE, I.C; and shende, S.S. (2011). "Acute phase Reactants in type II Diabetes Mellitus and Their Correlation with the Duration of Diabetes Mellitus" J. Of clinical and Research No.6 (5):1165–1168.

22. Donah, M.Y; Starling, J; Maedier, K; and Manruppoulsem, T. (2003). "Inflammatory mediators and islet beta cell failure": a link between type I and type II diabetes" J. Mol. Med. 81: 455–470.
23. Michael, L. Bishop; Edwared, P; Fody, and Larry Schoeff. (2005). "Clinical chemistry principle, procedures, correlation." 5th ed. Lippincott Williams and Wilkins. P :(194–195), P:(286–270) .
24. Veldman, C. M; and Cantorna, M.I.H.F. (2000). "Expression of 1, 25-dihydroxy vitamin D_3 receptor in the immune system". Arch. Biochem. Biophys 374:334–338 .
25. Kamen, D.L; and Tangpricha, V. (2010). "Vitamin D and Molecular actions on immunesystem" : modulation of innate and autoimmunity ; J. Mol. Med, 88 : 441 – 450 .
26. Von Essen, M.R; Kongsbak, M; Schjerling. P; Olgaard k; Odum. N; and Geisler. C. (2010). "Vitamin D controls T cell antigen receptor signaling and activation of human T cells"; Nat Immunol, 11:344–349.
27. Takiishi, T.; Gysemans, C.; Bouillon R.and Mathieu, C. (2010). Endocrinol. Metab. Clin. N. Am., 39:419–446.
28. Pittas, A. and Dawson-Hughes, B. (2010). "Vitamin D and diabetes". J. Sterol. Biochem. Mol. Biol. 121:425–424.

Table (1): Vitamin D_3, Albumin, CRP, ESR, IgG, IgM, IgA, $\propto_1$-antitypic and total protein (TP) levels in Sera of type II diabetes mellitus patients group and control

Biochemical parameters	Control N=20 Mean±SD	Patient group N=20 Mean ±SD	t- test	Significance
Vitamin D_3(ng/ml)	46.57±9.2	33.07±11.2	0.00001	Significant
Albumin (g/dl)	1.93±0.004	1.37±2.97	0.00002	Significant
CRP (m mol/dl)	----	3.6±0.12	0.0004	Significant
ESR (mmole/hr)	4.16±1.52	12.96±3.47	0.003	Significant
Ig G (mg/dl)	797.3±184	1214.64±101.22	0.003	Significant
Ig M (mg/dl)	34.68±1.98	15.4±28.92	0.001	Significant
Ig A (mg/dl)	151.14±39.31	347.28±57.38	0.0005	Significant
$\propto_1$-antitrypsin (mg/dl)	52.44±18.12	77.8±1.84	0.004	Significant
TP (g/l)	2.75±0.31	2.79±0.28	0.2	Not-significant

دراسة عن تأثيرات فيتامين D وسيط مناعي في مرضى داء السكري النوع الثاني

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استلم البحث في : 12 آيار 2013 ، قبل البحث في :24 تموز 2013

الخلاصة

صممت هذه الدراسة لبيان تأثيرات فيتامين D وسيط مناعي في مصل دم عشرين مريضا عراقيا يعانون من داء السكر النوع الثاني، وعشرين فردا من الأصحاء مجموعة سيطرة . وقد تم تصنيفها إلى مجموعتين:
المجموعة الأولى تتضمن عشرين مريضا بداء السكر النوع الثاني من كلا الجنسين وبمعدل عمر من (35 –65) سنة. والمجموعة الثانية تتضمن عشرين فردا من الاصحاء مجموعة سيطرة ومن كلا الجنسين بمعدل عمر (30–45) سنة وليس لديهم أي أمراض سابقة تؤثر في المتغيرات الحياتية التي تم قياسها في هذا البحث. حللت جميع نماذج الدم لقياس المتغيرات الحياتية مثل فيتامين D_3 و الألبومين و بروتين C التفاعلي و معدل ترسب كريات الدم الحمراء و الكلوبيولينات المناعية وهي(IgG, IgM, IgA,) ، بروتين الفا – 1- أنتي تربسين والبروتين الكلي .
اذ أظهرت النتائج انخفاضاً معنويا في كل من مستوى فيتامين D_3، الالبومين، الكلوبيولين المناعي IgM بينما أظهرت النتائج ارتفاعا معنويا في مستوى كل من بروتين C التفاعلي، معدل ترسب كريات الدم الحمراء والكلوبيولينات المناعية IgA , IgG وبروتين الفا – 1- انتي تربسين مقارنة مع الأصحاء مجموعة سيطرة بينما أظهرت النتائج عدم وجود فرق معنوي في مستوى البروتين الكلي لمرضى داء السكر النوع الثاني مقارنة مع الأصحاء مجموعة سيطرة، ومن خلال هذه النتائج توصلنا إلى أن النقصان الحاصل في مستوى فيتامين D له تأثير في تطور الحالة الالتهابية عند مرضى داء السكر النوع الثاني.

الكلمات المفتاحية : فيتامين D_3، مرض السكر النوع الثاني، الالتهاب، بروتينات الطور الحاد.