STUDY OF SERUM CATHELICIDIN AND VITAMIN D LEVELS IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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ABSTRACT

Cathelicidins are antimicrobial peptides that play a critical role in innate immune defense against invasive bacterial infections. Several studies have suggested the potential role of vitamin D in maintaining immune homeostasis and preventing the development of autoimmune processes e.g., diabetes mellitus. **The aim of the present work** was to study serum level of vitamin D and cathelicidin in a group of Egyptian children with type 1 diabetes mellitus and to know their relation to infection in those cases. **It was conducted at** Alzahraa University hospital on sixty children, aged 3-15 years of both sexes: forty of them known to have type 1 diabetes mellitus (type 1 DM) as defined by ADA, 2012 (twenty with acute bacterial infection and twenty without infection), and twenty were apparently healthy children age and sex matched taken as a control group. Patients were taken randomly from outpatient clinic of National Diabetes Institute during the period from January 2013 to June 2013. **All studied children were subjected to** careful history taking, thorough clinical examination and laboratory investigations including: CBC, CRP, measurement of serum 1,25(OH)2 vitamin D and serum cathelicidin levels by ELISA. For diabetic children fasting blood sugar, postprandial blood sugar and glycated hemoglobin (HbA1c) were also measured. **Results revealed** a significantly higher mean serum cathelicidin level in diabetic with infection when compared to controls (38.57±33.6 vs 18.67±25.07) while no significant difference was found between either diabetic without infection and controls or between diabetic with and without infection. The mean serum vitamin D levels in the diabetic patients (both with and without infection) were significantly lower than its mean serum level in controls (23.89±7.39 pg/ml and 24.49±5.37 versus 31.30±6.78 gp/ml respectively). Also, a significant positive correlation was found between serum cathelicidin and BMI while there was no significant correlation between serum cathelicidin or vitamin D and other studied parameters. ***In conclusion,*** serum cathelicidin level was significantly higher in diabetic children with acute bacterial infection when compared to controls, all studied diabetic children had significant lower mean serum vitamin D level in comparison to controls and a significant positive correlation was found between serum cathelicidin and BMI.

INTRODUCTION

Diabetes mellitus (DM) is group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels ***(ADA, 2012).***

Beside the known role of vitamin D in bone mineralization and calcium homeostasis there is now extensive evidence supporting its actions in immunity and inflammations and the expression of nuclear vitamin D receptors (VDR) by immune cells ***(Hughes and Nortas, 2009).***

Cathelicidins are a family of polypeptides found in macrophages and polymorphonuclear leukocytes after activation by bacteria, viruses, fungi or active form of vitamin D. It was found to have a critical role in innate immune defense against invasive bacterial infections ***(Girnita et al., 2012).***

AIM OF THE WORK

To study serum level of vitamin D and cathelicidin in a group of Egyptian children with type 1 diabetes mellitus and their relation to infection in those cases.

SUBJECTS AND METHODS

This is a case control comparative study that was carried out on 60 children including the following groups: ***Group I:*** twenty children with type 1 DM associated with acute bacterial infection (respiratory tract infection, skin infection or urinary tract infection). ***Group II:*** Twenty children with type 1 DM without associated infection. ***Group III:*** Twenty non diabetic apparently healthy age and sex matched children served as controls. All diabetic patients have fulfilled the diagnostic criteria of type 1 Dm as defined by ADA, 2012. Patients were taken randomly from outpatient clinic of National Diabetes Institute during the period from January 2013 to June 2013. They were 22 females and 18 males, their ages ranged from 3 to 15 years.

Children with all types of rickets, other types of diabetes and those on vitamin D therapy in the previous 6 months were excluded from the study. Informed consent was obtained from all patients and controls or their families before getting them involved in the study, and confidentiality of all data were ensured.

All studied children were subjected to the following:

- **Full medical history taking** according to a pre-designed questionnaire with stress on disease onset, duration of disease, mode of disease control and diabetic complications.
- **Thorough clinical examination** for detection or exclusion of infection and presence of complications.
- **The following investigation:**

  For all studied children:
  - ♦ Complete blood picture ***(Greer et al., 2009).***
  - ♦ C-reactive protein ***(Jialal et al., 2004)***.
  - ♦ Serum calcium and Phosphorus ***(Kuksis et al., 1980).***
  - ♦ Serum 1,25(OH)2 vitamin D by enzyme linked immunosorbent assay (ELISA) ***(Holick, 2009)***.
  - ♦ Serum cathelicidin by enzyme linked immuno-sorbent assay (ELISA) ***(Gambichler et al., 2012)***.

For diabetic: the following additional investigations had been done:

- ■ Fasting blood glucose and two hour postprandial ***(Wilson and Walker, 2005).***
- ■ Glycated hemoglobin (HbA1c) by HPLC method. TECO DIAGNOSTICS kit, (Cat number. 92807) ***(ISPAD, 2009).***

Statistical analysis:

Analysis of data was done by IBM computer using SPSS (Statistical package for social science version 12): Qualitative data were presented as: number (N) and percentage (%), while quantitative data as mean ($\overline{X}$), standard deviation (SD) and range. Normally distributed variables (parametric) between two study groups were analyzed using: Chi-square (X^2) test to compare of qualitative variables and unpaired (t) test to compare of quantitative variables, while Fisher exact test was used instead of chi-square when one expected cell or more was less than 5. Analysis of variance (ANOVA) for comparing more than two groups as regard quantitative variable followed by post hoc analysis using LSD method. Pearson correlation coefficient test (r) test. Was used to rank variables versus each others positively or inversely. For all tests a probability (p) was considered non significant if $\geq$ 0.05 and significant if $<$ 0.05 ***(Miller and Knapp, 1992).***

RESULTS

The results of this work are shown in tables (1-10).

Table (1): Clinical characteristics of all studied groups.

Group / Variables	Group (III) (Control, n=20)	Group (I) (Diabetic with infection, n=20)	P value	Group (II) (Diabetic without infection, n=20)	P-value
Age	8.70±3.25	10.70±2.39	0.037	9.80±2.44	0.233
Female: male ratio	12/8	8/12	0.343	10/10	0.751
Z score for height	0.02±1.00	-0.04±1.00	0.850	-0.20±1.00	0.490
Z score for weight	0.37±1.00	0.10±1.00	0.398	0.19±1.00	0.572

*P value for t-test. P> 0.05= non significant

No statistically significant difference was found between nth three studied groups regarding age, sex and anthropometric measurements.

Table (2): BMI percentile of studied groups

BMI centile	Group (I) (Diabetic with infection, n=20)		Group (II) (Diabetic without infection, n=20)		Group (III) (Control, n=20)		Chi-square test	
	No.	%	No.	%	No.	%	X^2	P-value
3rd	5	25%	2	10%	1	5%	20.724	0.294
5th	1	5%	0	0%	1	5%		
10th	0	0%	1	5%	0	0%		
25th	3	15%	1	5%	1	5%		
50th	3	15%	4	20%	1	5%		
75th	4	20%	6	30%	7	35%		
85th	1	5%	0	0%	3	15%		
90th	2	10%	4	20%	1	5%		
95th	1	5%	0	0%	2	10%		
97th	0	0%	2	10%	3	15%		
Total	20	100%	20	100%	20	100%		

No statistically significant difference was found in BMI percentile between the studied groups.

Table (3): Clinical data of studied diabetic children.

Variable	Mean	SD
Duration DM (years)	3.48	±2.5
Age onset DM (years)	6.77	±2.8
Variable	**No.**	**%**
Symptoms of DM		
Polyphagia	23	57.5%
Polydipsia	23	57.5%
Polyurea	39	97.5%
Acute complications of DM		
Hypoglycemia	14	35%
DKA	13	32.5%

Mean age of onset was 6.77 years, mean duration of disease was 3.48 years, 97.5% of them had polyurea and DKA occurred in 67.5%

Table (4): Comparison between diabetic with and without infection regarding laboratory parameters

Group / Parameter	Group (I) (Diabetic with infection, n=20)	Group (II) (Diabetic without infection, n=20)	Independent t-test	
	Mean±SD	Mean±SD	T	p-value
WBC (10^9/l)	19.54±4.39	6.95±2.27	11.386	**0.000**
RBC (10^{12}/l)	4.48±0.48	4.59±0.69	-0.594	0.556
HB (gm/dL)	12.23±1.83	11.85±1.46	0.726	0.472
PLT (10^9/L)	280.1±78.14	297.05±97.16	-0.60	0.547
CRP (mg/dl)	12.60±6.43	5.85±0.49	4.683	**0.000**

There was a statistically significant higher level of WBCs and CRP in diabetic with infection than those without infection.

Table (5): Diabetic laboratory parameters in studied cases.

Group / Parameter	Group (I) (Diabetic with infection, n=20)	Group (II) (Diabetic without infection, n=20)	Independent t-test	
	Mean±SD	Mean±SD	T	p-value
FBS	314.45±80.97	301.25±72.14	-0.544	0.589
2hr BS	212.31±45.72	203.12±52.13	0.504	0.618
HbA1c%	8.33±3.60	6.55±3.49	1.589	0.120

No statistically significant differences were found between the two studied diabetic groups regarding diabetic laboratory parameters.

Table (6): Comparison between all diabetic patients and controls regarding mean serum calcium and phosphorus levels

Group / Parameter	Diabetic group n=40	Control group n=20	Independent t-test	
	Mean±SD	Mean±SD	T	p-value
Ca (mg/dl)	9.21±0.67	9.49±0.93	1.337	0.186
P (mg/dl)	3.5±0.67	3.66±0.72	0.851	0.398

There was no statistically significant difference between diabetic group and control group regarding mean serum calcium and phosphorus levels.

Table (7): Serum cathelicidin values in the three studied groups

Groups	Serum cathelicidin (ng/dl)			One Way ANOVA	
	Mean±SD	Range	Normal reference	F	P-value
Group (I) (Diabetic with infection, n=20)	38.57±33.60	0.13 – 138.19	5 – 20 ng/dl	4.351	0.021
Group (II) (Diabetic without infection, n=20)	37.57±39.78	0.89 – 98.20			
Group (III) (Control, n=20)	18.67±25.07	1.31 – 86.29			
Post Hoc analysis: LSD test					
Group I vs II (Diabetic with infection versus diabetic without infection)	**Group I vs III** (Diabetic with infection versus control)		**Group II vs III** (Diabetic without infection versus control)		
0.932**	**0.040***		0.080**		

* Significant ** Non significant

A statistically significant higher level of serum cathelicidin was found in diabetics with infection than controls while no significant differences were found when comparing other groups.

Table (8): Serum vitamin D values in the three studied groups.

Groups	Serum vitamin D values pg/ml			One Way ANOVA	
	Mean±SD	Range	Normal reference	F	P-value
Group (I) (Diabetic with infection, n=20)	23.89±7.39	12.51 – 36.97	23.8 – 74.2 pg/ml	5.31	0.000
Group (II) (Diabetic without infection, n=20)	24.49±5.37	13.22 – 33.54			
Group (III) (Control, n=20)	31.30±6.78	20.29 – 42.72			
Post Hoc analysis: LSD test					
Group I vs II (Diabetic with infection versus diabetic without infection)	**Group I vs III** (Diabetic with infection versus control)		**Group II vs III** (Diabetic without infection versus control)		
0.771**	**0.002***		**0.001***		

* Significant ** Non significant

This table shows statistically significant decrease in the mean serum vitamin D level in diabetic patients with and without infections when compared to controls. While no statistically significant difference was found between diabetic with and without infection.

Table (9): Correlation between serum cathelicidin and the studied parameters.

Variables	Serum cathelicidin	
	r	p-value
Age onset DM (years)	-0.031	0.852
Duration DM (years)	-0.08	0.628
BMI (kg/m^2)	**0.372***	**0.02**
HB (mg/dl)	0.093	0.573
WBC (10^9/L)	-0.045	0.784
PLT (10^9/L)	0.147	0.372
HbA1c (%)	0.092	0.577
Vit D (pg/ml)	0.171	0.297
CRP (mg/dl)	-0.059	0.806

There was statistically significant positive correlation between serum cathelicidin and BMI while there was no significant correlation with other variables.

Table (10): Correlation between serum vitamin D and the studied parameters.

	Serum vitamin D	
	r	p-value
Age onset DM (years)	0.141	0.384
Duration DM (years)	0.064	0.695
BMI (kg/m^2)	0.125	0.443
WBC (10^9/L)	-0.099	0.544
PLT (10^9/L)	0.214	0.184
HbA1c (%)	0.114	0.482
Cathelicidin (ng/dl)	0.171	0.297
CRP (mg/dl)	0.057	0.812

No statistically significant correlation was found between serum vitamin D and other parameters.

DISCUSSION

Human cathelicidin (LL-37) is a multifunctional peptide with many important biological activities: anti-microbial activity, chemoattraction**,** dendritic cell differentiation, mast cell degranulaion**,** cytokine secretion, angiogenesis stimulation, and wound healing ***(Heilborn et al., 2003; Davidson et al., 2004; Yang et al., 2004).***

In our study, the mean serum cathelicidin level was significantly higher in diabetic with acute bacterial infection group when compared to controls. An explanation of this finding is that the presence of infection in diabetic children may be the cause of increased expression of serum cathelicidin level in those children in an attempt to enhance innate immune response and fight infection ***(Girnita et al., 2012)***.

In the present study, the mean serum vitamin D level was significantly lower in the diabetic patients either with or without infection when compared to controls (23.89±7.39, 24.49±5.37 versus 31.30±6.78 respectively). However, no statistically significant difference was found between diabetic children and controls regarding mean serum calcium and phosphorus levels.

Similar finding was reported by ***Vibhor et al. (2011)*** who studied serum vitamin D level in diabetic patients (T1DM) and observed significantly lower serum vitamin D level in diabetics than control subjects. The lower vitamin D level in diabetic children could be explained by the hypothesis that vitamin D is present in the insulin-producing beta cells, and in type 1 DM, T cells infiltrate the islet, causing insulitis and ultimately beta cells death. This may result in lower serum vitamin D level in type 1 DM ***(Sharif et al., 2010)***.

Vitamin D level in type 1 DM is very critical, as it is theorized that vitamin D has a role in both insulin secretion (as insulin secretion is calcium dependent and therefore indirectly vitamin D dependent) and insulin sensitivity for glucose homeostasis in type 1 DM ***(Alvarez and Ashraf, 2010).***

Evidence suggests that vitamin D deficiency in diabetes may be associated with hyperglycemia, increased HbA1c, insulin resistance, progression of diabetes as well as hypertension and cardiovascular disease ***(Penckofer et al., 2008)***.

Vitamin D has been shown to reduce the production of inflammatory cytokines in the body and hence may reduce complications ***(Sharif, 2010)***

The present study revealed statistically significant positive correlation between serum cathelicidin and BMI, while no significant correlation was found with other studied parameters. On the other hand, no significant correlations were found between serum vitamin D and other studied parameters.

Increased BMI (overweight and obesity) is associated with increased levels of inflammatory cytokines (e.g., CRP, IL6) throughout the body, which has been linked to the increased risk of developing several co-morbidities including cardiovascular disease ***(Wang et al., 2004).*** Diabetes may be complicated with infection that may lead to increased expression of serum cathelicidin.

In conclusion, our study demonstrated that

- Serum cathelicidin level was significantly higher in diabetic children with infection when compared to controls.
- All studied diabetic children had statistically significant lower mean serum vitamin D level when compared to controls.
- A significant positive correlation was found between serum cathelicidin and BMI.

From our study we recommend the following:

- Further large-scale studies to explore role of cathelicidin in childhood diabetes mellitus and its complications in general and its relation to infection in those children in particular. Further studies are recommended to explore the relation between increase BMI and cathelicidin.
- To study the effect of a trial of vitamin D supplementation for children with type 1 DM on preventing recurrent infections, assuring better glycemic control and reducing complications.

REFERENCES

1. **American Diabetes Association (ADA) (2012):** Diabetes management in correctional institutions. Diabetes Care; 35(1):S87-S93.
2. **Alvarez JA and Ashraf A (2010):** Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol; 351-385.
3. **Davidson DJ, Currie AJ, Reid GS, Bowdish DM, Macdonald KL, Ma RC, Hancock RE and Speert DP (2004):** The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol; 172:1146-1156.
4. **Gambichler T, Bechara FG, Scola N, Rotterdam S, Altmeyer P, et al. (2012):** Serum levels of antimicrobial peptides and proteins do not correlate with psoriasis severity and are increased after treatment with

fumaric acid esters. Arch Dermatol Res 304: 471-474.

5. **Girnita A, Zherg H, Gronberg et al. (2012):** Identification of the cathelicidin peptide LL37 as agonist for the type 1 insulin like growth factor receptor in oncogene, Journal, 19:31(3):352-365.

6. **Greer J, Foester J, Rodgers G, et al. (2009):** Winterobe's clinical heamatology., 12th ed, vol. 1, part 1, Ch. 1, p. 1-70.

7. **Heilborn JD, Nilsson MF, Kratz G, et al. (2003):** The cathelicidin antimicrobial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol; 120:379-389.

8. **Holick MF (2009):** Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 19: 73-78.

9. **Hughes DA and Norton R (2009):** Institute of food research, Norwich Research park, university of East Anglia. Norwich U.K. Clin Exp Immunol; 158(1):20-5.

10. **International Society for Pediatric and Adolescent Diabetes (ISPAD) (2009):** ISPAD clinical guidelines 2009 commending editors: Ragnor Hanas, Kim Donaghre, Georgeanng Klingen Smith and Peter GF swift. Pediatric Diabetes; 12:10:71-81.

11. **Jialal I, Devaraj S, Venugopal SK (2004):** C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 44: 6-11.

12. **Kuksis A, Myher JJ, Geher K, Shaikh NA, Breckenridge WC, et al. (1980):** Comparative determination of plasma phospholipids by automated gas--liquid chromatographic and manual colorimetric phosphorus methods. J Chromatogr 182: 1-26.

13. **Miller C and Knapp G (1992):** Clinical epidemiology and biostatistics, published by Williams & Wilkins, Maryland: 3rd edition.

14. **Penckofer S, Kouba J, Wallis D and Emanuele M (2008):** Vitamin D and Diabetes let the sunshine in. The diabetes Educator, 34: 9339.

15. **Sharif BM, et al. (2010):** Is there a role of vitamin D deficiency in type 1 Diabetes of Children? Am J Prev Med; 39(2): 189-190.

16. **Vibhor VB, Devidayal, Savita V, Bhalla AK (2010):** Low levels of vitamina D in North Indian children with newly diagnosed type 1 Diabetes. Pediatr Diabetes; 11(5): 345-50.

17. **Wang CC, Goalstone ML, Draznin B (2004):** Molecular mechanisms of insulin resistance that impact cardiovascular biology. Diabetes 53: 2735-2740.

18. **Wilson K and Walker J (2005):** Principle and technology of biochemistry and molecular biology. Six edition.

19. **Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ (2004):** Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxina in host defense. Annu Rev Immunol; 22:181-215.

دراسة مستوى فيتامين (د) والكاثيليسيدين في مصل الأطفال المصابين بمرض السكر النوع الأول

إن الكاثيليسيدين هي عائلة من الببتيدات المضادة للميكروبات والتي تلعب دور هام في تقوية المناعة الفطرية ضد العدوى البكتيرية، ولقد ذكرت العديد من الدراسات دور فيتامين (د) في الاتزان المناعي ومنع حدوث أمراض المناعة الذاتية مثل مرض السكر.

لذا كان الهدف من هذا البحث هو دراسة مستوى فيتامين (د) و الكاثيليسيدين في مصل مجموعة من الأطفال المصريين المصابين بمرض السكر النوع الأول ومعرفة علاقتهم بالعدوى في هذه الحالات.

وتم إجراء الدراسة بمستشفى الزهراء الجامعي على عدد 60 طفلاً من الجنسين تتراوح أعمارهم بين 3–15 سنة: 40 منهم مصابين بمرض السكر النوع الأول (20 مصابين أيضاً بالعدوى البكتيرية، و20 مصابين بمرض السكر فقط بدون عدوى) وكذلك 20 طفلاً صحيحاً كمجموعة ضابطة متساويين في العمر والجنس مع مجموعة مرضى السكر، وقد تم اختيار الحالات عشوائياً من العيادة الخارجية للمعهد القومي للسكر في الفترة من يناير 2013 وحتى يونيو 2013، وقد خضع جميع أطفال الدراسة لأخذ التاريخ المرضي كاملاً، والفحص الإكلينيكي الشامل وكذلك عمل الفحوصات المعملية التالية: صورة دم كاملة، البروتين التفاعلي سي، مستوى فيتامين (د) وكذلك مستوى الكاثيليسيدين بالمصل بطريقة إليزا، كما تم قياس مستوى السكر في الدم (صائم وبعد الأكل بساعتين) والهيموجلوبين السكري بالنسبة للأطفال المصابين بمرض السكر.

وقد أظهرت النتائج ارتفاع ذو دلالة إحصائية في مستوى الكاثيليسيدين بمصل الأطفال المصابين بالسكر ولديهم عدوى بكتيرية بالمقارنة في الأطفال الأصحاء بينما لا يوجد فرق ذو دلالة إحصائية في مستوى الكاثيليسيدين بالمصل بين الأطفال المصابين بمرض السكر وليس لديهم عدوى مقارنة بالأصحاء أو بين مجموعتي الأطفال المصابين بالسكر (بالعدوى مقارنة مع من بدون عدوى)، كما أظهرت انخفاض ذو دلالة إحصائية في مستوى فيتامين (د) في مصل

الأطفال المصابين بمرض السكر (سواء بعدوى أو بدونها) عنه في مصل الأطفال الأصحاء، وأيضاً أظهرت وجود علاقة طردية ذات دلالة إحصائية بين مؤشر كتلة الجسم ومستوى الكاثيليسيدين بالمصل بينما لم يكن هناك أي علاقات أخرى ذات دلالة إحصائية.

لذا استنتجنا وجود ارتفاع ذو دلالة إحصائية في مستوي الكاثيليسيدين بمصل الأطفال المصابين بمرض السكر ولديهم عدوى بكتيرية مقارنةً بالأصحاء، ووجود انخفاض ذو دلالة إحصائية في مستوى فيتامين (د) بمصل الأطفال المصابين بمرض السكر مقارنة بالأصحاء، وكذلك وجود علاقة طردية بين مؤشر كتلة الجسم ومستوى الكاثيليسيدين بالمصل.