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ORIGINAL ARTICLE

The Correlation Between Vitamin D Deficiency and Vitamin D Receptor Polymorphisms and The Frequency of Type 2 Diabetes Mellitus Complications Among Egyptian Patients

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KEYWORDS

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ABSTRACT: Diabetes mellitus and obesity are a growing overall medical issues, particularly in developed nations. Vitamin D deficiency is pandemic that has been involved in a different kind of disease. This study was intended to detect the effect of vitamin D deficiency and vitamin D receptor polymorphisms on type 2 diabetes mellitus (T2DM) patients through induction of obesity**.** 80 Egyptians T2DM patients and 20 non diabetic subjects were included in our research. Diabetic cases were categorized into two subgroups according to diabetic complications. PCR-RFLP for vit D receptor polymorphisms and vit D level were assessed to all subjects. The deficiency of vitamin D was more prevalent in diabetic cases than in control cases. There was significant difference between control and diabetic patients in genotyping of FokI (VDR 2228570 C) and TaqI (VDR rs731236 T) polymorphisms. The results of our study revealed that vitamin D deficiency could be a contributing factor for T2DM rising among Egyptian patients. Additionally, there was a genetic variation in the VDR genes FokI and TaqI related to diabetic mellitus in Egyptian patients.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder distinguished by changing carbohydrate, protein, and lipids metabolism. DM could result from defective insulin secretion or action. Obesity can affect the metabolism by secreting hormones, leptin, adiponectin, and proinflammatory cytokines that

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initiate the process of insulin resistance and subsequent DM development [1]

recently, Vitamin D3 has been given an extra attention due its skeletal and other non-skeletal biological responses involving inhibition of cancer cell progression; effects on the cardiovascular system; and protective role against multiple autoimmune diseases including inflammatory bowel disorder and multiple sclerosis [2, 3].

There is a developing proof that vitamin D deficiency is an enhancing factor in the progress of type 2 diabetes through the following mechanisms; i: pancreatic β-cell contains VDRs as well as the 1 alpha hydroxylase enzyme needed for vitamin D activation [4]. ii: vitamin D controls insulin secretion through calcium. iii: vitamin D induces insulin receptor gene expression, enhancing insulin mechanism for glucose uptake. iv: additionally, systemic inflammation improvement through cytokines [5, 6]. furthermore, many studies showed that vitamin D decreases the incidence of obesity. Vitamin D lowers the formation of new fat cells and decreases the storage of fat cells, effectively reducing fat accumulation [7]. Interestingly, vitamin D may elevate levels of, neurotransmitter serotonin. Serotonin can increase satiety, decrease body weight [8].

vitamin D can modify the circulating lipid through increasing the activity of lipoprotein lipase and decreasing the level of LDL [9].

The biological activities of the dynamic type of 1,25(OH) 2 D3, are intervened by the VDR, is a chromatin-associated intracellular receptor that belongs to steroid hormone receptor superfamily [10] There are more than 60 VDR polymorphisms that are situated in the promoter, in and around exons 2-9 and in the 3'UTR region. As a result, just few polymorphisms of this enormous gene have been contemplated. A huge portion of them are restriction fragment length polymorphisms (RFLP) with an anonymous functional action. Sometimes in several cases, it has been indicated that they could be connected to truly functional polymorphisms somewhere else in the VDR gene (or in a nearby gene), which explains some of the associations observed. Examples of these include the Fok1, BsmI, ApaI, TaqI, Tru9I, Cdx2 and poly (A) microsatellite and. Fok1 polymorphism in exon II, Bsm1 and Apa1 in intron VIII, Taq1 in exon IX, Tru91 in intron VIII, Cdx2 in exon I, and the poly(A) mononucleotide repeat in the 3'-untranslated region (3'-UTR) part of the gene [11].

Subjects and Method

A case – control study was designed to assess 80 Egyptians patients with type 2 diabetic mellitus of both sex and their aged around 40 - 60 years old and 20 Egyptians healthy as control group matched in age and sex with the diabetic patients. Diabetic cases were recruited from Diabetic clinic in the memorial Souad Kafafi University Hospital, and normal cases were recruited from internal medicine clinic in the memorial Souad Kafafi University Hospital. All cases fulfilled informed consent after explaining aim of study. Diabetic cases were then divided according to the presence of complication in to diabetic patients with and without complications. All cases were interviewed for demographic data collection including: age – sex - residence - Occupation - BMI - sun exposure average per day, and medical history including: duration of diabetes - current treatment of diabetes – type of diabetic complications including: cardiovascular diseases, kidney disease (diabetic nephropathy), nerve disease (diabetic neuropathy), eye disease (diabetic retinopathy). The study was directed according to the principles of the Declaration of Helsinki. Institutional Review Board (IRB) study approval was obtained prior to commencement of the study and signed informed consent was collected from all study patients at the point of recruitment and before randomization.

Ethical consideration

Written informed consent was acquired from each patient and the research protocol was approved according to the ethical guidelines of research Ethical Committee, faculty of medicine, Beni-suef University.

Biochemical investigations of blood samples

Venous blood samples were withdrawn from all subjects to assess the following a) Vitamin D serum level assessment by ELISA technique according to kit instructions of manufactures (DRG, international Inc., USA). The data are expressed as ng/ml. Grading of vitamin D levels was done as follows: (≥30ng/ml): normal, (>10 - <30ng/ml): insufficiency, (≤10ng/ml): deficiency. b) Measurement of Creatinin, ALT, albumin, Calcium and Phosphorous in serum by were detected using kits provided by Randox Laboratories Limited (Country Antrim, UK). c)Measurement of Lipid profile (total cholesterol, Triglyceride, and HDL- C) in serum by fully automated procedures and LDL cholesterol was calculated as follows: LDL = TC − HDL − TG \ 5. d) Determination of microalbuminuria in urine sample by performing albumin creatinine ratio. e) HbA1c was detected using kits provided by Stanbio Laboratory (Boerne, TX, USA).

DNA extraction

DNA extraction was performed by using GeneJET Whole Blood Genomic DNA Purification Kit. The extracted DNA was amplified and followed by restriction fragment length polymorphism (RFLP) to detect genotyping for four VDR SPNs FokI (VDR rs2228570 C>T)), BsmI (VDR rs1544410 A>G) TaqI (VDR rs731236) and ApaI (VDR rs7975232) [12,13].

RFLP –PCR

Amplification reaction was done in a 50 µL reaction mixture as following: (25 µL of Taq PCR master mix (Bioline), 100 ng of extracted DNA, 25 nM forward primer, 25 nM reverse primer (Operon Biotechnologies, Inc.) and 19 µL RNAase free water added in each tube in a gradient thermal Master cycler (Biometra), according to thermal profile: 35 cycles at 94°C for one minute, 60°C for one minute, 72°C for one minute and one final cycle of extension at 72°C for five minutes and finally were visualized by gel documentation system. Amplification reactions were set up separately for FokI, BsmI and TaqI polymorphic sites of VDR gene according to primers sequence. Table 1.

Table 1. PCR-RFLP pattern of FokI, BsmI TaqI and ApaI of VDR gene.

Restriction enzymes	Primers	Annealing temp (C)	PCR-RFLP products
FokI intron 8 and exon 2	F:5′AGCTGGCCCTGGCACTGACTCTGCTCT-3′ R:5′ATGGAAACACCTTGCTTCTTCTCCTCCCTC-3′	58 °C	TT :196 - 69 bp TC :265,196,69 bp CC :265 bp
BsmI intron 8	F:5′CAACCAAGACTACAAGTACCGCGTCATGA-3′ R:5′AACCAGCGGGAAGAGGTCAAGGG-3′	63 °C	GG :650, 175 bp GA:825, 650, 175 bp AA:825 bp
TaqI Exon 9	F: 5′-CAGAGCATGGACAGGGAGCAA-3′ R: 5′-GCAACTCCTCATGGCTGAGGTCTC-3′	63 °C	TT:495,245 bp TC:495,290,245,205 bp CC :290,245,205 bp
ApaI	F: 5′- CAGAGCATGGACAGG GAGCAA-3′, R: 5′- GCAACTCCTCATGGCTGAGGTCTC -3′.	62°C	TT : 490 bp TG : 490, 280, 210 bp GG : 280 and 210 bp

Statistical analysis

Statistical analysis was done using program IBM SPSS Statistics version 22. Descriptive data were explained as mean and standard deviation. Independent t test is used to compare the means of biochemical values when comparing two groups and ANOVA when comparing more than two groups. Chi square test (X2) is used to compare the genotypes frequency. Multinomial logistics regression, odds ratios and 95 confidence intervals (CI) was calculated to assess the relationship between the diabetic and genotypes. Statistical significance was considered when p values were <0.05.

RESULTS

The demographic data of participating subjects revealed non-significant difference between the control cases and diabetic cases according to the age, sex, occupation (p value>0.05), but it has been observed that diabetic patients have significantly higher BMI than the control (p value <0.001) and most of diabetic patients (81%) lack the sun exposure in compare to control (25%) (p value <0.001) Table 2.

Table 2. Demographic data for all studied subjects**.**

Parameter	Control, N=20	Diabetic, N= 80	P value
Age	51.2 ± 5.16	52.12 ± 6.94	0.579
Sex			
Male	10 (50%)	31 (39%)	0.418
Female	10 (50%)	49 (61%)	
BMI	20.81 ± 3.33	30.74 ± 5.58	<0.001
Occupation			
Employee	9 (45%)	19 (24%)	0.471
No job or Retired	11 (55%)	61 (76%)	
Sun exposure			
20-35min daily	15 (75%)	15 (19%)	<0.001
No exposure	5 (25%)	65 (81%)	
Duration of diabetes			
Less than 10 years		34 (42%)	
More than 10 years		46 (58%)	
Current treatment			
Insulin		28 (35%)	
Oral therapy		52 (65%)	

Regarding the biochemical data, there was a significant decrease in vitamin D level (Figure 1A), HDL, platelet count in diabetic patients compared to the control (p value (<0.001, <0.001, 0.017) respectively. But there was no significant difference in vitamin D level between diabetic patients without complication and those having complication (p value= 0.5) (Figure 1B). Finally, there was non-significant difference in vitamin D level between different types of complications (p value =0.2) (Figure 1C).

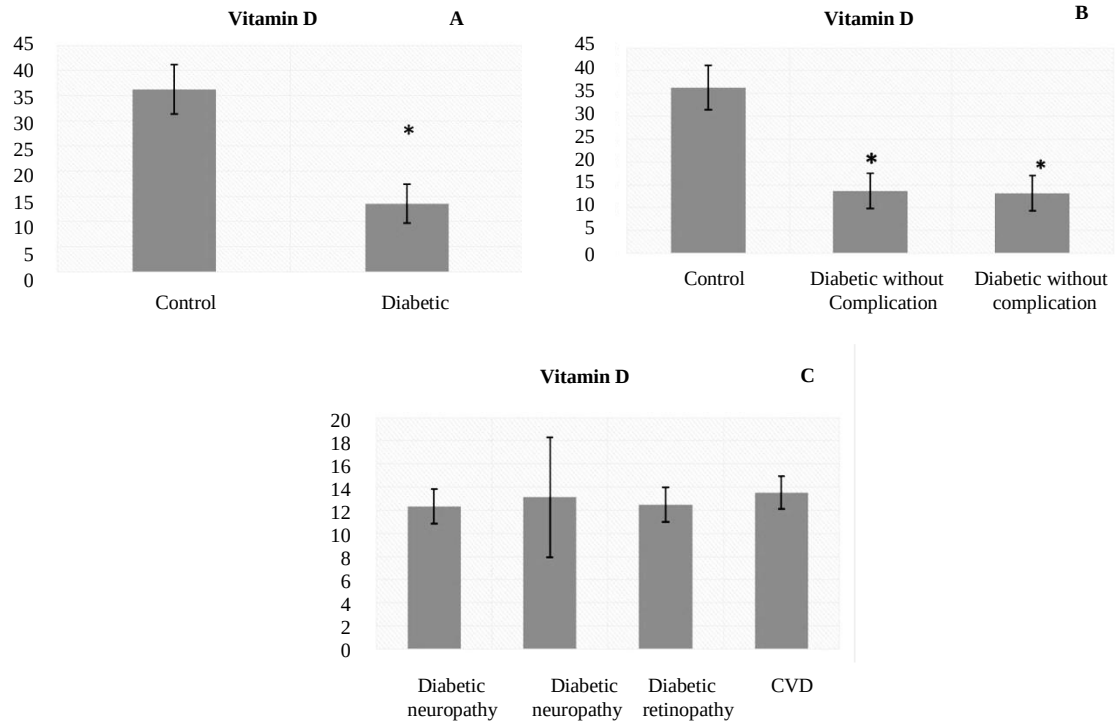

Figure 1A. significant decrease in vitamin D level in diabetics compared to control (p value=0.001). B: significant decrease in vitamin D level in diabetics with and without complication compared to control (p value=0.001), but no significant difference between the two diabetic groups (p value=0.842). C: no significant difference in vitamin D levels between different types of complications (p value=0.744).

There was a significant increase was found in (HbA1c, creatinine, micro albuminuria, TG, LDL-cholesterol) in diabetic patients compared to the control (p value (<0.001,0.038, 0.003, <0.001, <0.001) respectively (Table 3).

Table 3. Analysis of biochemical results for all studied subjects.

Parameter	Control, N = 20	Diabetic, N = 80	P value
Vitamin D (ng/dl)	36.25 ± 4.87	13.53 ± 3.87	<0.001
HbA1c (%)	4.82 ± 0.23	6.97 ± 0.405	<0.001
Creatinine (mg/dl)	0.80 ± 0.15	0.877 ± 0.134	0.038
Albumin (mg/dl)	3.98 ± 0.35	3.90 ± 0.223	0.230
Micro albuminuria (µg\mg creatinine)	9.1 ± 4.96	42.77 ± 49.37	0.003
SGPT (IU/L)	22.06 ± 5.58	18.13 ± 5.16	0.316
Calcium (mg/dl)	9.0 ± 0.743	8.86 ± 0.35	0.424

Phosphorous (mg/dl)	3.505 ± 0.47	3.52 ± 0.503	0.888
INR %	1.004 ± 0.016	1.003 ± 0.012	0.705
Cholesterol (mg/dl)	172.13 ± 32.37	169.66 ± 53.43	0.844
TG (mg/dl)	142.63 ± 24.17	208.23 ± 50.01	<0.001
HDL-C (mg/dl)	60.6 ± 13.79	48.15 ± 12.62	<0.001
LDL-C (mg/dl)	80.82 ± 29.47	110.65 ± 47.50	0.009
Hb (g/dl)	12.24 ± 0.42	11.46 ± 2.49	0.174
RBCs ($c\times10^6$/ml)	4.38 ± 0.52	4.66 ± 1.121	0.277
WBCs ($c\times10^3$/ ml)	6.64 ± 1.81	29.34 ± 67.35	0.136
Platelets ($c\times10^3$/ ml)	320.55 ± 31.95	261.69± 107.03	0.017

Genotyping and allele distributions and frequencies for four studied VDR gene polymorphisms at FokI, BsmI, TaqI and ApaI in all studied cases revealed that VDR FokI CC genotype significantly increase the risk of type II diabetes with frequency (68%) in diabetic patients compared the control (40%) (p value =0.005) OR =0.39, 95% CI (0.173- 0.915). In addition, VDR TaqI TC genotype significantly increase the risk of type II diabetes with frequency (46%) in diabetic patients compared the control (30%) (p value =0.035) OR =2.2, 95% CI (1.07- 4.5). While no significant difference in the distribution of VDR BsmI and ApaI genotypes between diabetic patients and control (p value =0.139, 0.053). Table 4.

Table 4. Analysis of genotyping for VDR gene polymorphisms at FokI, BsmI, TaqI and ApaI in all subjects.

Genotype	Control, N = 20, %	Diabetic, N = 80	P value	OR	95% CI
FokI (VDR 2228570)					
CC	8 (40%)	54 (68%)			
CT	12 (60%)	19 (19%)	0.005	0 .398	(0.173- 0.915)
TT	0 (0%)	7 (8%)			
BsmI (VDR 1544410)					
AA	6 (40%)	10(56%)			
GA	10 (50%)	55(24%)	0.139	1.154	(0.564 - 2.359)
GG	4 (20%)	15 (19%)			
TaqI (VDR rs731236)					
TT	10 (50%)	17 (21%)			
TC	6 (30%)	37 (46%)	0.035	2.213	(1.077- 4.549)
CC	4 (20%)	26 (33%)			
ApaI (VDR rs7975232)					
TT	16 (80%)	71 (89%)			
TG	0 (0%)	5 (6%)	0.053	0.354	(0.135 - 0.924)
GG	4 (20%)	4 (5%)			

The frequency distributions of VDR genotype (FokI, BsmI, TaqI and ApaI) shows non-significant difference between diabetic patients without complications and those having complication (p value >0.05). By comparing four VDR gene polymorphism between diabetic patients with several types of complications, there was non-significant difference in their distribution (p value >0.05) except CT (43%) genotype is most commonly happening in diabetic nephropathy in compare to diabetic patient without complications (27%) (p value 0.013) Table 5.

Table 5. Genotyping analysis for VDR gene polymorphisms at FokI, BsmI, TaqI and ApaI in between different types of complications.

Genotyping	Diabetic without complication	Diabetic Nephropathy	Diabetic neuropathy	Diabetic retinopathy	Cardiovascular disease	p-value
	37(73%)	4 (57%)	4(66%)	8(100%)	14 (87%)	0.401*
FokI	14 (27%)	3 (43%)	1 (17%)	0 (%)	2 (13%)	0.013**
	0 (0%)	0	1 (17%)	0	0	0.090***
						0.221****
	11(22)	2(29%)	1(17%)	2 (25%)	2 (13%)	0.543*
BsmI	33(65%)	5 (71%)	4(66%)	6 (75%)	12 (74%)	0.972**
	7 (14%)	0	1 (17%)	0	2 (13%)	0.531***
						0.786****
	44(86)	6(86%)	5(83)	8(100%)	14 (87%)	0.554*
ApaI	3 (6%)	1 (14%)	1(17%)	0 (%)	2 (13%)	0.505**
	4 (8%)	0	0	0	0	0.536***
			0 (0%)			0.373****
	10(50%)	3 (42%)	5 (83%)	1 (12%)	2 (13%)	0.564*
TaqI	6 (30%)	2 (29%)	1(17%)	4 (50%)	10 (62%)	0.206**
	4 (20%)	2 (29%)		3 (38%)	4 (25%)	0.688***
						0.468****

*Comparison between diabetic patients without complications and diabetic patients with nephropathy.
**comparison between diabetic patients without complications and diabetic patients with neuropathy.
***comparison between diabetic patients without complications and diabetic patients with retinopathy.
****comparison between diabetic patients without complications and diabetic patients with CVD.

DISCUSSION

Type 2 diabetes mellitus is one of the most common chronic disorder globally, characterized by insulin resistance and defective insulin secretion. Vitamin D has a huge role in glucose homeostasis. It enhances β-cell function and enhances insulin sensitivity of the target cells (skeletal muscle, liver, and adipose tissue).

This study was conducted to evaluate if there is a correlation between VitD deficiency together with VDR gene polymorphisms and diabetes mellitus, diabetic complications; among Egyptian patients.

The present study revealed that the significant deficiency of vitamin D in T2DM cases was more than in control group. This is agreed with previous studies revealed significant decrease in Vit D levels in diabetic cases when compared with control group [14-16].

We recorded a significant elevated TG and LDL-C and a significant decrease in HDL-C in diabetic patients comparing to control group, this coincides with a cross-sectional study revealed that significantly elevated mean serum levels of TC, TG and LDL and

significantly decrease mean serum levels of HDL were observed in patients with diabetes [17]. More studies detected in their results that diabetic patients presented serum lipid abnormality. Increasing triglyceride (TG) levels and lowering high-density lipoprotein cholesterol (HDL-C) [18, 19].

Some recent studies evaluated the relationship between T2DM and platelets counts. A statistically significant difference in platelet counts of diabetics and controls subjects was detected" these results were in harmony with present study findings which included a significant difference in platelets count between diabetic patients and control group [20]. Additionally, the platelet test could be helpful in the early detection of long-term complications in diabetic patients, because it is a simple and cheap tool [20]. Otherwise, authors detected The PDW (platelet distribution width) and platelets count were not significantly different between diabetic group and non-diabetic group [21].

The microalbuminuria results showed a significant increase in diabetic patients when comparing with control group. Researchers detected an elevated level of microalbuminuria was significantly related to chronic micro/macro-vascular complications of diabetes mellitus; microalbuminuria levels screening provides a predictive value for a presence of chronic micro/macro-vascular complications in patients with T2DM [22].

The BMI value was evaluated in diabetic patients' group and its subgroups and control group, the results revealed that diabetic patients with different diabetic durations and patients with and with no complications, all in obese. A recent study carried out on Chinese elderly with T2DM concluded that elevated BMI was related to high insulin resistance and low insulin sensitivity in elderly Asian population with type 2 diabetes mellitus [23] Another study evaluated the relationship between obesity and controlled blood glucose among patients with type 1 (T1DM) or type 2 diabetes mellitus, this study concluded that, T2DM patients with overweight and obese have greater HbA1c value than those in normal body weight. While obese individuals with T2DM have lack glycemic control were more likely than overweight individuals with T2DM [24].

In the present study, we evaluated the four functional SNPs, FokI (VDR 2228570), BsmI (VDR 1544410), TaqI (VDR rs731236) and ApaI (VDR rs7975232) of the VDR gene in all study groups.

By comparing genotyping frequencies and allelic distributions of four studied SNPs for diabetic patients and control group, the results revealed that there was a significant difference between control subjects and diabetic patients in genotyping of FokI and TaqI polymorphism, while there is no observed significant difference in VDR BsmI and ApaI genotypes between patient and control subjects.

Many recent studies were carried out on VDR polymorphisms and its relation to T2DM; the results of these studies were differed according to ethnic populations. Some of these studies were carried out in South Asian countries and reported a strong relation between T2DM and FokI polymorphism and its genotypes regarding VDR Bsm-I genotype there was no any statistical difference between the control and T2DM patient [25]. Additionally, there was a significant difference between patients and controls in TaqI and BsmI polymorphism in the frequency of their homozygous and heterozygous genotyping [26]. In Pakistani population, ApaI was investigated in T2DM patients; the results revealed that there was no any significant relationship of ApaI with the T2DM onset and metabolic parameters [27]. Another study investigated four SNPs of the VDR gene ((BsmI,) (Tru9I,), (TaqI,) and (BglI,) and their correlation to susceptibility to T2DM in Chinese Han and Hui populations. the results showed that the VDR BglI and BsmI gene were correlated to susceptibility to T2DM in the Chinese Han population but not in the Chinese Hui ethnicity in this region. In addition, the G allele of the BsmI may be a risk factor of T2DM in the Chinese Han population [28]. The results of case–control study which applied on older people living in community in

Santiago de Chile revealed that the C allele of the VDR-FokI gene may be a risk factor for T2D in older people living in a community in Santiago de Chile [29].

Another similar study was carried out in Arab countries include a case control study conducted in Obese Iraqi Population which evaluated VDR TaqI gene polymorphisms in T2DM Iraqi patients against control and the results revealed T allele of Taq1 polymorphism in VDR gene is related to higher risk of diabetic disease in type 2 diabetic Iraqi patients. Taq1 genotype may be used as a risk marker to predict the diabetes complication [30]

In Saudi population, the results reported a significant difference in ApaI VDR gene polymorphisms genotypes and allele frequencies between T2DM patients and control groups [31].

In the Tunisian population the study revealed non-significant relation of the FokI polymorphism in type 2 diabetes mellitus [32], While the study carried out in Moroccans population reported that f allele of Fok-I polymorphism may have a protective effect against diabetes [33].

In this study, by comparing the genotyping frequencies of four studied VDR (FokI, BsmI, TaqI and ApaI) genes polymorphisms between diabetic patients without complications and patients with different types of diabetic complications, there was significant different in FokI polymorphism genotyping between diabetic patient without complications and diabetic patients with neuropathy. Otherwise, there wasn't any significant difference in the other polymorphism in patients without complications and patients with different complications.

CONCLUSIONS

Our finding revealed that vitamin D deficiency related to T2DM among Egyptian patients most probably through induction of obesity. There was a genetic variation in the VDR gene related to diabetic mellitus, FokI and TaqI SNPs were associated with susceptibility to T2DM in Egyptian patients.

Limitations

The major limitation of the present study is the low sample size, and shortage of financial.

CONFLICT OF INTERESTS

All authors declare that no conflict of interest.

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