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Original Article

Serum vitamin D status in type 2 diabetic patients from Gaza Strip

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ABSTRACT

Objective: To assess serum vitamin D status and its relations to other biochemical parameters in type 2 diabetic patients from Gaza Strip.
Materials and methods: This case-control study included 58 type 2 diabetic patients as well as 58 non-diabetic controls. Patients and controls were matched for age and gender. Data were obtained from questionnaire interview, and biochemical analysis of blood samples.
Results: Serum vitamin D was significantly lower in diabetic patients compared to non-diabetic controls (25.9 ± 11.0 *versus* 34.6 ± 13.8 ng/dl, % difference = 28.8%, $P < 0.001$). The number of patients having vitamin D deficient, insufficient and sufficient were 6 (10.4%), 35 (60.3%) and 17 (29.3%) compared to controls of 3 (5.2%), 16 (27.6%) and 39 (67.2%), respectively ($\chi^2 = 14.672$, $P < 0.001$). Serum glucose, glycated hemoglobin (HbA1c), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triglycerides were significantly higher in patients than in controls whereas serum insulin, high density lipoprotein cholesterol (HDL-C) and calcium were significantly lower in patients. Serum vitamin D showed significant negative correlations with HbA1c ($r = -0.186$, $P = 0.046$), ALT ($r = -192$, $P = 0.040$) and AST ($r = -0.188$, $P = 0.044$) whereas significant positive correlations were found with HDL-C ($r = 0.188$, $P = 0.044$) and calcium ($r = 0.239$, $P = 0.010$).
Conclusion: The significant negative and positive correlations of vitamin D with HbA1c and calcium, respectively suggests that vitamin D supplementation would be of potential therapeutic value in clinical settings for controlling of type 2 diabetes and more importantly its complications. However, a well-designed clinical trials are needed to define the contribution of vitamin D status and therapy in the global diabetes problem.

1. Introduction

Diabetes is a heterogeneous condition characterized by hyperglycemia. Historically patients with diabetes have been classified into two main categories: type 1 diabetes mellitus, characterized by a near-absolute deficiency of insulin secretion and type 2 diabetes mellitus where the cause is a combination of insulin resistance and an insulin secretory defect [1]. Diabetes complications are more common among type 2 diabetic patients and are responsible for significant morbidity and mortality. The chronic complications of type 2 diabetes are broadly divided into microvascular and macrovascular complications. Microvascular complications include neuropathy, nephropathy, and retinopathy, while macrovascular complications consist of cardiovascular disease, stroke, and peripheral artery disease [2].

Lack of insulin action and/or secretion in type 2 diabetes results in hyperglycemia and promotes lipolysis in adipose tissue leading to elevated circulating levels of free fatty acids. In addition, excess fatty acids in serum of diabetic patients are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins [3]. In addition, disturbance in liver and kidney functions was also reported in type 2 diabetic patients [4,5].

Vitamin D is a fat-soluble prohormone that plays an essential role in calcium homeostasis and the maintenance of normal function in multiple tissues. Humans obtain vitamin D either directly from the diet or through exposure to solar ultraviolet B radiation [6]. In addition to its well-recognized effects on skeletal health, vitamin D has been suggested to have a potential role in other

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disease states and health conditions including type 2 diabetes, cardiovascular disease, autoimmune disorders and cancer [7,8].

Regarding diabetes, several studies have found that patients with type 2 diabetes or impaired glucose tolerance are significantly more likely to have a lower serum vitamin D concentration compared to those without diabetes [9,10]. In this context, some authors revealed a strong negative correlation of serum vitamin D with insulin resistance [11,12], and others reported a positive association with β-cell function [13,14]. In Gaza Strip, the published studies on vitamin D are very limited and restricted just to identifying the risk factors associated with its deficiency in children [15]. To our best knowledge, no previous study linked vitamin D to diabetes mellitus. Therefore, this study is the first to assess serum vitamin D status and its relations to other biochemical parameters in type 2 diabetic patients from Gaza strip.

2. Materials and methods

2.1. Study design and study population

The present study was a case-control design. The study population included 58 type 2 diabetic patients (29 males and 29 females) who were referred to Diabetic Care Unit at Al Rimal Medical Center (the representative clinic for diabetic patients in Gaza Strip) and were previously diagnosed according to the current World Health Organization diagnostic criteria for diabetes [16]. Patients with type 1 diabetes, pregnant women and patients who take hormone replacement therapy or corticosteroid therapy were excluded. A total of 58 apparently healthy individuals (29 males and 29 females) with no personal history of diabetes were selected randomly from general population and served as a control group. All patients and controls were matched for age (40–65 years old) and gender.

2.2. Ethical consideration

The research was undertaken according to the Declaration of Helsinki and after the Local Research Ethics Committee had approved the study. All participants provided written informed consent prior to the study.

2.3. Questionnaire interview

A meeting interview was used for filling in the questionnaire which designed for matching the study need for both patients and controls. All interviews were conducted *face to face* by only one researcher himself. During the survey the interviewer explained any of the questions that were not clear. The questionnaire was based on diabetic clinic questions of the Palestinian Ministry of Health with some modifications [17]. Most questions were the yes/no type, which offer a dichotomous choice [18]. A questionnaire was validated, and piloted with 8 individuals not included in the population sample, and modified as necessary. The questionnaire included questions on age, education, employment, family income, smoking, family history of diabetes, physical activity, and diet.

2.4. Patients' records

Clinical data including duration of diabetes and diagnosed diabetic complications (retinopathy, cardiovascular disease and neuropathy) were obtained from the patients' records.

2.5. Blood sampling and processing

Fasting blood samples (about 8 ml each) were collected from patients and controls. About 2 ml blood was placed into EDTA vacutainer tube to perform HbA1c. The remainder quantity of blood (about 6 ml) was placed into a plastic tube and was left for a while without anticoagulant to allow blood to clot. Then, serum samples were obtained by centrifugation at 4000 rpm/10 min using a Rotina 46 Hettich Centrifuge, Japan.

2.6. Biochemical analysis

Serum Vitamin D was determined by enzyme-linked immunosorbent assay [19]. The glucose oxidase/glucose peroxidase (POD) method was used to measure serum glucose using Labkit Kits, Spain [20]. HbA1C was determined by the colorimetric determination of glycated hemoglobin in whole blood using Stanbio Kit, Texas-USA [21]. Serum insulin was measured by microparticle enzyme immunoassay, using Abbott IMx Insulin assay [22]. Serum ALT and AST were measured by using optimized UV-test according to International Federation of Clinical Chemistry and Laboratory Medicine using DiaSys reagent kits [23]. Serum urea and creatinine were determined by the urease glutamate dehydrogenase/UV method and by the alkaline picrate method, respectively, using the BioSystems kit, Spain [24,25]. Serum cholesterol and triglycerides were measured by the cholesterol oxidase/POD method and by the glycerol phosphate oxidase/POD method, respectively, using the BioSystems kit, Spain [26,27]. High-density lipoprotein cholesterol was determined by the precipitating method using Labkit kit, Spain [28]. Low-density lipoprotein cholesterol was calculated using the empirical relationship of Friedewald [29]. Serum calcium was assayed following instructions of Randox reagent kit manual [30]. Serum phosphorus was determined by phosphomolybdate UV end point, using Amonium Molybdate Diagnostic kit [31].

2.7. Statistical analysis

Data were analyzed using Statistical Package for Social Science Inc., Chicago, IL (SPSS) computer program version 23 for windows. A Simple distribution of the study variables and cross tabulation was applied. Chi-square (χ^2) was used to identify the difference between variables. Yates's continuity correction test, $\chi^{2}_{(corrected)}$, was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small. The independent sample *t*-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between patients and controls vitamin D. Pearson's correlation test was applied. The results were accepted as statistically significant when $P < 0.05$. The percentage difference was calculated according to the formula: Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

$$\text{Percent difference} = (|\,(V1 - V2)\,|/((V1 + V2)/2)) \times 100.$$

3. Results

3.1. Clinical and socio-demographic characters

Table 1 revealed no significant differences between diabetic patients and controls for age, education, smoking and physical activity ($P > 0.05$). However, employment and family income/month were significantly lower among patients compared to controls ($\chi^2 = 4.275$, $P = 0.039$ and $\chi^2 = 8.309$, $P = 0.016$, respectively) whereas family history of diabetes and diet were significantly

Table 1
Clinical and socio-demographic characters of controls and diabetic patients.

Character	Controls (n = 58)	Patients (n = 58)	Test	P-value
Age (year)	51.9 ± 7.5	51.8 ± 7.3	t = 0.063	0.950
Education (year)				
1–9	17 (29.3)	26 (44.8)	$\chi^2 = 2.993$	0.084
>9	41 (70.7)	32 (55.2)		
Employment				
Yes	30 (51.7)	19 (32.8)	$\chi^2 = 4.275$	0.039
No	28 (48.3)	39 (67.2)		
Family income/month (NIS)				
<1000	12 (20.7)	9 (15.5)	$\chi^2 = 8.309$	0.016
1000–2000	15 (25.9)	30 (51.7)		
>2000	31 (53.4)	19 (32.8)		
Smoking				
Yes	9 (15.5)	12 (20.7)	$\chi^2 = 0.523$	0.470
No	49 (84.5)	46 (79.3)		
Family history of diabetes				
Yes	21 (36.2)	35 (60.3)	$\chi^2 = 6.767$	0.009
No	37 (63.8)	23 (39.7)		
Physical activity				
Yes	17 (29.3)	27 (46.6)	$\chi^2 = 3.662$	0.056
No	41 (70.7)	31 (53.4)		
Diet				
Yes	9 (15.5)	19 (32.8)	$\chi^2 = 4.708$	0.030
No	49 (84.5)	39 (67.2)		

NIS: new Israeli Shekel (~0.27 $US).
Values are n (%) except age where values are expressed as means ± SD.
P < 0.05: Significant, P > 0.05: not significant.

higher among patients ($\chi^2 = 6.767$, P = 0.009 and $\chi^2 = 4.708$, P = 0.030, respectively).

3.2. Duration of diabetes and diabetic complications

The mean diabetes duration among patients was 7.2 ± 6.0 years, distributed as follows: patients with diabetes ≤ 5 years were 28 (48.3%), whereas those with diabetic duration of 6–11 years were 19 (32.7%). The rest of patients 11 (19.0%) had diabetes for more than 11 years. Table 2 pointed out that retinopathy and CVD were significantly higher in patients compared to controls ($\chi^2 = 6.294$, P = 0.012 and $\chi^2 = 3.980$, P = 0.046, respectively). On the other hand, neuropathy showed no significant difference between the two groups ($\chi^2 = 3.356$, P = 0.067).

3.3. Serum vitamin D and its categories in controls and diabetic patients

As indicated in Table 3, serum vitamin D was significantly lower in diabetic patients with respect to controls (25.9 ± 11.0 *versus* 34.6 ± 13.8 ng/dl, % difference = 28.8%, P < 0.001). The number of patients having vitamin D deficient, insufficient and sufficient were 6 (10.4%), 35 (60.3%) and 17 (29.3%) compared to controls of 3 (5.2%), 16 (27.6%) and 39 (67.2%), respectively ($\chi^2 = 14.672$ and P < 0.001).

Table 2
Diabetic complications in controls and diabetic patients.

Complication	Controls (n = 58)	Patients (n = 58)	χ^2	P-value
Retinopathy				
Yes	4 (6.9)	15 (25.9)	6.294	0.012
No	54 (93.1)	43 (74.1)		
CVD				
Yes	3 (5.2)	11 (19.0)	3.980	0.046
No	55 (94.8)	47 (81.0)		
Neuropathy				
Yes	1 (1.7)	7 (12.1)	3.356	0.067
No	57 (98.3)	51 (87.9)		

CVD: Cardiovascular disease.
Values are n (%).
P-value of χ2(corrected) test.
P < 0.05: Significant, P > 0.05: not significant.

3.4. Metabolic profile of controls and diabetic patients

Table 4 illustrates that serum glucose, HbA1c, serum ALT, AST and triglycerides were significantly higher in patients (208.2 ± 113.0 mg/dl, 7.9 ± 1.7%, 20.9 ± 14.8 U/L, 22.6 ± 10.6 U/L and 284.7 ± 120.2 mg/dl) than in controls (100.5 ± 24.4 mg/dl, 5.3 ± 0.8%, 16.5 ± 6.2 U/L, 17.9 ± 6.0 U/L and 234.2 ± 134.6 mg/dl) with P < 0.001, P < 0.001, P = 0.045, P = 0.004 and P = 0.035, respectively. In contrast, serum insulin, HDL-C and calcium were significantly lower in patients compared to controls (13.0 ± 13.7 μIU/ml, 34.5 ± 7.1 mg/dl and 9.0 ± 0.7 mg/dl *versus* 18.0 ± 8.7 μIU/ml, 41.3 ± 10.9 mg/dl and 9.4 ± 0.7 mg/dl, P = 0.030, P < 0.001 and P = 0.002, respectively).

3.5. Serum vitamin D in relation to the studied parameters

As shown in Table 5, Pearson correlation test revealed significant negative correlations of serum vitamin D with HbA1c (r = - 0.186, P = 0.046), ALT (r = - 192, P = 0.040) and AST (r = - 0.188, P = 0.044). Conversely, significant positive correlation of serum vitamin D was found with HDL-C (r = 0.188, P = 0.044) and calcium (r = 0.239, P = 0.010).

4. Discussion

Despite the high morbidity and mortality cases of diabetes mellitus in Gaza Strip [32], there is under-diagnosis and under-reporting of the disease. Biochemical tests of diabetes are restricted to monitoring blood glucose level when the patient visits the clinic. This necessitated further assessment of other biochemical parameters in blood such as vitamin D, aiming to speculate its possible role, if present, in this metabolic disorder. Vitamin D deficiency has been recently linked to type 2 diabetes [33,34]. This study is the first to assess serum vitamin D status and its relations to other metabolic parameters in type 2 diabetic patients in Gaza strip.

Type 2 diabetes was more frequent among unemployed patients and among low-income families. Similar results were previously documented [35,36]. It was addressed that poverty increases type 2 diabetes incidence and inequality of care despite universal health coverage [37]. Family history of diabetes and diet were significantly higher in patients compared to controls. Indeed, family history is well recognized as a risk factor for type 2 diabetes [38]. The finding that about half of patients (48.3%) had diabetes for 5 years or less does confirm the idea that type 2 diabetes has a long asymptomatic pre-clinical phase which frequently goes undetected. At the time of diagnosis, the patient could have one or more diabetes complications. Retinopathy and CVD were significantly higher in patients compared to controls. It is worth mentioning that CVD associated with diabetes is the leading cause of morbidity and mortality; diabetic patients have up to a 3 times increase in mortality than non-diabetics [39].

Serum vitamin D was significantly lower in patients with respect to controls. When categorized, the number of patients having vitamin D deficient and insufficient was significantly higher than controls. This does support the finding that hypovitaminosis D is prevalent in type 2 diabetic patients [10,40]. The precise role of vitamin D in the pathophysiology of type 2 diabetes still ambiguous and needs further clarification. The proposed mechanisms to explain how vitamin D deficiency promotes type 2 diabetes

Table 3
Serum vitamin D and its categories in controls and diabetic patients.

Category	Controls (n = 58)	Patients (n = 58)	Test	P-value
Vitamin D (ng/dl) *(min - max)*	34.6 ± 13.8 *(10–70)*	25.9 ± 11.0 *(3.8 - 55)*	t = 3.780	<0.001
Deficient (<10 ng/dl)	3 (5.2)	6 (10.4)	$\chi^2 = 14.672$	<0.001
Insufficient (10–30 ng/dl)	16 (27.6)	35 (60.3)		
Sufficient (>30 ng/dl)	39 (67.2)	17 (29.3)		

Values are n (%) except vitamin D where values are expressed as means ± SD.
P-value of χ2(corrected) test.
P < 0.05: Significant.

Table 4
Metabolic profile of controls and diabetic patients.

Category	Controls (n = 58)	Patients (n = 58)	% Difference	*t*-test	P-value
Glucose (mg/dl)	100.5 ± 24.4	208.2 ± 113.0	69.8	7.097	<0.001
HbA1c (%)	5.3 ± 0.8	7.9 ± 1.7	39.4	10.757	<0.001
Insulin (μIU/ml)	18.0 ± 8.7	13.0 ± 13.7	−32.2	2.028	0.030
ALT (U/L)	16.5 ± 6.2	20.9 ± 14.8	23.0	2.032	0.045
AST (U/L)	17.9 ± 6.0	22.6 ± 10.6	23.2	2.962	0.004
Urea (mg/dl)	33.7 ± 9.0	36.9 ± 10.9	9.1	0.817	0.087
Creatinine (mg/dl)	0.8 ± 0.3	0.7 ± 0.4	−11.8	1.726	0.416
Cholesterol (mg/dl)	175.2 ± 48.7	185.0 ± 64.9	5.4	0.927	0.356
Triglycerides (mg/dl)	234.2 ± 134.6	284.7 ± 120.2	9.7	2.130	0.035
HDL-C (mg/dl)	41.3 ± 10.9	34.5 ± 7.1	−17.9	3.975	<0.001
LDL-C (mg/dl)	86.5 ± 70.4	95.0 ± 40.1	9.4	0.767	0.445
Calcium (mg/dl)	9.4 ± 0.7	9.0 ± 0.7	−4.3	3.246	0.002
Phosphorus (mg/dl)	4.4 ± 0.9	4.3 ± 0.6	−2.3	0.076	0.940

HbA1c: Glycated hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol.
Values are expressed as means ± SD.
P < 0.05: Significant, P > 0.05: not significant.

Table 5
Serum vitamin D in relation to the studied parameters.

Parameter	Serum vitamin D (ng/dl)	P-value
	Pearson's correlation (r)	
Glucose (mg/dl)	−0.143	0.130
HbA1c (%)	−0.186	0.046
Insulin (μIU/ml)	0.137	0.181
ALT (U/L)	−0.192	0.040
AST (U/L)	−0.188	0.044
Urea (mg/dl)	−0.104	0.286
Creatinine (mg/dl)	- 0.097	0.351
Cholesterol (mg/dl)	−0.081	0.390
Triglycerides (mg/dl)	−0.150	0.109
HDL-C (mg/dl)	0.188	0.044
LDL-C (mg/dl)	−0.123	0.190
Calcium (mg/dl)	0.239	0.010
Phosphorus (mg/dl)	−0.102	0.448

HbA1c: Glycated hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol.
The correlation was analyzed using Pearson's correlation coefficient (normally distributed data).
P < 0.05: Significant, P > 0.05: not significant.

included; 1) vitamin D appears to enhance directly insulin synthesis via the nuclear vitamin D receptor (VDR) in β-pancreatic cells [41], 2) vitamin D may also promote morphological improvement in pancreatic islet cells, decrease apoptosis, and have nongenomic effects mediated by messenger VDR [42], and 3) vitamin D acts to reduce inflammation, which is a major process in inducing insulin resistance [14].

Serum glucose, HbA1c, serum ALT, AST and triglycerides were significantly higher in patients than in controls whereas serum insulin, HDL-C and calcium were significantly lower in patients. Such findings are in agreement with that reported by several authors [43–45]. HbA1c has been used as an objective marker of average glycemic control for many years, has an accepted place in the monitoring of patients with diabetes, and is relied on for significant management decisions, such as initiation of insulin therapy [46]. Hypoinsulinemia observed in the present study minimizes the utilization of glucose by the cell leading to hyperglycemia. Under such circumstances, insulin resistance which is a well-established feature in type 2 diabetes may exacerbate the condition [47]. Lipid metabolism will also be disturbed in the form of elevated triglyceride levels and low HDL-C levels [48,49]. Elevation of ALT and AST in patients could be explained by hepatotoxicity of excess fatty acids and by oxidative stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells [4]. Association between liver enzymes and incident type 2 diabetes have been recently admitted [50,51]. The observed hypocalcaemia with the resultant hypoinsulinemia may be a possible mechanism to explain the association between calcium insufficiency and the risk of diabetes [52].

In this study, serum vitamin D showed significant negative correlations with HbA1c, ALT and AST whereas significant positive correlations were found with HDL-C and calcium. Similar findings were reported in the literature [53,54]. The negative correlation between vitamin D and HbA1c throughout the course of the disease indicates the involvement of vitamin D deficiency in pathophysiology of type 2 diabetes. This may have therapeutic implications as vitamin D supplementation may improve glycemic control in type 2 diabetes. In this regard, further research is recommended to assess serum vitamin D status and its relation to HbA1c in different stages of diabetic nephropathy. The significant positive correlation of vitamin D with calcium implies that vitamin D deficiency is associated with hypocalcaemia in type 2 diabetic patients. As insulin secretion is a calcium-dependent process [55], hypocalcaemia will decrease its secretion from β-pancreatic cells. The significant

decrease in insulin level observed in diabetic patients does support this view. Therefore, one can say that cautious combined supplementation with both vitamin D and calcium would be beneficial in optimizing glucose metabolism in type 2 diabetes.

5. Conclusions

Serum vitamin D was significantly lower in diabetic patients compared to non-diabetic controls. Serum glucose, HbA1c, serum ALT, AST and triglycerides were significantly higher in patients than in controls whereas serum insulin, HDL-C and calcium were significantly lower in patients. Serum vitamin D showed significant negative correlations with HbA1c, ALT and AST whereas significant positive correlations were found with HDL-C and calcium.

Conflicts of interest

The authors declare no conflicts of interest.

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References

[1] Karalliedde J, Gnudi L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrol Dial Transplant 2016;31:206–13.
[2] Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of diabetes 2017. J Diabetes Res 2018:3086167.
[3] Tomkin GH, Owens D. Diabetes and dyslipidemia: characterizing lipoprotein metabolism. Diabetes Metab Syndr Obes 2017;10:333–43.
[4] Atiba AS, Oparinde DP, Babatunde OA, Niran-Atiba TA, Jimoh AK, Adepeju AA. Liver enzymes and lipid profile among type 2 diabetic patients in Osogbo, Nigeria. GJMS 2013;3(5):174–8.
[5] Bamanikar SA, Bamanikar AA, Arora A. Study of serum urea and creatinine in diabetic and non-diabetic patients in a tertiary teaching hospital. JMR 2016;2(1):12–5.
[6] Holick MF. Vitamin D: evolutionary, physiological and health perspectives. Curr Drug Targets 2011;12(1):4–18.
[7] Basit S. Vitamin D in health and disease: a literature review. Br J Biomed Sci 2013;70(4):161–72.
[8] Zmijewski MA. Vitamin D and human health. Int J Mol Sci 2019;20:145.
[9] Gorham ED, Garland CF, Burgi AA, Mohr SB, Zeng K, Hofflich H, et al. Lower prediagnostic serum 25-hydroxyvitamin D concentrationis associated with higher risk of insulin-requiring diabetes: a nested case-control study. Diabetologia 2012;55(12):3224–7.
[10] Mahmood Y, Shahid SM, Fawwad A, Basit A, Azhar A. Association of vitamin D with type 2 diabetes mellitus in Karachi, Pakistan. Int J Biol Biotechnol 2018;15(2):201–5.
[11] Manju M, Mishra S, Toora BD, Vithiyavathy S. Relationship between serum Vitamin D and insulin resistance in pre-diabetic and diabetic states- A comparative study. Int J Clin Biochem Res 2018;5(1):91–6.
[12] Dhas Y, Banerjee J, Damle G, Mishra N. Association of vitamin D deficiency with insulin resistance in middle-aged type 2 diabetics. Clin Chim Acta 2019;492:95–101.
[13] Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Rheum Dis Clin N Am 2012;38(1):179–206.
[14] Berridge MJ. Vitamin D deficiency and diabetes. Biochem J 2017;474(8):1321–32.
[15] Yassin MM, Lubbad AH. Risk factors associated with nutritional rickets among children aged 2 to 36 months old in the Gaza Strip: a case control study. IJFNPH 2010;3(1):33–45.
[16] World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia Report of a WHO/IDF Consultation. Geneva: WHO; 2006.
[17] Palestinian Ministry of Health. Diabetic questionnaire, Diabetic clinic records. Palestine: Gaza Strip; 2006.
[18] Backestrom C, Hursh-Cesar G. Survey research. Pennsylvania, United States: Literary Licensing, LLC; 2012.
[19] Bikle DD. Vitamin D and the skin. J Bone Miner Metab 2010;28(2):117–30.
[20] Trinder P. Determination of glucose in blood using glucose oxidase. Ann Clin Biochem 1969;6:24–33.
[21] Trivelli LA, Ranney HM, Lai HT. Hemoglobin components in patients with diabetes mellitus. N Engl J Med 1971;284(7):353–7.
[22] Travis JC. Clinical radioimmunoassay - state of the art. Scientific Newsletters, Inc. Radioassay-Ligand Assay. Anaheim: Publishers; 1980. p. 89–92.
[23] Thomas L. Alanine aminotransferase (ALT), aspartate aminotransferase (AST). Clinical laboratory diagnostics. first ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55–65.
[24] Bergmeyer HU. Methods of enzymatic analysis. second ed. Weinheim: Verlag Chemie: New York: Academic Press; 1974.
[25] Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. Clin Chem 1971;17:696–700.
[26] Meiatlini F, Prencipe L, Bardelli F, Giannini G, Tarli P. The 4-hydroxybenzoate/4aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. Clin Chem 1978;24(12):2161–5.
[27] Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973;19:476–82.
[28] Grove TH. Effect of reagent pH on determination of HDL cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin Chem 1979;25:560–4.
[29] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of LDL-C in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[30] Barnett RN, Skodon SB, Goldberg MN. Performance of "Kits" used for clinical chemical analysis of calcium in serum. Am J Clin Pathol 1973;59:836–45.
[31] Tietz NW. Specimen collection and processing; sources of biological variation. Textbook of clinical chemistry. second ed. Philadelphia, USA: WB Saunders; 1994.
[32] Palestinian Ministry of Health. Health annual report, Palestine 2016. Palestinian Health Information Center, Diabetes mellitus; July 2017. p. 99–102.
[33] Nakashima A, Yokoyama K, Yokoo T, Urashima M. Role of vitamin D in diabetes mellitus and chronic kidney disease. World J Diabetes 2016;7(5):89–100.
[34] Gao Y, Zheng T, Ran X, Ren Y, Chen T, Zhong L, et al. Vitamin D and incidence of prediabetes or type 2 diabetes: a four-year follow-up community-based study. Dis Markers 2018;2018:1926308.
[35] Vaz NC, Ferreira AM, Kulkarni MS, Vaz FS. Prevalence of diabetes mellitus in a rural population of Goa, India. Natl Med J India 2011;24(1):16–8.
[36] Shaikh A, Hari Kumar KVS. Diabetes and poverty: a primer for resource poor countries. J Soc Health Diabetes 2018;6(1):11–4.
[37] Hsu CC, Lee CH, Wahlqvist ML, Huang HL, Chang HY, Chen L, et al. Poverty increases type 2 diabetes incidence and inequality of care despite universal health coverage. Diabetes Care 2012;35(11):2286–92.
[38] Geetha A, Gopalakrishnan S, Umadevi R. Study on the impact of family history of diabetes among type 2 diabetes mellitus patients in an urban area of Kancheepuram district, Tamil Nadu. Int J Community Med Public Health 2017;4(11):4151–6.
[39] Li YW, Aronow WS. Diabetes mellitus and cardiovascular disease. J Clinic Experiment Cardiol 2011;2:114.
[40] Yu JR, Lee SA, Lee JG, Seong GM, Ko SJ, Koh G, et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. Chonnam Med J 2012;48(2):108–15.
[41] Razip N, Khaza'ai H. Review on potential vitamin D mechanism with type 2 diabetes mellitus pathophysiology in Malaysia. Curr Res Nutr Food Sci 2018;6(1):1–11.
[42] Wolden-Kirk H, Overbergh L, Christesen HT, Brusgaard K, Mathieu C. Vitamin D and diabetes: its importance for beta cell and immune function. Mol Cell Endocrinol 2011;347(1–2):106–20.
[43] Yassin MM, Altibi HI, El Shanti AF. Clinical and biochemical features of type 2 diabetic patients in Gaza Governorate, Gaza Strip. W Afr J Med 2011;30(1):51–6.
[44] Yassin MM, Lubbad AH, Saadallah NM, AbuTaha AJ. Biochemical and hematological parameters in relation to Helicobacter pylori infection among type 2 diabetic patients in Gaza Strip. JAMR 2014;4(1):10–20.
[45] Hassan SA, Elsheikh WA, Abdel Rahman NI, ElBagir NM. Serum calcium levels in correlation with glycated hemoglobin in type 2 diabetic Sudanese patients. Adv Diabetes Metab 2016;4(4):59–64.
[46] d'Emden MC, Shaw JE, Colman PG, Colagiuri S, Twigg SM, Jones GR, et al. The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Med J Aust 2012;197(4):220–1.
[47] American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes – 2019. Diabetes Care 2019;42(Suppl 1):S13–28.
[48] Yassin MM, AbuMustafa AM, Abujami SM, Jaber EA. Leptin status and biochemical parameters in type 2 diabetic males from Gaza strip. AMBSJ 2017;3(1):4–10.
[49] Rajendran B, Mallampati S, Jha JS, Hakkim S. Lipid profile in type 2 diabetes mellitus: a case-controlled study. Int J Adv Med 2019;6(2):1–6.
[50] Wang Y-L, Koh W-P, Yuan J-M, Pan A. Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women. BMJ Open Diabetes Res Care 2016;4:e000296.
[51] Abro MUR, Butt A, Baqa K, Waris N, Khalid M, Fawwad A. Association of serum liver enzyme Alanine Aminotransferase (ALT) in patients with type 2 diabetes. Pak J Med Sci 2018;34(4):839–43.
[52] Kanchana N, Saikumar P. Serum calcium levels in type 2 diabetes mellitus. IOSR-JDMS 2014;13(8):1–3.
[53] Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P. Vitamin D and glycemic control in diabetes mellitus type 2. Ther Adv

Endocrinol Metab 2013;4(4):122–8.
[54] Shenoy V, Datta P, Prabhu K, Singh K. Association between vitamin D, fasting blood glucose, HbA1c and fasting lipid profile in euglycemic individuals. J Res Diabetes 2014;2014:929743.
[55] Muñoz-Garach A, García-Fontana B, Muñoz-Torres M. Vitamin D status, calcium intake and risk of developing type 2 diabetes: an unresolved issue. Nutrients 2019;11:642.