Serum Vitamin D, Renal Biomarkers, Protein Profile and Some Electrolytes in Chronic Kidney Disease Patients With a Clinical Trial of Vitamin D Therapy

Maged M. Yassin1*, Said S. Al-Ghora1, Mohamed M. Laqqa2, Saleh N. Mwafy3, Soha A. Abdallah2

1 Faculty of Medicine, Islamic University of Gaza, Gaza, Palestine
2 Faculty of Health Sciences, Department of Laboratory Medical Sciences, Islamic University of Gaza, Gaza, Palestine
3 Faculty of Science, Al Azhar University of Gaza, Gaza Strip, Palestine

*Corresponding Author: Maged M. Yassin, Ph.D., Professor, Faculty of Medicine, Islamic University of Gaza, P.O. Box 108, Gaza, Palestine. Tel: +970-82856230, Email: myassin@iugaza.edu.ps

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Abstract
Background: Vitamin D deficiency is involved in a broad spectrum of diseases including chronic kidney disease (CKD).
Objectives: This study was designed to assess serum vitamin D, renal biomarkers, protein profile, and electrolytes in CKD patients with a clinical trial of vitamin D therapy.
Methods: This case-control follow-up interventional study comprised 42 CKD patients and 42 apparently healthy controls. Patients and controls were matched for age and gender. Patients were assigned to receive, a weekly oral dose of vitamin D3 (50,000 IU) for 3 successive months. The follow-up therapy was conducted under direct and full physician supervision.
Results: Vitamin D was significantly lower in CKD patients compared to controls (29.6 ± 12.4 versus 35.2 ± 9.9 ng/dL, \(P=0.033\)). Significant increases were shown in the urea, creatinine, and calcium were significantly lower in patients. A significant improvement was noted for vitamin D and calcium where they registered mean values of 43.8 ± 9.1 ng/dL and 9.65 ± 0.70 mg/dL at the end of the therapeutic period compared to 29.6 ± 12.4 ng/dL and 8.61 ± 0.77 mg/dL in patients before vitamin D therapy (\(P=0.028\) and \(P=0.033\), respectively).
Conclusion: General amelioration of the metabolic profile of CKD patients in response to vitamin D therapy has been shown. Besides a significant improvement in vitamin D and calcium. Consequently, vitamin D is a useful candidate in clinical settings for the improvement of renal function and controlling of CKD, and more importantly its complications.
Keywords: Vitamin D, CKD, Renal Biomarkers, Metabolic Profile, Therapeutic Trial

1. Background
The kidneys are vital organs that specialized for filtration or purification of blood from natural waste products, chiefly urea and creatinine, as well as foreign substances such as drugs. Chronic kidney disease (CKD) is recently defined as decreased kidney function shown by glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m², or markers of kidney damage, or both, of at least 3 months duration, regardless of the underlying cause.\(^1\) The severity of CKD is categorized into five stages according to GFR level, with stage 1 (GFR 90 mL/min/1.73 m²) being the mildest and stage 5 (GFR <15 mL/min/1.73 m² or dialysis) being a severe illness.\(^2\) Kidney damage can also be ascertained by the presence of proteinuria, disturbances of serum urea and creatinine, kidney biopsy, and abnormalities on imaging.\(^3,4\) Despite that these CKD markers are being in use, diagnosis is commonly made after chance findings from screening tests (urinary dipstick or blood tests), or when symptoms become severe.
Most epidemiological information on CKD originates from data available on end-stage renal disease (ESRD), as many patients are often asymptomatic or have non-specific symptoms in the earlier stages of CKD.\(^6\) However, the global prevalence of CKD is estimated at 13.4% and a mortality rate at 1.2 million per year.\(^2\) Diabetes and hypertension are the more common causes of CKD in adults.\(^7,8\) Other risk factors include heart disease, obesity, a family history of CKD, inherited kidney disorders, past damage to the kidneys, and older age.\(^8,10\) Complications of CKD include anemia and mineral bone disorders caused by disturbed vitamin D, calcium, and phosphate metabolism.\(^11\) As the kidney function worsens towards the ESRD (CKD stages 4-5), the risk of mortality rises exponentially and is largely attributable to cardiovascular disease (CVD) complications.\(^12,13\)

Vitamin D is a seco-steroid with an endocrine mechanism of action. In humans, the major natural source of vitamin D is the dermal synthesis following exposure to ultraviolet light.\(^14\) Few foods contain also vitamin D.\(^15\) Vitamin D obtained from sun exposure and food undergo two steps of activation-hydroxylation in the body. The first hydroxylation occurs in the liver,
the multiple choice questions, which offer several fixed alternatives. The questionnaire was validated and piloted. The interviewer asked questions about age, education, employment, family income/month and family history of CKD. Diagnosed CKD and its duration were obtained from the patients’ records. The body mass index (BMI) was calculated as kilogram (kg) body weight / height in meter squared. The body weight and height were measured using a carefully calibrated balance (Detecto, CAP-180 kg, USA) and vertical measuring rod, respectively.

### 3.3. Blood Sampling and Analysis

Fasting venous blood samples of around 8 mL each were drawn from all the study population into plastic tubes and were left for a while without anticoagulant to allow blood to clot. Then, serum samples were separated by centrifugation at 4000 rpm/10 min using a Rotina 46 Hettich Centrifuge, Japan. Serum vitamin D was determined by enzyme-linked immunosorbent assay. Urea and creatinine were determined by the urease glutamate dehydrogenase/UV method and by the alkaline picate method, respectively, using the BioSystems kit, Spain. Uric acid was determined by enzymatic photometric test with TRHBA (2, 4, 6-tribromo-3-hydroxybenzoic acid) using DiaSys reagent kits. eGFR was calculated by Schwartz equation: eGFR (mL/min/1.73 m²) = 0.55 x length/serum creatinine. Total serum protein and albumin were measured by means of the Biuret reaction and with bromocresol green, respectively, using DiaSys reagent kits. Serum globulin was calculated according the following formula: Globulin = Total protein – Albumin. Serum calcium was assayed following instructions of Randox reagent kit manual. Serum phosphorus was determined by phosphomolybdate UV end point, using Ammonium Molybdate Diagnostic kit.

### 3.4. Follow up Therapy Trail of the Patients

The follow up therapeutic part of the study was conducted under direct and full physician supervision at Al-Shifa hospital and Nasser Medical Complex in Gaza Strip. Patients were assigned to receive, a weekly oral dose of vitamin D3 (50000 IU) for 3 successive months. Throughout this period, patients were inquired about their general health and/or any problem that they may had. At the end of the third month, relevant metabolic profile of patients was assessed and compared with their metabolic profile before vitamin D therapy.

### 3.5. Statistical Analysis

Data were analyzed using Statistical Package for Social Science Inc., Chicago, IL (SPSS) computer program version 24 for windows. A Simple distribution of the study variables and cross tabulation were applied. Chi-square ($\chi^2$) was used to identify the difference between variables. Yates’s continuity correction test, $\chi^2$ (formula/study) 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. Different means of vitamin D level at various CKD duration intervals were compared using the one-way analysis of variance (ANOVA). Pearson’s correlation test was applied. The results were accepted as statistically significant at $P<0.05$.

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. Percent difference = $|(V1 - V2) / ((V1 + V2)/2)) X 100$.

4. Results

4.1. Clinical and Socio-demographic Aspects

Table 1 showed no significant differences between CKD patients and controls for age, BMI and education ($P>0.05$). However, employment and family income/month were significantly lower among patients with respect to controls ($\chi^2=4.941, P=0.026$ and $\chi^2=7.939, P=0.019$, respectively) whereas family history of CKD was significantly higher among patients ($\chi^2=4.200, P=0.040$).

4.2. Serum Vitamin D Levels of the Study Population

As illustrated in Table 2, the mean level of vitamin D was significantly lower in CKD patients than controls (29.6 ± 12.4 versus 35.2 ± 9.9 ng/dL, $t=2.171, P=0.033$).

4.3. Duration intervals of CKD by Serum Vitamin D Levels

Table 3 revealed a progressive decrease in the level of serum vitamin D with increase duration of CKD, registering mean values of 36.0 ± 11.8, 33.7 ± 10.3 and 25.4 ± 12.2 ng/dL at time intervals of 1-10, 11-20 and 21-34 years, respectively. However, the mean difference of vitamin D level at various CKD duration intervals was not significant ($F=2.048, P=0.143$).

4.4. Renal Biomarkers, Protein Profile and Electrolytes of CKD Patients Versus Controls

As depicted from Table 4, renal biomarkers including serum urea, creatinine and uric acid were significantly higher in CKD patients (84.6 ± 47.4, 1.90 ± 1.20 and 7.92 ± 2.29 mg/dL) than controls (35.7 ± 13.5, 0.81 ± 0.27 and 5.18 ± 2.31 mg/dL, respectively) with $P<0.001$ for all whereas GFR was significantly lower in patients (62.4 ± 32.5 versus 124.6 ± 45.4 (mL/min/1.73 m²) = 0.55 × length/Scr, $P<0.001$). There were significant decreases in serum total protein, albumin and calcium in patients compared to controls (7.0 ± 0.50, 5.2 ± 0.40 and 8.61 ± 0.77 mg/dL versus 7.3 ± 0.60, 5.4 ± 0.59 and 9.12 ± 0.69 mg/dL, $P=0.005$, $P=0.023$ and $P=0.003$, respectively).

4.5. Serum vitamin D in Relation to the Studied Parameters

Table 4. Serum Vitamin D in Relation to the Studied Parameters

Pearson correlation test presented in Table 5 displayed significant negative correlations of serum vitamin D with
Table 4. Renal Biomarkers, Protein Profile and Electrolytes of Chronic Kidney Disease Patients Versus Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 42)</th>
<th>Patients (n = 42)</th>
<th>% Difference</th>
<th>T Test</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>35.7 ± 13.5</td>
<td>84.6 ± 47.4</td>
<td>81.3</td>
<td>6.428</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.81 ± 0.27</td>
<td>1.90 ± 1.20</td>
<td>- 40.4</td>
<td>5.875</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR</td>
<td>124.6 ± 45.4</td>
<td>62.4 ± 32.5</td>
<td>- 54.2</td>
<td>7.114</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.18 ± 3.31</td>
<td>7.92 ± 2.29</td>
<td>29.5</td>
<td>5.428</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>7.3 ± 0.60</td>
<td>7.0 ± 0.50</td>
<td>3.6</td>
<td>2.928</td>
<td>0.005</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>5.4 ± 0.59</td>
<td>5.2 ± 0.40</td>
<td>- 3.8</td>
<td>2.316</td>
<td>0.023</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>1.92 ± 0.60</td>
<td>1.82 ± 0.60</td>
<td>- 5.3</td>
<td>0.728</td>
<td>0.469</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.12 ± 0.69</td>
<td>8.61 ± 0.77</td>
<td>- 5.8</td>
<td>3.221</td>
<td>0.003</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.49 ± 0.85</td>
<td>4.72 ± 0.94</td>
<td>9.0</td>
<td>1.186</td>
<td>0.023</td>
</tr>
</tbody>
</table>

GFR: Glomerular filtration rate was calculated from Schwartz equation (National Kidney Foundation, 2002); GFR (mL/min/1.73 m²) = 0.55 × length/Scr.

Values of all parameters are expressed as means ± standard deviation.

P<0.05: Significant, P>0.05: not significant.

Table 5. Serum Vitamin D in Relation to the Studied Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum Vitamin D (ng/dL)</th>
<th>Pearson’s Correlation (r)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>- 0.302</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>- 0.343</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>0.258</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>- 0.249</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>0.283</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>0.278</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>0.159</td>
<td>0.156</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>0.562</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>- 0.168</td>
<td>0.125</td>
<td></td>
</tr>
</tbody>
</table>

GFR: Glomerular filtration rate was calculated from Schwartz equation (National Kidney Foundation, 2002); GFR (mL/min/1.73 m²) = 0.55 × length/Scr.

The correlation was analyzed using Pearson’s correlation coefficient (normally distributed data).

P<0.05: Significant, P>0.05: not significant.

urea (r = -0.302, P = 0.005), creatinine (r = -0.343, P = 0.001) and uric acid (r = -0.249, P = 0.022). Conversely, significant positive correlations of serum vitamin D was found with GFR (r = 0.258, P = 0.020), total protein (r = 0.283, P = 0.011), albumin (r = 0.278, P = 0.012) and calcium (r = 0.562, P = 0.001).

4.6. Clinical Therapeutic Follow-up Trial of Patients

Oral administration of 50 000 IU of vitamin D3 at weekly basis for three successive months provoked general amelioration of the metabolic profile of CKD patients at the end of the third month of the trial. The significant improvement was noted for vitamin D and calcium where they registered mean values of 43.8 ± 9.1 ng/dL and 9.65 ± 0.70 mg/dL in patients at the end of the third month of vitamin D therapy compared to 29.6 ± 12.4 ng/dL and 8.61 ± 0.77 mg/dL in patients before vitamin D therapy (P = 0.028 and P = 0.033, respectively) (Table 6).

5. Discussion

CKD is prevalent in Gaza Strip, and its effect not only progression to ESRD and then renal failure, but also increased risk of CVD, and the overall mortality rate continues to be unacceptably high. Therefore, the dual key point here is the proper management of the gap between the initial stage of CKD and the renal failure as well as the control of the CVD, which both is expected to improve and prolong patient’s life. The laboratory markers currently available to assess kidney function in Gaza Strip hospitals are the routine traditional tests, urea and creatinine, when the patient visited the hospital. This necessitates more understanding of the disease and searching for other markers, besides the traditional ones to strengthen the diagnostic and even the therapeutic strategies of CKD. Vitamin D has been recently linked to CKD in terms of its deficiency as a putative contributor and/or an indicator, and its supplementation as a cure, without testing its relation with other biochemical parameters. This study was carried out to speculate the status of serum vitamin D and its correlations with renal impairment indicators, and as a trial of therapy in CKD patients from Gaza Strip.

Besides the selection of the inclusion and exclusion criteria in this research, both controls and CKD patients were matched for age, gender and BMI to avoid the impact of these confounder variables on the various studied parameters. The frequency of CKD was significantly higher among unemployed patients and low-income families. Association of such socio-demographic factors with the prevalence of CKD was previously reported among different populations. Family history is another socio-demographic factor found in this study to be associated with CKD. In a small or a large population-based family studies, a positive family history was strongly associated with increased risk of CKD.

CKD patients showed a significant decline in serum vitamin D compared to controls. As the kidney plays a central role in vitamin D metabolism and regulation, it is expected that impaired renal function in CKD patients will lead to vitamin D deficiency. Defects in renal uptake of vitamin D substrate 25-hydroxyvitamin D to the renal enzyme 1α-hydroxylase, downregulation and/or unavailability of the enzyme were proposed to be important contributors to vitamin D deficiency in CKD.
The different categories of vitamin D level displayed that the number of patients having vitamin D deficient and insufficient was significantly higher than controls. This coincides with the findings that hypovitaminosis D is prevalent in CKD patients and/or suggested to be a sensible predictor of renal worsening function.47,48

In this study, more than two thirds of CKD patients had CKD for 10 years or less. This confirm the idea that CKD has long asymptomatic preclinical phase which frequently goes undetected, particularly in developing countries where CKD is prevalent and most of people did not had routine medical examination.49 Additionally, an inverse relationship was found between duration intervals of CKD and vitamin D level. This means that vitamin D level is decreasing with progression of CKD and supports the finding that vitamin D is an inverse predictor of CKD progression.50 Therefore, supplementation of vitamin D may slow worsening of kidney function. This point was tested in this study as a small clinical trial of vitamin D therapy. However, further investigation is needed on vitamin D therapy at a larger scale.

The metabolic profile of patients showed significant increases in the mean levels of urea, creatinine and uric acid whereas GFR, total protein, albumin and calcium levels were significantly decreased with respect to controls. Similar trends were documented elsewhere in the literature.51,52 Gathering of urea, creatinine and uric acid in the blood is a predictable consequence of progressive decline of GFR as CKD proceeded. Loss of serum albumin in urine (albuminuria) is associated with CKD.53 Additionally, serum proteins may breakdown to form urea that further contributing to elevation of serum urea concentration observed in CKD patients. Hypocalcemia registered in CKD patients may be explained by phosphate retention as a result of decreasing GFR which increases fibroblast growth factor-23 secretion from bone that inhibits 1α-hydroxylase, therefore, the synthesis of vitamin D will decrease. Decreased levels of vitamin D results in reduced calcium absorption in the intestine, increased calcium excretion, and decreased calcium resorption from bone, and thus, blood calcium levels will decrease.54 Hypovitaminosis D and hyperphosphatemia registered in the present study do support this pathophysiologic scenario.

When related to serum vitamin D, urea, creatinine and uric acid showed significant negative correlations whereas GFR, total protein, albumin and calcium displayed significant positive correlations with vitamin D. Such findings are in agreement with that reported by other authors,51,56 and implies that vitamin D deficiency is linked to impairment of renal function. Recently, the synergistic interactions of vitamin D deficiency with these parameters were even reported to be associated with the severity of CKD staging.57 Therefore, the progressive decline of vitamin D accompanied with the successive fall of GFR towards the ESRD, and the subsequent disturbances of the metabolic parameters in the blood will be more likely to increase the mortality rate in CKD patients. In this case, vitamin D therapeutic strategy will be designed to suite the various stages of CKD. However, such strategy was beyond the scope of this study.

Oral administration of 50 000 IU of vitamin D3 at weekly basis for three successive months ameliorate the metabolic profile of patients at the end of the third month of the trial. Serum vitamin D levels were even exceeded the control values. Nephropathies studies reported that active vitamin D protects the kidneys through its anti-inflammatory action and inhibitory effects on progression to renal interstitial fibrosis and/or tubular atrophy as well as its role in reduction of proteinuria through decrease the expression of renin-angiotensin system.58,59 Therefore, one can say that supplementation of vitamin D could be a promising therapy to decelerate the transitional changes of CKD towards ESRD and finally to renal failure. This will no doubt improve survival in patients with CKD. The therapeutic role of vitamin D in CKD was pointed out by several authors.60,61 However, the mount of vitamin D dose, frequency and duration of dosing are important determinants in the therapeutic protocol of CKD. Although this study knocked a small scale clinical therapeutic trial, the results should

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**Table 6. Metabolic Profile of Controls, Patients and Vitamin D-Treated Patients (50 000 IU/wk) for 3 Successive Months**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 42)</th>
<th>Patients (n = 42)</th>
<th>Follow-up Vitamin D Therapy</th>
<th>% Difference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (mg/dL)</td>
<td>35.2 ± 9.9</td>
<td>29.6 ± 12.4</td>
<td>43.8 ± 9.1</td>
<td>38.7</td>
<td>0.028</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>35.7 ± 13.5</td>
<td>8.6 ± 47.4</td>
<td>65.4 ± 20.2</td>
<td>- 25.6</td>
<td>0.362</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.81 ± 0.27</td>
<td>1.90 ± 1.20</td>
<td>1.53 ± 0.38</td>
<td>- 21.5</td>
<td>0.395</td>
</tr>
<tr>
<td>GFR</td>
<td>124.6 ± 45.4</td>
<td>62.4 ± 32.5</td>
<td>81.7 ± 27.2</td>
<td>26.8</td>
<td>0.291</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.18 ± 2.31</td>
<td>7.92 ± 2.29</td>
<td>6.86 ± 1.75</td>
<td>- 14.3</td>
<td>0.387</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>7.3 ± 0.60</td>
<td>7.0 ± 0.50</td>
<td>7.2 ± 0.30</td>
<td>2.8</td>
<td>0.292</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>5.4 ± 0.59</td>
<td>5.2 ± 0.40</td>
<td>5.3 ± 0.21</td>
<td>1.9</td>
<td>0.403</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>1.92 ± 0.60</td>
<td>1.82 ± 0.60</td>
<td>1.91 ± 0.20</td>
<td>4.3</td>
<td>0.471</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.12 ± 0.69</td>
<td>8.61 ± 0.77</td>
<td>9.65 ± 0.70</td>
<td>11.4</td>
<td>0.033</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.49 ± 0.85</td>
<td>4.72 ± 0.94</td>
<td>4.36 ± 0.38</td>
<td>- 7.9</td>
<td>0.392</td>
</tr>
</tbody>
</table>

GFR: Glomerular filtration rate was calculated from Schwartz equation (National Kidney Foundation, 2002); GFR (mL/min/1.73 m²) = 0.55 × length/Scr.

Values of all parameters are expressed as means ± standard deviation.

P < 0.05: Significant, P > 0.05: not significant.
stimulate future studies looking for the impact of different doses of vitamin D on various stages of CKD.

6. Conclusion
Serum vitamin D was significantly lower in CKD patients compared to healthy controls. Serum urea, creatinine and uric acid were significantly higher in patients compared to controls whereas GFR, serum total protein, albumin and calcium were significantly lower in patients. Vitamin D exhibited significant negative correlations with urea, creatinine and uric acid whereas significant positive correlations were found with GFR, total protein, albumin and calcium. Oral administration of 50,000 IU of vitamin D3 at weekly basis for three successive months provoked general amelioration of the metabolic profile of CKD patients at the end of the three month of the therapeutic trial.

Author Contributions
MMY designed the study, wrote the protocol, helped in the statistical analysis and wrote the first draft of the manuscript. SSA helped in data analysis and revised the final draft of the manuscript. MML involved in writing and reading the manuscript. SNM managed the literature searches. SAA performed the experimental work.

Conflict of Interest Disclosures
The authors declare no conflicts of interest.

Ethical Approval
This work was carried out in accordance with the written approval issued by Helsinki Committee in the Palestinian Ministry of Health under the ethical number PHRC/HC/37/20. Patients and controls received a complete explanation about the intended research and then they signed a written consent to participate in the study.

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References