Homocysteine, Renal Biomarkers, Glucose, Glycated Hemoglobin and Lipid Profile in Diabetic Nephropathy Patients from Gaza Strip

Yassin MM1, Alghora SS1, Alnajjar MK2, Yasin MM3

1 Faculty of Medicine, The Islamic University of Gaza, Gaza Strip, Palestine; 2 Faculty of Health Sciences, The Islamic University of Gaza, Gaza Strip, Palestine; 3 Faculty of Medicine, October 6 University, Cairo, Egypt.

ABSTRACT

Introduction: Homocysteine is recently speculated as an indicator for the development of diabetic nephropathy.

Objective: To assess homocysteine in various stages of diabetic nephropathy and its correlation with other biochemical parameters.

Methods: A mini questionnaire, and analysis of blood and urine were employed in 120 patients with type 2 diabetes (40 normo-, 40 micro- and 40 macroalbuminuric) and 40 non-diabetic controls.

Results: Urinary albumin was significantly elevated in patients with normoalbuminuria (22.3±5.2 mg/g), microalbuminuria (146.7±80.7 mg/g) and macroalbuminuria (348.0±37.6 mg/g) than controls (15.9±4.8 mg/g), in microalbuminuria and macroalbuminuria versus normoalbuminuria, and in macroalbuminuria versus microalbuminuria. There were also significant increases in serum urea and creatinine in diabetic groups. Serum homocysteine behaves like urinary albumin in being significantly higher in diabetic groups (17.1±4.8, 20.3±5.9 and 22.9±5.5 µmol/l, respectively) than controls (13.4±3.7 µmol/l), in microalbuminuria and macroalbuminuria versus normoalbuminuria, and in macroalbuminuria versus microalbuminuria. Serum glucose, HbA1c, cholesterol, triglycerides and LDL-C were significantly increased whereas HDL-C was significantly decreased in diabetic groups. There were significant positive correlations of serum homocysteine with urinary albumin (r=0.564, P<0.001), urea (r=0.654, P<0.001), creatinine (r=0.561, P<0.001), glucose (r=0.465, P<0.001), HbA1c (r=0.517, P<0.001), and triglycerides (r=0.320, P<0.001) whereas HDL-C showed significant negative correlation (r=-0.517, P<0.001).

Conclusion: Increment of serum homocysteine accompanied with elevation of urinary albumin, urea, creatinine and HbA1c, could nominate homocysteine as a novel biomarker for trans change to diabetic nephropathy.

Key Words: Serum homocysteine, Renal parameters, Glycated hemoglobin, Lipids, Diabetic nephropathy, Gaza Strip
10 to 20 years, making it one of the main causes of end-stage renal disease. Therefore, early detection and proper treatment of diabetic nephropathy are particularly important for patients' prognosis.

Homocysteine is a sulphur containing intermediary amino acid which is derived by the demethylation of the essential amino acid methionine. The main metabolic organs of homocysteine are liver and kidney. The normal range of serum homocysteine level is between 4.4 and 10.8 µmol/l. A condition of increasing levels of serum homocysteine beyond 15µmol/L is named as hyperhomocysteinemia. Elevation of serum homocysteine may be due to genetic insufficiencies of the enzymes needed for its metabolism, to nutritional deficits in vitamins B12, B6 and folic acid, or to medical conditions such as CVD. Several studies have also been indicated that homocysteine is closely related to the development of diabetic nephropathy. Hyperhomocysteinemia is reported to be a risk factor for diabetic nephropathy; which can directly produce cytotoxicity, lead to oxidative stress and synergistic glycation end products, and thereafter damage vascular endothelium and induce microvascular injury.

Although type 2 diabetes and its complications account for approximately 5.7% of all Palestine mortalities, there is a lack of diagnosis and/or under-reporting of the disease and its progression towards the end-stage renal disease. This necessitates searching for other diagnostic markers for diabetic nephropathy, besides the traditional ones. The published articles on diabetic nephropathy among Palestinians are few and recent. There is no previous study investigates homocysteine status among diabetic nephropathy patients in Gaza Strip. To our best knowledge, this is the leading investigation of homocysteine level and its correlations with other biochemical parameters in diabetic nephropathy among type 2 diabetic patients in Gaza Strip. Understanding of homocysteine status and its variation, as well as its relation during trans changes to diabetic nephropathy, could offer diagnostic and management values on the progression of this life-threatening disease.

**MATERIALS AND METHODS**

**Type of study design and target population**

This is a case-control investigation. A total of 120 patients with type 2 diabetes were chosen from the main and representative Diabetic Care Unit in Gaza Strip (Al Rimal Medical Center); previously diagnosed according to the World Health Organization diagnostic criteria for diabetes. The diabetic patients were three groups: Group I comprised 40 patients (20 males and 20 females) with normoalbuminuria (Urinary albumin<30 mg/g), Group II included 40 patients (20 males and 20 females) with microalbuminuria (Urinary albumin=30-300 mg/g) and Group III comprised 40 patients (20 males and 20 females) with macroalbuminuria (Urinary albumin>300 mg/g). The exclusion criteria were urinary tract infection, hypertensive patients (Blood pressure ≥140/90 mmHg), pregnant women, and women under hormonal therapy. Forty non-diabetic healthy individuals (20 males and 20 females) were chosen randomly from general population in Gaza Strip, and constituted the control group. Patients and controls were age matched (40 to 65 years old).

**Ethical consideration**

Helsinki committee provided us with written approval to perform this study under the ethical number PHRC/HC/28/13 in Gaza Strip, and an informed consent form was signed by all patients and controls prior to commence the study.

**Questionnaire interview and patients’ records**

Patients and controls filled in a mini questionnaire designed to suit the purpose of the study. The questions were simple, clear, direct, and depend on diabetes clinic questions adopted by the Palestinian Ministry of Health with some modifications. The majority of the questions were of the yes/no category, which provide a dichotomous choice. The questionnaire was piloted with 12 individuals (3 from each study group) not involved in the population sample. The questions were related to age, education, employment, family history of diabetes and diet. Clinical data including diagnosed diabetic complications and duration of diabetes were obtained from the patients’ records.

**Urine and blood analysis**

Blood and random urine samples were obtained from all participants. A fraction of blood was placed into EDTA vacutainer tube to determine HbA1c. The remainder quantity of blood was placed into a plastic tube, and left for a short period without anticoagulant to allow blood clotting. Blood samples in plastic tubes and urine samples were centrifuged at 4000 rpm/10 min using a Rotina 46 Hettich Centrifuge, Japan. Then, the obtained serum and urine samples were analyzed. Albumin concentration in urine was measured by Immunoturbidimetry-Latex assay using BioSystems kit, Spain. Serum homocysteine was quantitatively analyzed by enzymatic colorimetric method, using Globe diagnostics kit, Italy. The urease glutamate dehydrogenase/UV and the alkaline picrate procedures were employed for serum urea and creatinine measurement, using the BioSystems kit, Spain. The method employed for determination of serum glucose was glucose oxidase/glucose peroxidase (POD), using Labkit Kits, Spain. HbA1C was estimated through determination of glycated hemoglobin in blood as a whole with the use of Stanbio Kit, Texas-USA. The cholesterol oxidase/POD and the glycerol phosphate oxidase/POD assays were followed in the determination of serum cholesterol and triglycerides, using the BioSystems kit, Spain.
The precipitating procedure was applied for measurement of high-density lipoprotein cholesterol, using Labkit kit, Spain, and finally, low-density lipoprotein cholesterol was calculated using the empirical relationship of Friedewald.

**Statistical analysis**

The obtained data were computer analyzed using IBM SPSS statistics version 22. A simple frequency of the study variables was presented. Chi-square ($\chi^2$) was applied to show the difference between variables. Yates’s continuity correction test, $\chi^2$ (corrected), was employed when not more than 20% of the cells had an expected frequency of < 5. The continuous variables were expressed as mean ± standard deviation and compared using the independent one-way analysis of variance (ANOVA) to test the relationship between various diabetic groups and controls. Bonferroni test served to test the difference within different studied groups. To examine the correlations between homocysteine and other studied parameters, the test of Pearson’s correlation was used. The level of significance was considered when the probability values (P)<0.05.

**RESULTS**

**Socio-demographic characteristics of the study population**

There were no significant differences between diabetic groups and control with respect to age and diet (Table 1). On the other hand, education, employment and family history of diabetes revealed significant differences among the groups ($\chi^2=21.756$, P=0.040; $\chi^2=15.726$, P=0.001 and $\chi^2=18.912$, P<0.001, respectively). Within groups, the educational level was significantly lower in group III versus control group (P<0.05). Employment was significantly lower whereas family history of diabetes was significantly more frequent among diabetic groups when compared to control group. Also, family history was significantly higher in group II than group I.

**Diabetic complications and duration of diabetes**

As illustrated in Table 2, diabetic complications namely retinopathy, CVD and neuropathy displayed significant differences among the different groups ($\chi^2=22.828$, P<0.001; $\chi^2=8.067$, P=0.045 and $\chi^2=20.104$, P<0.001, respectively). Within groups, in general, retinopathy, CVD and neuropathy were significantly more frequent in diabetic groups compared to control one (P<0.05). However, no significant differences were found among the diabetic groups (P>0.05). When related to duration of the disease (Table 3), diabetic complications showed a significantly increasing trend with increasing duration of diabetes ($\chi^2=7.957$, P=0.019 for retinopathy; $\chi^2=7.323$, P=0.026 for CVD and $\chi^2=6.432$, P=0.040 for neuropathy).

**Urinary albumin, serum urea and creatinine**

As depicted from Table 4, urinary albumin, serum urea and serum creatinine exhibited significant differences among various groups (F=220.2, P<0.001; F=7.888, P<0.001 and F=4.347, P=0.006, respectively). Within groups and in general, Bonferroni test revealed significant increases in these biomarkers of groups I, II and III compared to control group (P<0.05). Significant increase in urinary albumin was also registered in groups II and III against group I, and in group III against group II. Additionally, serum urea and creatinine exhibited significant increments in group III with respect to group I.

**Serum homocysteine, glucose, HbA1c, and lipid profile**

Serum homocysteine level shows an increasing trend upon disease progression, with significant differences among various groups (F=21.563, P<0.001), as indicated in Table 5. Like urinary albumin, homocysteine was significantly elevated in diabetic groups than control group, and in groups II and III versus group I (P<0.05). Also, homocysteine level was significantly higher in group III than group II. Significant differences among the groups was recorded for glucose and HbA1c (F=21.530, P<0.001 and F=88.666, P<0.001, respectively), with progressive significant increments in diabetic groups than control one, in group III than group I. Also, HbA1c was significantly higher in group II than group I. Lipid profile in the serum represented by cholesterol, triglycerides, HDL-C and LDL-C showed significant differences among the studied groups (F=6.329, P=0.001; F=8.570, P<0.001; F=7.234, P<0.001 and F=4.576, P=0.005, respectively). Cholesterol, triglycerides and LDL-C were significantly increased in diabetic groups in comparison to control one (P<0.05). In contrast, the level of HDL-C was significantly lower in diabetic groups than control, and in groups II and III versus group I (P<0.05).

**Correlations of serum homocysteine with studied parameters**

Table 6 summarized correlations between serum homocysteine and the other studied parameters. Significant positive correlations of serum homocysteine with urinary albumin (r=0.564, P<0.001), urea (r=0.654, P<0.001), creatinine (r=0.561, P<0.001), glucose (r=0.465, P<0.001), HbA1c (r=0.517, P<0.001), and triglycerides (r=0.320, P=0.001) were obtained upon applying Pearson correlation test. Conversely, HDL-C showed significant negative correlation with homocysteine (r=-0.517, P<0.001).
**DISCUSSION**

Diabetic nephropathy is a complicated life-threatening disease usually develops to end stage renal disease followed by renal failure. However, these trans changes are somewhat interdigitated and their onsets are difficult to be determined. Therefore, the proper management of the gap between the initial stage of diabetic nephropathy and the renal failure is expected to improve and prolong patient’s life. This necessitates searching for other biomarkers besides the traditional ones to strengthen the diagnostic process of diabetic nephropathy. Serum homocysteine was recently innominate to be an indicator for transition to diabetic nephropathy, without testing its relation to diabetic parameters. Consequently, the present study assessed serum homocysteine level and examined its relation to metabolic profile in various stages of diabetic nephropathy among type 2 diabetic patients in Gaza Strip. Evaluation of homocysteine level with diabetic parameters and their correlations in diabetic nephropathy could offer a potential diagnostic tool for the progression of the disease for the seek of its management and protection.

In general, the educational level and employment were significantly lower, whereas family history of diabetes was significantly more frequent among diabetic groups in comparison to controls. This implies that low educational level, unemployment and family history are all risk factors of type 2 diabetes and its complication. The higher educated diabetic patients had a good management of the disease. Unemployment raises poverty which consequently participates largely in raising type 2 diabetes incidence and inequality of care in spite of global health coverage. In addition, many authors indicated that family history is a risk factor of diabetes, particularly in type 2. The frequent diabetic complications recorded included retinopathy, CVD and neuropathy, were more prevalent in diabetic patients in comparison to controls. Such complications showed a significantly increasing trend with increasing duration of diabetes, implying that patients with long periods of diabetes are more likely to develop nephropathy. This is will be accelerated in uncontrolled disease. Similar results were previously reported.

It is not surprising that urinary albumin level was markedly and obviously raised in diabetic groups than controls. Even within diabetic groups this traditional biomarker is still increasing with the progression of the disease, reaching its maximal level in macroalbuminuric group. Progressive elevation of urinary albumin is largely attributed to frequent disturbance of renal filtration capacity, and implies that employment of urinary albumin concentration as a marker for different stages of diabetic nephropathy in this study is convenient and satisfactory. Clinical screening and diagnosis of diabetic nephropathy is chiefly based on the presence of albuminuria. The significant increase in serum urea and creatinine in patients with diabetes than controls, and in macroalbuminurics than normoalbuminurics do confirm the occurrence of renal impairment towards the progression of diabetic nephropathy. Higher serum urea and creatinine levels were found in patients with diabetic nephropathy than in patients without.

Serum homocysteine behaves like urinary albumin in being progressively elevated in diabetic patients, reaching its maximal level in macroalbuminuria stage of diabetic nephropathy. In this context, the significant positive correlation of serum homocysteine with urinary albumin found in this study do support this view. Hyperhomocysteinemia was recorded in diabetic nephropathy or even in hemodialysis patients, and was proposed to be associated with renal endothelial and mesangial cells dysfunction in glomerular capillaries, chronic inflammation and oxidative stress. These findings strongly nominate homocysteine to be an outstanding marker for transition into diabetic nephropathy stage, and may be valuable in diagnostic and even in therapeutic strategies. Just recently, one study supports such conclusion. Consequently and from clinical point of view, serum homocysteine level must be kept under control in patients with diabetic nephropathy, and this can be achieved by administration of vitamins B12, B6 and folic acid supplements i.e. medications can delay the progress of diabetic nephropathy. However, such clinical trials are not in the range of this investigation and needs further assessment.

It is expected and of logic that hyperglycemia is overt in all diabetic groups, and even reached its maximal value in macroalbuminuria. Hyperglycemia was reported to be a modifiable risk factor that promotes the development of diabetic kidney disease in type 2 diabetes. In parallel, blood HbA1c was significantly higher in diabetic groups than controls as well as in micro- and macroalbuminuric than normoalbuminuric diabetic patients. Therefore, intensive glycemic control can reduce the risk of the onset of diabetic nephropathy and slows its progression when it has occurred. Serum cholesterol, triglycerides and LDL-C were significantly increased whereas HDL-C was significantly decreased in the three diabetic groups with respect to control group. In addition, HDL-C showed significant lower levels in microalbuminuria and macroalbuminuria than normoalbuminuria. Such findings are in concord with other studies. It is recently accepted that hyperglycemia is, in part, involved in the determination of the initial step in renal injury, whereas high triglycerides and low HDL-C levels, might be the actual triggers of the onset and progression of diabetic kidney disease.

Serum homocysteine showed significant positive correlations with urinary albumin, serum urea, creatinine, glucose, HbA1c, and triglycerides whereas significant negative correlation was found with HDL-C. Such findings are in the line of that obtained by other authors. However, few studies have investigated these associations. Alterations of serum homocysteine...
level throughout transitional changes to diabetic nephropathy and its strong correlations with various metabolic parameters reflect its involvement in the pathophysiology of the disease. The increment of serum homocysteine accompanied with increments of urinary albumin, urea, creatinine and HbA1c, could make homocysteine a novel indicator for transition to diabetic nephropathy. To complete the scenario, further research is recommended to assess serum homocysteine status and its relations to other biochemical parameters in end-stage renal disease or even in hemodialysis patients. In combination with other biomarkers, homocysteine will no doubt open new avenues in diagnosis, management and therapeutic strategies of diabetic kidney diseases.

CONCLUSION

Serum homocysteine and urinary albumin have similar trend in being significantly higher in diabetic patients than controls, in micro- and macroalbuminuria than normoalbuminuria, and in macroalbuminuria than microalbuminuria. In general, serum urea, creatinine, glucose, HbA1c, cholesterol, triglycerides, LDL-C, were significantly increased in diabetic groups compared to non-diabetics, and reaching their maximum change in macroalbuminuria, whereas HDL-C showed significant decrease and reached its maximum decrease in macroalbuminuria. Serum homocysteine showed significant positive correlations with urinary albumin, urea, creatinine, glucose, HbA1c, and triglycerides, whereas with HDL-C it showed significant negative correlation.

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Authors’ contribution

Prof. Dr. Maged Yassin designed the study, wrote the protocol, helped in the statistical analysis and wrote the first draft of the manuscript. Dr. Said Alghora performed data analysis and revised the final draft of the manuscript. Dr. Mahmoud Almajjar performed the experimental work. Dr. Mohammed Yasin managed the literature searches.

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Table 1: Socio-demographic characters of control and diabetic (normoalbuminuria and diabetic nephropathy) groups

| Characteristic          | Control group (n=40) | Group I Normo-albuminuria (n=40) | Group II Micro-albuminuria (n=40) | Group III Macro-albuminuria (n=40) | Test | P-value |
|-------------------------|----------------------|----------------------------------|-----------------------------------|-----------------------------------|------|---------|
| Age (year)              | 56.9 ± 6.2           | 57.7 ± 7.1                       | 57.8 ± 5.4                        | 58.5 ± 6.3                        | F = 0.263 | 0.852   |
| Education               |                      |                                  |                                   |                                   |      |         |
| University              | 8 (20.0)             | 0 (0.0)                          | 4 (10.0)                          | 0 (0.0)                          | χ² = 21.756 | 0.040** |
| Secondary school        | 14 (35.0)            | 12 (30.0)                        | 6 (15.0)                          | 6 (15.0)                         |      |         |
| Preparatory school      | 6 (15.0)             | 10 (25.0)                        | 8 (20.0)                          | 16 (40.0)                        |      |         |
| Primary school          | 7 (17.5)             | 12 (30.0)                        | 12 (30.0)                         | 10 (25.0)                        |      |         |
| Illiterate              | 5 (12.5)             | 6 (15.0)                         | 10 (25.0)                         | 8 (20.0)                         |      |         |
| Employment              |                      |                                  |                                   |                                   |      |         |
| Yes                     | 23 (57.5)            | 11 (27.5)*                       | 10 (25.0)*                        | 8 (20.0)*                         | χ² = 15.726 | 0.001   |
| No                      | 17 (42.5)            | 29 (72.5)                        | 30 (75.0)                         | 32 (80.0)                        |      |         |
| Family history          |                      |                                  |                                   |                                   |      |         |
| Yes                     | 10 (25.0)            | 19 (47.5)*                       | 28 (70.0)**                       | 25 (62.5)*                       | χ² = 18.912 | < 0.001 |
| No                      | 30 (75.0)            | 21 (52.5)                        | 12 (30.0)                         | 15 (37.5)                        |      |         |
| Diet                    |                      |                                  |                                   |                                   |      |         |
| Yes                     | 3 (7.5)              | 10 (25.0)                        | 4 (10.0)                          | 10 (25.0)                        | χ² = 5.480 | 0.140** |
| No                      | 37 (92.5)            | 30 (75.0)                        | 36 (90.0)                         | 30 (75.0)                        |      |         |

Values are presented as n (%), and age as mean ± standard deviation.
Significant (P < 0.05): *compares diabetic groups versus control group, **compares groups II & III versus group I.
* P-value of χ² (corrected), test.
Table 2: Diabetic complications among control and diabetic (normoalbuminuria and diabetic nephropathy) groups

| Complication   | Control group (n=40) | Group I Normoalbuminuria (n=40) | Group II Microalbuminuria (n=40) | Group III Macroalbuminuria (n=40) | Test | P-value |
|---------------|----------------------|---------------------------------|----------------------------------|----------------------------------|------|---------|
| Retinopathy   |                      |                                 |                                  |                                  |      |         |
| Yes           | 6 (15.0)             | 22 (55.0)*                      | 20 (50.0)*                       | 26 (65.0)*                       | χ² = 22.828 | < 0.001 |
| No            | 34 (85.0)            | 18 (45.0)                       | 20 (50.0)                        | 14 (35.0)                        |      |         |
| CVD           |                      |                                 |                                  |                                  |      |         |
| Yes           | 2 (5.0)              | 8 (20.0)                        | 12 (30.0)*                       | 12 (30.0)*                       | χ² = 8.067 | 0.045** |
| No            | 38 (95.0)            | 32 (80.0)                       | 28 (70.0)                        | 28 (70.0)                        |      |         |
| Neuropathy    |                      |                                 |                                  |                                  |      |         |
| Yes           | 4 (10.0)             | 22 (55.0)*                      | 16 (40.0)*                       | 22 (55.0)*                       | χ² = 20.104 | < 0.001* |
| No            | 36 (90.0)            | 18 (45.0)                       | 24 (60.0)                        | 18 (45.0)                        |      |         |

CVD: Cardiovascular disease.
Values are presented as n (%).
Significant (P < 0.05): *compares diabetic groups versus control group.
** P-value of χ² (corrected) test.

Table 3: Diabetic complications in relation to duration of diabetes among various diabetic groups (n = 120)

| Complication   | Diabetes duration (year) | χ² | P-value |
|---------------|--------------------------|----|---------|
|               | < 5 (n=30)               |    |         |
| Retinopathy   |                          |    |         |
| Yes           | 13 (43.3)                |    |         |
| No            | 17 (56.7)                |    |         |
| CVD           |                          |    |         |
| Yes           | 5 (16.7)                 |    |         |
| No            | 25 (83.3)                |    |         |
| Neuropathy    |                          |    |         |
| Yes           | 8 (26.7)                 |    |         |
| No            | 22 (73.3)                |    |         |

CVD: Cardiovascular disease.
Values are presented as n (%).

Table 4: Urinary albumin, serum urea and serum creatinine of control and diabetic (normoalbuminuria and diabetic nephropathy) groups

| Variable                  | Control Group (n=40) | Group I Normoalbuminuria (n=40) | Group II Microalbuminuria (n=40) | Group III Macroalbuminuria (n=40) | F    | P-value |
|---------------------------|----------------------|---------------------------------|----------------------------------|----------------------------------|------|---------|
| Urinary albumin (mg/g)    | 15.9 ± 4.8           | 22.3 ± 5.2*                     | 146.7 ± 80.7*                   | 348.0 ± 37.6*                    | 220.2| < 0.001 |
| Serum urea (mg/dl)        | 26.0 ± 7.5           | 48.7 ± 38.1*                    | 54.6 ± 40.0*                    | 72.1 ± 62.6*                     | 7.888| < 0.001 |
| Serum creatinine (mg/dl)  | 0.72 ± 0.16          | 0.89 ± 0.62                     | 1.23 ± 1.25*                    | 1.68 ± 1.87*                     | 4.347| 0.006   |

Values are presented as means ± standard deviation.
Significant (P < 0.05): *compares diabetic groups versus control group, †compares groups II & III versus group I, and ‡compares group III versus group II.
Table 5: Serum homocysteine, glucose, glycated hemoglobin, and lipid profile of control and diabetic (normal albuminuria and diabetic nephropathy) groups

| Parameter                | Control group (n=40) | Group I Normo-albuminuria (n=40) | Group II Micro-albuminuria (n=40) | Group III Macro-albuminuria (n=40) | F       | P-value |
|--------------------------|----------------------|----------------------------------|----------------------------------|-----------------------------------|---------|---------|
| Homocysteine (µmol/l)    | 13.4 ± 3.7           | 17.1 ± 4.8*                     | 20.3 ± 5.9**                    | 22.9 ± 5.5**                     | 21.563  | < 0.001 |
| Glucose (mg/dl)          | 109.7 ± 14.8         | 225.6 ± 51.1†                   | 251.3 ± 104.3†                  | 288.7 ± 176.2**                  | 21.530  | < 0.001 |
| HbA1c (%)                | 4.7 ± 0.6            | 7.6 ± 1.1†                      | 8.6 ± 1.3*                      | 8.8 ± 1.4*                       | 88.666  | < 0.001 |
| Cholesterol (mg/dl)      | 182.2 ± 31.2         | 290.9 ± 141.8†                  | 227.9 ± 55.4**                  | 253.5 ± 157.2†                   | 6.329   | 0.001   |
| Triglycerides (mg/dl)    | 133.6 ± 69.2         | 248.0 ± 146.8†                  | 256.8 ± 148.3†                  | 269.8 ± 160.2†                   | 8.570   | < 0.001 |
| HDL-C (mg/dl)            | 46.3 ± 7.1           | 42.9 ± 4.8†                     | 38.0 ± 10.7**                   | 37.7 ± 10.0*                     | 7.234   | < 0.001 |
| LDL-C (mg/dl)            | 113.8 ± 27.9         | 196.1 ± 123.3†                  | 137.1 ± 45.5**                  | 159.8 ± 136.9†                   | 4.576   | 0.005   |

HbA1c: Glycated hemoglobin, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol.
Values are expressed as means ± standard deviation.
Significant (P < 0.05): *compares diabetic groups versus control group, †compares groups II & III versus group I, and ¥compares group III versus group II.

Table 6: Correlation of serum homocysteine with the studied parameters

| Serum homocysteine (µmol/l) (n=160) | Pearson correlation (r) | P-value |
|------------------------------------|-------------------------|---------|
| Urinary Albumin (mg/g)             | 0.564                   | < 0.001 |
| Urea (mg/dl)                       | 0.654                   | < 0.001 |
| Creatinine (mg/dl)                 | 0.561                   | < 0.001 |
| Glucose (mg/dl)                    | 0.465                   | < 0.001 |
| HbA1c (%)                          | 0.517                   | < 0.001 |
| Cholesterol (mg/dl)                | 0.147                   | 0.138   |
| Triglycerides (mg/dl)              | 0.320                   | 0.001   |
| HDL-C (mg/dl)                      | - 0.517                 | < 0.001 |
| LDL-C (mg/dl)                      | 0.114                   | 0.252   |

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