**ORIGINAL ARTICLE**

# HAPTOGLOBIN AND LIPID PROFILE IN DIABETIC NEPHROPATHY IN ILORIN, KWARA STATE

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## ABSTRACT

***Background:*** *Diabetic Nephropathy (DN) is a common cause of abnormal lipoprotein metabolism and can be influenced by impairment of renal function and metabolic controls in diabetes.*
***Aim:*** *The aim of this study is to determine the level of Haptoglobin and Lipid Profile in Diabetic Nephropathy Patients.*
***Subjects and Method:*** *A prospective case-controlled study was carried out among Diabetic Nephropathy Patients and Control with a total number of 50 DN Patients and 50 Non-Diabetics control subjects respectively. Serum Glucose estimation was analyzed using Glucose Oxidase Peroxidase (GOD) method, Serum Haptoglobin was determined using a Nephelometric method while the Lipid Profile (Total cholesterol, Triglycerides, and HDL-cholesterol) was assayed using an enzymatic method of estimation, while LDL-cholesterol was calculated by Friedewald equation. Body Mass Index (BMI, kgm-2) was calculated from height and weight which were obtained from a questionnaire used to record the demographic features of all the participants/subjects.*
***Result:*** *The results obtained show that serum glucose was significantly increased in Diabetic Nephropathy Patients (8.62 ± 1.34, $p<0.05$) when compared with control subjects (3.02 ± 0.88, $p<0.05$). There was also a significant increase ($p<0.05$) in mean Serum Haptoglobin (38.25 ± 6.67) in Diabetics when compared with control subjects (19.40 ± 3.92). A significant increase was also observed in Triglycerides in DN Patients with a mean of (0.77 ± 0.53, $p<0.05$) when compared to control subjects (0.63 ± 0.26). However, there were no significant increases in Total Cholesterol and LDL-Cholesterol, with their mean value of (4.15 ± 1.27 and 1.95 ± 0.72) when compared to control subjects (3.59 ± 1.04 and 3.59 ± 1.04) respectively. While an insignificant decrease was observed in DN Patient's HDL-cholesterol mean value (0.81 ± 0.34) when compared to control subjects (1.18 ± 0.19). In this study, a strong statistically significant positive correlation was observed in Haptoglobin and Total cholesterol (R= 0.939, P= 0.015), HDL-C (R= 0.897, P= 0.025).*
***Conclusion:*** *This study showed increased levels of Fasting Blood Glucose, Serum Haptoglobin, and Triglycerides increased Diabetic Nephropathy in Ilorin. Lipid control appears to be important in the prevention and treatment of Diabetic Nephropathy. This study suggests that serum Hp levels may be used as a potential biomarker for the early diagnosis of Diabetic Kidney Diseases in Diabetes Patients.*

**Keywords:** *Haptoglobin, Lipid Profile, Diabetes, Nephropathy*

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## INTRODUCTION

Diabetic Nephropathy (DN) or Diabetic Kidney Disease (DKD) is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of Glomerular Filtration Rate (GFR) in diabetics (1). High blood glucose levels (Hyperglycaemia) due to diabetes can damage the glomerulus of the nephron, making filtration of blood difficult. The damaged filter becomes 'leaky' and lets protein into the urine. For some individuals, diabetic nephropathy can progress to chronic kidney disease and kidney failure (2). Diabetes Mellitus (DM) is the most frequent cause of chronic kidney failure in both developed and developing countries (2). Diabetes Nephropathy is one of the microvascular complications of Diabetes Mellitus. Diabetic Nephropathy (DN) or Diabetic Kidney Disease (DKD) is a syndrome characterized by the presence of pathological quantities of albumin in urine, diabetic glomerular lesions, and loss of Glomerular Filtration Rate (GFR) in diabetics (1). Having high blood glucose levels due to diabetes can damage the part of the kidneys that filters your blood. The damaged filter becomes 'leaky' and results in a protein found in urine. For some individuals, diabetic nephropathy can progress to chronic kidney disease and kidney failure. However, most individuals with diabetes do not develop kidney disease that progresses to kidney failure. Diabetes mellitus (DM) is the most frequent cause of chronic kidney failure in both developed and developing countries (3).

The haptoglobin (hp) gene in humans codes for the protein hemoglobin. Haptoglobin reduces the harmful oxidative activity of free hemoglobin by binding to it with a high affinity in blood plasma (4). The majority of haptoglobin is made by hepatic cells, although it is also made by tissues such as the skin, lungs and kidney. In addition, both murine and human adipose tissue express the haptoglobin gene. Ahaptoglobinemia and hypohaptoglobinemia are caused by mutations in this gene or its regulatory regions. Additionally, the incidence of coronary artery disease in type 1 diabetes and diabetic nephropathy has also been associated with this gene (5).

A significant clinical abnormality in people with Diabetes Mellitus is dyslipidemia. High plasma triglyceride levels. Low high-density lipoprotein cholesterol levels and an increase in the number of small, dense, low-density lipoprotein particles are the hallmarks of diabetic dyslipidemia. Insulin resistance induces the liver to mobilize free fatty acid from adipose tissue, which increases the generation of triglycerides. Dysfunction of lipoprotein lipase due to long-standing diabetes would raise triglyceride levels and lead to the buildup of large triglyceride-rich very low-density lipoprotein particles (6). Diabetic nephropathy is an established risk factor for cardiovascular events as well as mortality (7). Individuals with diabetic nephropathy


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are known to experience lipoprotein abnormalities; the majority of these patients die of cardiovascular events before their renal dysfunction advances to end-stage renal disease (Go et al., 2004).

## SUBJECTS AND METHODS

### Subjects

This study was conducted in Ilorin, Kwara State. Kwara State is located in the north-central geopolitical zone of Nigeria; Kwara State is one of the most peaceful states in Nigeria. It had a population of 777,667, making it the 7th largest city by population in Nigeria. The local Governments in Ilorin are Ilorin East, Ilorin South, and Ilorin West.

The comparative analytical case-control study was conducted over a period of four (4) months. Before sample collection, ethical approval was sought and obtained from the ethical committee of Kwara State Ministry of Health, Kwara State prior to the commencement of this study. The procedure for Sample collection was explained to the patient using an information sheet prepared by the Researcher (MOH/KS/EU/777/601). Each patient was required to give informed consent before been recruited to the study. Questionnaires were administered containing the details of the subjects and control group such as age, sex, place of residence, ethnicity, marital status, occupation, and other information. Laboratory investigations such as Fasting Blood Sugar (FBS), Serum Haptoglobin, Lipid profile {serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) were done, while LDL cholesterol was calculated using the friedwald formula.

***Inclusion criteria***

Patients having DN, Individuals who give consent, and apparently healthy individuals as control.

***Exclusion criteria***

The individual with no history of Diabetes nephropathy as a case study, Individuals who refuse to give consent, and Smokers and Alcoholic Individual

### Methods

***Sample collection and storage***

Five milliliter (ml) of venous blood was collected into Plain tubes using a sterile needle and syringe from the medial cubital vein in the antecubital fossa. Thereafter, all samples were centrifuged at 3000rev for five minutes, after which serum were aspirated in small aliquots into clean vials and stored at -20°C until the analysis is done.

***Laboratory method***

Determination of Diabetic nephropathy was done by the assessment of microalbuminuria, while serum haptoglobin was determined with a Spectrophotometric method, and Plasma Lipids such as Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL) were estimated by Enzymatic Method. While Low Density Lipoprotein (LDL) was calculated by Friedewald Equation (8).

### Statistical Analysis

The data generated from this study were analyzed using a statistical package for social science (SPSS) version 16 (SPSS Inc. Chicago Illinois) for Windows. Statistical testing was used to determine Haptoglobin and Lipid Profiles in Diabetics with Nephropathy in Ilorin, Kwara State. Categorical variables were compared using the chi-square test while for the comparison of continuous variables; the student T-test was used. P-value $< 0.05$ was taken to be statistically significant.

## RESULTS



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The Basic characteristics of enrolled Participants are presented in Table 1. A total of Fifty (50) Diabetic Nephropathy patients were recruited for this study. 50 non-diabetic individuals were used as negative controls. Participants with an age greater than fifty (50) for both the test and control are 36 and 14 respectively while participants with an age less than fifty (50) for both the test and control are 17 and 33 respectively. Participants that are females for both the test and control are 42 and 25 respectively while participants that are males for both the test and control are 13 and 20 respectively.

**Comparison of FBS, HAPTO, TC, TRIG, HDL and LDL between Control and DN group**

The concentration of FBS, HAPTO, TRIG, TC, HDL, and LDL in apparently healthy controls were compared with that of Diabetic Nephropathy patients, the result was presented in Table 2. As shown in the table, there were no significant differences between the levels of FBG, TC, HDL, and LDL in both the control and DN group ($p = 0.130$), ($p = 0.706$), ($p = 0.854$) and ($p = 0.\ 0.090$) respectively, while there were significant differences between the levels of HAPTO and TRIG in both Control and Study group ($p = 0.010$) and ($p = 0.020$) respectively.

**Comparison of HAPTO level with respect to Age, Sex and Duration of disease**

The mean value of HAPTO was compared among the diabetic patients with respect to age, sex and duration of disease. With respect to the duration of the disease, the value of HAPTO was higher ($p=0.087$) among the patients with a duration of disease higher than 5 years in relation to the patients with a duration of disease lower than 5 years. However, with respect to sex and age, there were no significant differences.

**Correlation between different parameters among diabetic patients**

The concentration of FBS, HAPTO, TRIG, TC, HDL and LDL were correlated. The results showed a significant correlation between serum LDL and TC alone ($R = 0.923^*$, $p = 0.000$). There were no significant correlations between other parameters in this study.

## DISCUSSION

Diabetic Nephropathy (DN) is a clinical syndrome characterized by persistent albuminuria, a relentless decline in glomerular filtration rate, raised arterial blood pressure and increased relative mortality from cardiovascular disease. The study population consisted of adult Nigerians with a history of Diabetic Mellitus, the age range of the participants were was 35-57 years. The control group comprised of apparently healthy individuals matched for age and sex. All the participants were residents of Ilorin, a North-central part of Nigeria. A standard questionnaire and interview method were used to gather relevant information from the participants after an informed consent has been sought. The WHO consent template was slightly modified and used for this study.


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An examination of the male/female ratio revealed that a little above half of the participants were females (approximately 56 %). This may imply and support the notion that DMNP affects women and men similarly (9). Also, it could be attributed to the genetic pattern and sedimentary lifestyles among the population studied. However, some Authors have reported a higher female ratio (10).

The mean values of Fasting Blood Glucose (FBG) are usually lower in non-diabetic individuals in with respect to those with DM. Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates (11). The pattern of FBG observed in this study was similar to a study of by Pillay et al., (12), which showed similar patterns for sex and age. Greater percentages of DMNP subjects study have their FBG within the normal range, probably due to compliance with treatment as at the time of collecting the sample. There is growing interested in non-traditional biomarkers of hyperglycemia, particularly for use in settings where (2–4 weeks) glycemic control is of interest or where traditional measures (glucose) are problematic such as in heamoglobinopathy.

In this study, a significant increase in Haptoglobin level (38.25± 6.67) (p= 0.10) (Table 2) was observed in DN subjects when compared with the Control subjects (19.40 ± 3.92 mg/dl). This is similar to the findings of Huang et al. (13) as they observed an increased Serum Haptoglobin level in Diabetic Nephropathy patients when compared with the control subjects. This could be due to the inflammation of the kidney, inducing an increase Haptoglobin levels in the DN subjects. Haptoglobin being the principal hemoglobin-binding protein in the blood is increased in inflammation, independently of the degree of hemolysis associated with the tissue injury process (14). However, serum Haptoglobin is known to be a good marker of kidney injury, especially in Diabetic Nephropathy subjects and it is a good prognostic marker. Haptoglobin being is an Acute Phase Protein Reactant which is elevated during inflammation or infection (15).

There was no significant difference in TC level between the two groups (p=0.706) (Table 4.2). However, an increased Total Cholesterol level was seen in the mean value of the DN group (4.15 ± 1.27 mmol/l) (p= 0.706) but not significant when compared to the mean value of the Control group (3.59 ± 1.04 mmol/l). This goes in contrary to Jenkins et al., (2003) which may be due to the drug consumption of the DN patients, thereby lowering its concentration. The insignificant TC may also be due to the decreased BMI in the DN group (18.5 – 24.5 kg/m2) when compared to Control (25 – 29.5kg/m2).

According to Bonnet and Cooper (16), Diabetic Nephropathy was said to be connected with an abnormal lipid profile with a distinctive increase in triglyceride triglyceride-rich lipoproteins as soon as renal disease progresses. In this study, the mean value of TRIG was higher in Diabetic Patients in respect to


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the control group (p=0.020) (Table 2). In relation to different duration of the disease, it was observed that TRIG was significantly higher (p=0.022) in patients with the duration of the disease higher than 5 years. Also, there was a significant increase in triglyceride concentration in Diabetic Nephropathy subjects with a mean value of 0.77 ± 0.53 mmol/l when compared with the control subjects with a mean value of 0.63 ± 0.26 mmol/l and there was no significant association in total cholesterol, LDL-C and HDL-C of the DN group (4.15 ± 1.27 mmol/l), (1.95 ± 072 mmol/l) and (0.81 ± 0.34 mmol/l) when compared with that of controls (3.59 ±1.04 mmol/l), (1.84 ± 0.67), (1.18 ± 0.19), with the P values of (0.706), (0.854) and (0.90) respectively.

In comparison between the test and control groups, there was no significant difference in LDL levels between the two groups (p= 0.854) (Table 2). The level of LDL was compared among the diabetic patients with respect to age, sex and duration of disease (Table 3). With respect to sex, duration of disease and age, there were no significant differences reported. In this study, as shown in Table 4, it was observed that the value LDL was significantly correlated with TC (r = 0.000, p = 0. 923**). This study is similar to that of Seongyul et al., (17), which stated that Type-2 Diabetes Patients do not always have higher LDL levels than non-diabetic individuals. However, their sdLDL particles are markedly increased (18).

## CONCLUSION

Diabetic Nephropathy (DN) is a common cause of abnormal lipoprotein metabolism and can be influenced by the impairment of renal function and metabolic control of diabetes. DN and ESRD remains a significant problem despite best efforts to limit the impact of the disease on such end-organ damage. This study showed that Fasting Blood Glucose (FBG), Haptoglobin, Total cholesterol, Triglycerides, low-density lipoprotein cholesterol, and Body Mass Index increased in the patients with Diabetic Nephropathy when compared to controls. While there was a decrease in HDL of the Nephropathy Patients when compared to controls. This is a result of the connection between diabetes and insulin resistance, which causes the body to use fat as a source of energy. Among all the increased parameters, those that were significant include FBG, Haptoglobin and Triglycerides.

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**Table 1:** Demographic characteristics of studied groups

| Character | | Test | Control |
|---|---|---|---|
| **N** | | 50 | 50 |
| **Age ( Years)** | < 50 | 17 | 33 |
| | > 50 | 36 | 14 |
| **Sex** | Female | 42 | 25 |
| | Male | 13 | 20 |
| **BMI (Kg/m$^2$)** | 18.5 – 24.5 | 13 | 15 |
| | 25 – 29.5 | 27 | 25 |
| **Duration of Disease** | <5 years | 25 | - |
| | >5 years | 25 | - |

The values are the number of participants with each characteristic.

**Table 2:** Comparison of FBS, HAPTO, TC, TRIG, HDL and LDL between Control and DN group

| Parameters | Test | Control | P-value |
|---|---|---|---|
| FBG (mmol/L) | 8.62 ± 1.34 | 3.02 ± 0.88 | 0.030* |
| HAPTO (mg/dl) | 38.25 ± 6.67 | 19.40 ± 3.92 | 0.010* |
| TRIG (mg/dl) | 0.77 ± 0.53 | 0.63 ± 0.26 | 0.020* |
| TC (mmol/L) | 4.15 ± 1.27 | 3.59 ± 1.04 | 0.706 |
| HDL (mmol/L) | 0.81 ± 0.34 | 1.18 ± 0.19 | 0.090 |
| LDL (mmol/L) | 1.95 ± 0.72 | 1.84 ± 0.67 | 0.854 |

**Table 3:** Comparison of HAPTO level with respect to Age, Gender and Duration of disease

| Character | | Mean ± SD | P. value |
|---|---|---|---|
| **Age** | < 50 | 38.57 ± 6.72 | 0.640 |
| | > 50 | 37.62 ± 6.88 | |
| **Gender** | Female | 38.27 ± 6.78 | 0.668 |
| | Male | 38.19 ± 6.80 | |
| **Duration of disease** | <5 years | 36.99 ± 7.65 | 0.038* |
| | >5 years | 39.51 ± 5.50 | |

The values are mean ± SD, p-value were determined by Student's t-test as appropriate, $p < 0.05$ was considered significantly different, *****when there is an intergroup significant difference.


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**Table 4:** Correlation of different parameters among diabetic patients

| Correlation | | FBS | HAPTO | TRIG | TC | HDL | LDL |
|---|---|---|---|---|---|---|---|
| FBS(mmol/L) | R | | | | | | |
| | P | 1 | | | | | |
| HAPTO (mg/dl) | R | 0.364 | | | | | |
| | P | 0.172 | 1 | | | | |
| TRIG (mg/dl) | R | 0.345 | 0.056 | | | | |
| | P | -0.179 | -0.352 | 1 | | | |
| TC (mmol/L) | R | 0.774 | 0.939 | 0.982 | | | |
| | P | 0.055 | 0.015 | 0.004 | 1 | | |
| HDL (mmol/L) | R | 0.581 | 0.897 | 0.833 | 0.045 | | |
| | P | -0.105 | 0.025 | 0.040 | 0.368 | 1 | |
| LDL (mmol/L) | R | 0.479 | 0.784 | 0.639 | 0.923 | 0.124 | |
| | P | 0.134 | 0.052 | -0.089 | 0.000** | 0.287 | 1 |

The table shows the relationship between different parameters among the DN, * is used to connote a significant correlation between the two parameters.


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