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# Evaluation of neuronal inflammation and oxidative DNA damage in different haptoglobin phenotypes of Nigerian type-2 diabetes mellitus population

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**Abstract**

*Background*: Oxidative stress is a major factor in the pathogenesis and progression of the clinical condition type 2 Diabetes Mellitus (DM) related to adverse biochemical/molecular interactions. *Aim and Objectives:* To determine whether haptoglobin phenotypes predispose DM patients to vascular complications and neuronal damage. *Material and Methods*: A total of 74 subjects were assessed out of which 31 had treated and untreated diabetes complicated with hypertension, 26 had treated and untreated uncomplicated DM and 17 were apparently healthy subjects who served as controls. Body Mass Index (BMI), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP), serum Glucose (GLU), protein S100B and 8-Hydroxy-2-deoxyguanosine (8-OHdG) were determined in all subjects alongside the characterization of Haptoglobin (Hp) phenotypes. *Results*: BMI, SBP, DBP, GLU, protein S100B and 8-OHdG in treated and untreated complicated and uncomplicated DM patients were higher when compared to controls ($p < 0.05$). Hp 2 allele (Hp 2-1 and Hp2-2) was seen to be associated with poor glucose control, higher blood pressure and increased neuronal damage in both complicated and uncomplicated DM. It was also seen that the possession of Hp 2 gene was associated with a lower response to treatment. *Conclusion:* The Hp 2 allele could be a predisposing factor in developing diabetes related complications like hypertension and neuronal damage.

**Keywords:** Diabetes Mellitus, Hypertension, Neuronal damage, Oxidative DNA damage, Haptoglobin

## Introduction

Diabetes Mellitus (DM) is a chronic disorder of carbohydrates, fats and protein metabolism. It is characterised by high levels of blood sugar [1]. DM is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1]. The hyperglycaemia of long duration seen in DM is linked with damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Quite a handful of pathophysiologic processes are involved in the development and progression of DM. These include destruction of the insulin secreting cells of the pancreas resulting in insulin deficiency to abnormalities that result in resistance to insulin action [1, 2].

Haptoglobins (Hp) are plasma glycoproteins. They bind to free haemoglobin from destroyed red blood cells to form haemoglobin-haptoglobin complex [3]. In humans, two alleles namely Hp 1 and Hp 2 exist for the Haptoglobin gene. These genes combine to result in three major phenotypes which

are Hp 1-1, Hp 2-1, and Hp 2-2 [4]. Most methods of haptoglobin phenotyping are based upon electrophoretic separation in a gel medium according to size. Larger molecules are impeded more than small molecules in their migration through the gel. Hp 1-1 has a single, fast-moving band, whilst Hp 2-1 and Hp 2-2 each have multiple bands, which differ in size and electrophoretic mobility. This is because the Hp 2 product is capable of producing homopolymers (Hp 2-2) and heteropolymers (Hp 2-1) [5]. Studies recently showed evidence that haptoglobin phenotype may serve as a predisposing factor for vascular complications to Type 2 DM (T2DM). The haptoglobin phenotypes have different alleles which differ in their activity or capability [5]. In T2DM patients, oxidative stress is a major factor in the pathogenesis and progression of the clinical condition [16]. It has been recognized that oxidative stress can manifest as oxidative DNA damage which more often than not result in cytotoxicity and is implicated in the pathogenesis of numerous diseases including metabolic [7] neurodegenerative diseases, cardiovascular diseases, chronic inflammatory diseases and cancers. 8-hydroxy-2′-deoxyguanosine (8-OHdG) derived from hydroxyl radical attack of deoxyguanosine residues has been commonly chosen as a biomarker of oxidative damage to DNA, assessing both antioxidants status and DNA damage [8].

Neuronal damage can be caused by pressure, stretching, or cutting. An injury to a neuron can stop the signals transmitted to and from the brain, causing muscles to not work properly or a loss of feeling in an injured area [9]. The loss or a cut in blood supply has been known to be the cause of damage to any organ, while the adverse effects of abnormal biochemical or molecular interactions also cannot be ruled out as a cause of neuronal damage [10]. As genetic factors have been known to be a predisposing factor, coupled with the fact that the likelihood of stroke is high in DM, this research was designed to assess neuronal damage, oxidative DNA damage in different haptoglobin phenotypes with T2DM.

**Material and Methods**

**Study design:** A cross sectional design using a case-control sampling method was used. Stratification was by therapy and the presence or absence of vascular complications.

**Study area:** The study area was Ido Ekiti and its environs. Ido Ekiti is a town in Ekiti state in south-west Nigeria. The state is mainly an upland zone, rising over 250 meters above sea level. Its coordinates are $7^0$ 40'N $5^0$ 15'E. The study involved both out patients and admitted patients at Federal Teaching Hospital, Ido Ekiti, Ekiti State.

**Sample size:** A total of 74 subjects were used for this research. Thirty-one of these had DM complicated with hypertension while 26 had uncomplicated DM. The remaining 17 were apparently healthy subjects without DM or hypertension. These were used as controls.

**Inclusion and exclusion criteria:** Males and females with T2DM whether on therapy or not were enrolled in this study. The inclusion criteria was based on the cut off of at least 7.8 mmol/L for fasting to 10 mmol/L. Subjects below the age of 18 years, pregnant women, type 1 DM, lactating mothers, cancer patients, hypertensive patients and sufferers of other chronic systemic conditions were excluded.

**Ethical clearance:** It was obtained from the Ethics and Research Committee, Federal Teaching Hospital Ido, Ido Ekiti, Ekiti State. The nature and purpose of research was explained to each participant using an informed consent for literate participants and verbal explanation for illiterate participants. Patients were not forced to answer questions, but at their own free will. The patients were assured of confidentiality.

**Sample collection:** After an overnight fasting of 12 hours, venous blood sample of 5 ml was collected from the cubital fossa using 22G needle and syringe. Out of this, 2 ml was dispersed into a fluoride oxalate bottle and 3 ml into a plain bottle. The blood in the anticoagulant bottle was centrifuged at 12000 rpm for 5 minutes to separate plasma from cells, while the blood in the non-anticoagulant bottle was allowed to clot and then centrifuged at 12000 rpm for 5 minutes to separate the serum from cells. The plasma from the fluoride oxalate bottle was used for glucose determination while the serum sample derived from above was placed in a plain bottle and stored at a temperature of -20°C before it was used for haptoglobin characterization, and assessment of oxidative DNA and neuronal damage.

**Methods of determination of parameters**

**Body Mass Index (BMI)** was derived from the height and weight using the formula: BMI= Weight (kg) / Height$^2$ (m$^2$). It is expressed in kg/m$^2$ [11].

**Blood Pressure** was determined using digital sphygmomanometer. Estimation of plasma glucose levels was estimated spectrophotometrically using Randox glucose kit by using Glucose Oxidase Peroxidase method as stated by Oloruntoba *et al*. [12].

Characterization of serum haptoglobin phenotypes was done by protein electrophoresis using polyacrylamide gel and visualized using specific peroxidase staining [13]. Estimation of 8OHdG as a marker of Oxidative DNA damage and protein S100B as a marker of neuronal damage were estimated using Sandwich ELISA technique

**Statistical analysis**

Results obtained were subjected to statistical analysis using SPSS (version 21.0 software, SPSS Inc. Chicago, Illinois, USA). Values were expressed as Mean ± SD. The student 't' test was the tool of choice in comparing means and p values were computed. The value of $p < 0.05$ was considered as statistically significant.

**Results**

**Figure 1: Distribution of all subjects according to gender.** Out of 74 subjects, there were 16 female with DM and 10 male with DM; 15 female with complicated DM and 16 male with complicated DM; 8 female control subjects and 9 male control subjects

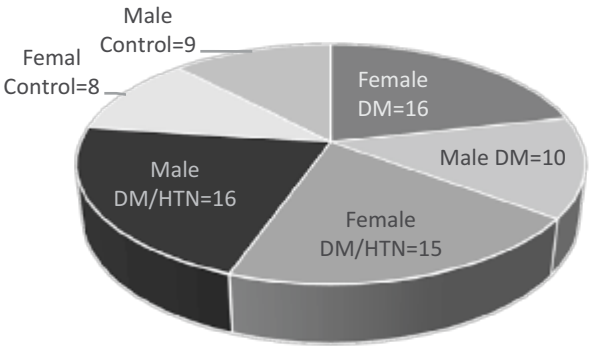



**Figure 1: Distribution of all subjects according to gender**

**Table 1:** There was statistically significant variation in the systolic (mmHg) blood pressure, glucose concentration (mmol/L) and 8-OHdG (ng/ml) ($p=0.0002$), ($p<0.0001$), ($p<0.0001$) respectively when untreated subjects with DM were compared with controls. In treated patients with DM, there was significant difference in the 8-OHdG levels (ng/ml) ($p<0.0001$)) as compared to the control group.

**Table 2**: Significantly higher BMI (kg/m$^2$), systolic (mmHg) blood pressure, diastolic (mmHg) pressure and 8-OHdG (ng/ml) levels ($p=0.0005$), ($p<0.0001$), ($p<0.0001$), ($p<0.0001$) were observed in patients with complicated DM as compared with controls. BMI (kg/m$^2$), systolic (mmHg) blood pressure, diastolic (mmHg) blood pressure, glucose (mmol/L) and 8-OHdG (ng/ml) were significantly higher ($p=0.0005$), ($p<0.0001$), ($p<0.0001$), ($p<0.0001$), ($p<0.0001$) respectively, in patients with untreated complicated DM when compared with the controls.

**Table 1: Treated and untreated diabetic subjects compared with control subjects**

| Group (n) | Treated DM Mean ± SD | Untreated DM Mean ± SD | Control Mean ± SD |
|---|---|---|---|
| **BMI (kg/m$^2$)** | 26.29 ± 7.09 | 27.71 ± 3.81 | 25.76 ± 0.46 |
| **SBP (mmHg)** | 104.6 ± 7.99 | 125.27 ± 9.19$^b$ | 109.53 ± 5.06 |
| **DBP (mmHg)** | 71.87 ± 3.09 | 78.45 ± 5.87 | 73.18 ± 7.56 |
| **Glucose (mmol/L)** | 5.05 ± 0.84 | 9.13 ± 1.51$^c$ | 4.74 ± 0.75 |
| **8-OHdG (ng/ml)** | 1.77 ± 0.29$^c$ | 3.58 ± 0.59$^c$ | 0.79 ± 0.12 |
| **S100B** | 0.05 ± 0.2$^{ad}$ | 0.10 ± 0.03$^c$ | 0.04 ± 0.01 |

*a, b and c = statistically significant relative to control at $p<0.05$, $p< 0.005$ and $p< 0.0001$ respectively*
*d, e and f = statistically significant relative to untreated at $p< 0.05$, $p< 0.005$ and $p< 0.0001$ respectively*
*BMI-Body Mass Index, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, 8-OHdG -8-Hydroxy-2-deoxyguanosine*

**Table 2: Treated and untreated hypertensive diabetic subjects compared with control subjects**

| Group (n) | Treated DM/HTN Mean ± SD | Untreated DM/HTN Mean ± SD | Control Mean ± SD |
|---|---|---|---|
| **BMI (kg/m$^2$)** | 31.23 ± 5.81[b] | 30.90 ± 5.43[b] | 25.76 ± 0.46 |
| **SBP (mmHg)** | 142.06 ± 10.79[c] | 152.5 ± 9.90[c] | 109.53 ± 5.06 |
| **DBP (mmHg)** | 93.0 ± 5.95[c] | 96.79 ± 6.76[c] | 73.18 ± 7.56 |
| **Glucose (mmol/L)** | 4.99 ± 1.04 | 10.02 ± 3.46[c] | 4.74 ± 0.75 |
| **8-OHdG (ng/ml)** | 3.48 ± 1.18[c] | 4.69 ± 1.50[c] | 0.79 ± 0.12 |
| **S100B** | 0.07 ± 0.03[ad] | 0.17 ± 0.07[c] | 0.04 ± 0.01 |

*a, b and c = statistically significant relative to control at p <0.05, p <0.005 and p <0.0001 respectively*
*d, e and f = statistically significant relative to untreated at p <0.05, p <0.005 and p <0.0001 respectively*
*BMI-Body Mass Index, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, 8-OHdG -8-Hydroxy-2-deoxyguanosine*

**Tables 3 and 4** compared all estimated parameters so as to assess the effect of complication on each. Therefore all parameters were compared between complicated and non-complicated DM (treated and untreated). All BMI, S100B, 8-OHdG and S100B levels were significantly higher in the event of complications

**Table 3: Complicated and uncomplicated treated diabetic subjects compared with control subjects**

| Group (n) | Treated DM Mean ± SD | Treated DM/HTN Mean ± SD | Control Mean ± SD |
|---|---|---|---|
| **BMI (kg/m$^2$)** | 26.29 ± 7.09 | 31.23 ± 5.81 | 0.000 |
| **SBP (mmHg)** | 104.6 ± 7.99 | 142.06 ± 10.79 | 0.000 |
| **DBP (mmHg)** | 71.87 ± 3.09 | 93.0 ± 5.95 | 0.000 |
| **Glucose (mmol/L)** | 5.05 ± 0.84 | 4.99 ± 1.04 | 0.8581 |
| **8-OHdG (ng/ml)** | 1.77 ± 0.29 | 3.48 ± 1.18 | 0.000 |
| **S100B** | 0.05 ± 0.2 | 0.11 ± 0.03 | 0.000 |

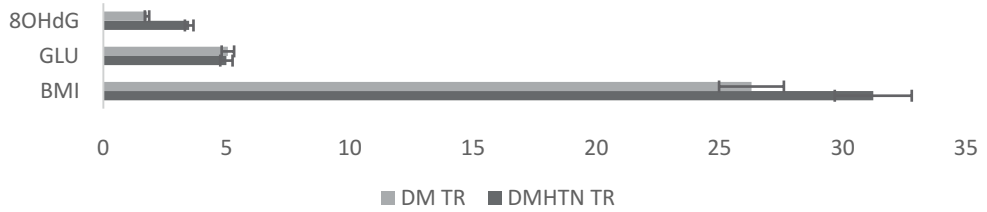
*BMI-Body Mass Index, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, 8-OHdG -8-Hydroxy-2-deoxyguanosine*

**Table 4: Complicated and uncomplicated untreated diabetic subjects compared with control subjects**

| Group (n) | Untreated DM Mean ± SD | Untreated DM/HTN Mean ± SD | Control Mean ± SD |
|---|---|---|---|
| **BMI (kg/m$^2$)** | 27.71 ± 3.81 | 30.90 ± 5.43 | 0.000 |
| **SBP (mmHg)** | 125.27 ± 9.19 | 152.5 ± 9.90 | 0.000 |
| **DBP (mmHg)** | 78.45 ± 5.87 | 96.79 ± 6.76 | 0.000 |
| **Glucose (mmol/L)** | 9.13 ± 1.51 | 10.02 ± 3.46 | 0.399 |
| **8-OHdG (ng/ml)** | 3.58 ± 0.59 | 4.69 ± 1.50 | 0.022 |
| **S100B** | 0.10 ± 0.03 | 0.17 ± 0.07 | 0.000 |

*BMI-Body Mass Index, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, 8-OHdG -8-Hydroxy-2-deoxyguanosine*

**Figure 2:** BMI, GLU and 8-OHdG levels between treated complicated and treated uncomplicated DM There was a significant increase in S100B, 8-OHdG and BMI levels in treated complicated DM was compared with treated uncomplicated DM, thereby indicating that treated complication of DM with hypertension caused a significant increase in 8-OHdG and BMI levels and made the parameters not responsive to treatment. The chart also showed no significant increase in glucose when treated complicated DM was compared with treated uncomplicated DM.




** = significant increase in parameters when compared*
*DMHTN UNTR: Untreated DM complicated with hypertension; DM UNTR: Untreated uncomplicated DM; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure*

**Figure 2: BMI, GLU and 8OHdG between treated complicated and treated uncomplicated diabetics**

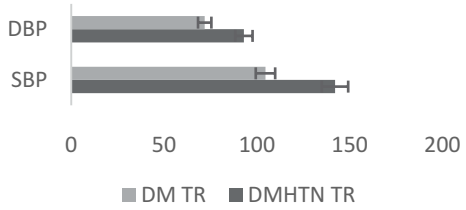
**Figure 3:** Systolic blood pressure and diastolic blood pressure of treated complicated DM was significantly higher than treated uncomplicated DM patients

**Figure 4:** A significantly higher systolic and diastolic blood pressure was observed when untreated complicated DM was compared with untreated uncomplicated DM, untreated complication of DM with hypertension caused an increased level in the above parameters and made an untreated case look more severe.

**Figure 5:** BMI, GLU and 8-OHdG levels between untreated complicated and untreated uncomplicated DM patients.

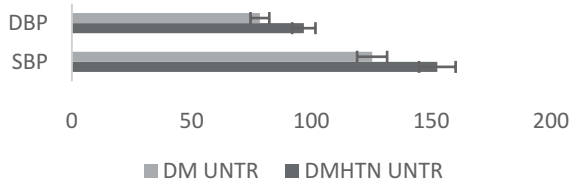
There was a significant increase in 8-OHdG, BMI and an insignificant increased glucose when untreated complicated DM was compared with untreated uncomplicated DM. Untreated complication of DM with hypertension accelerated the above parameters.

**Table 5:** Haptoglobin phenotypes in treated and untreated DMHTN and DM subjects. Haptoglobin 2 phenotypes (2-1 and 2-2) appear to be higher than the Hp 1 (1-1) phenotype in both complicated and uncomplicated DM. However, among the controls, the Hp 1 phenotype population appears to be significantly higher than the Hp 2 phenotype. It seems Hp phenotype predisposes to the development of DM and perhaps the likelihood of hypertension in DM



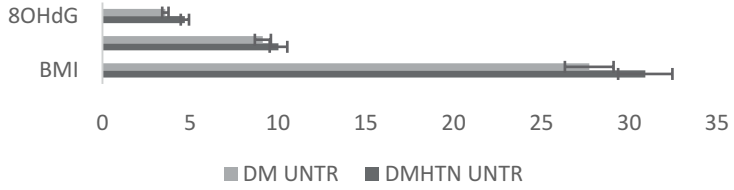

** = significant increase in parameters when compared DMHTN UNTR: Untreated DM complicated with hypertension; DM UNTR: Untreated uncomplicated DM; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure*

**Figure 3: SBP and DBP between treated complicated and treated uncomplicated diabetics**




** = significant increase in parameters when compared DMHTN UNTR: Untreated DM complicated with hypertension; DM UNTR: Untreated uncomplicated DM; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure*

**Figure 4: SBP and DBP between untreated complicated and untreated uncomplicated diabetics**




** = significant increase in parameters when compared DMHTN UNTR: Untreated DM complicated with hypertension; DM UNTR: Untreated uncomplicated DM; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure*

**Figure 5: BMI, GLU and 8-OHdG between untreated complicated and untreated uncomplicated diabetics**

**Table 5: Haptoglobin phenotypes in treated and untreated DMHTN and DM subjects**

| Group (n) | N (%) | N (%) | N (%) |
|---|---|---|---|
| | 1-1 | 2-1 | 2-2 |
| **DMHTN (n = 31)** | 4/ 31 (12.9) | 16/31 (51.6) | 11/31 (35.5) |
| **DM (n =26)** | 6/26 (23.1) | 12/26 (46.2) | 8/26 (30.8) |
| **Control (n= 17)** | 8/17 (47.1) | 8/17 (47.1) | 1/17 (5.8) |

*DMHTN: DM complicated with hypertension DM: uncomplicated DM*

**Discussion**

DM is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Symptoms of marked hyperglycaemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision [1].

Hypertension on the other hand is common among patients with DM, with the prevalence depending on type and duration of DM, age, sex, race/ethnicity, BMI, history of glycaemic control, and the presence of kidney disease. Hypertension is a strong, modifiable risk factor for the macrovascular and microvascular complications of DM [14] and as both conditions have been associated with lowered antioxidant status and systemic damage, this study was carried out to determine whether haptoglobin phenotypes predispose both complicated and uncomplicated patients with DM to vascular complications and neuronal damage It was also designed to assess whether Hp phenotypes determine the response to treatment.

BMI is a measure of weight adjusted for height, calculated as weight in kilograms divided by the square of height in meters ($kg/m^2$). It ranges as follows: below 18.5 $kg/m^2$ indicates underweight,18.5 $kg/m^2$ to 24.9 $kg/m^2$ indicates normal, 25.0 $kg/m^2$ to 29.9 $kg/m^2$ indicates overweight and 30.0 $kg/m^2$ and above indicates obesity. Obesity accelerates the likelihood of developing type 2 DM [15]. In this study, BMI was significantly higher when treated and untreated complicated DM was compared to control, BMI was insignificantly higher in treated and untreated uncomplicated DM when compared to control. When treated complicated DM was compared with treated uncomplicated DM, BMI was significantly higher in treated complicated DM than in treated uncomplicated DM and also in untreated complicated DM when compared with untreated uncomplicated DM. This finding support the work of Zhao *et al.* [16] where BMI was significantly higher when untreated DM was compared to controls. Furthermore, the likelihood of obesity appears to be higher when DM is complicated with hypertension. BMI appears to normalize in response to treatment as evidenced when treated DM were compared with controls.

SBP is the pressure exerted by the blood flowing through the arteries when blood is pumped and it is also the pressure when the heart beats while the heart muscle is contracting and pumping oxygen-rich blood into the blood vessels, it ranges between 90 mmHg to 120 mmHg and DBP is the pressure that indicates how much pressure the blood is exerting against the artery walls while the heart is resting between beat, it ranges between 60 mmHg to 80 mmHg [17]. This research study shows that SBP and DBP in treated and untreated complicated and uncomplicated DM were significantly higher when compared to controls. It was also found that SBP and DBP in untreated and treated complicated DM were higher when compared to untreated and treated uncomplicated DM. These findings are similar to Ko *et al.* [18] where SBP and DBP were significantly higher in DM. The logical rationale for these findings could not be farfetched as hypertension have been known to be a co-morbidity of DM, increasing the likelihood of metabolic syndrome as high blood pressure and hyperglycaemia co-exist in a large percentage of those with the metabolic syndrome [19]. Much more interesting is the fact that blood pressure was found to be higher in complication of DM with hypertension, a finding that is in line with the works of Dhas *et al*. [20].

Glucose ($C_6H_{12}O_6$), also known as d-glucose, dextrose, or grape sugar is a simple monosaccharide found in plants. It is one of the three dietary monosaccharides, along with fructose and galactose, that are absorbed directly into the bloodstream during digestion and approximately 4 grams of glucose are present in the blood of a 70-kilogram human at all times [21]. The level of blood glucose is the concentration of glucose found in human blood, the normal range is between 3.9 and 7.1 mmol/L (70 to 130 mg/dL). In this study, glucose in treated as well as untreated complicated ($p=0.43$) and uncomplicated DM ($p=0.28$) were insignificantly higher when compared to controls while glucose in untreated complicated and uncomplicated DM were significantly higher when compared to controls, glucose in untreated complicated diabetics was higher when compared to untreated uncomplicated diabetics, and there was no significant increase in glucose level when treated complicated diabetics was compared to treated uncomplicated diabetics, these findings are similar Patel *et al.* [22] where glucose was significantly higher in untreated DM when compared to controls. The implication of these above findings is that significant hyperglycemia tends to be milder as well as respond better to treatment in the absence of complication with hypertension [23]. Glucose in treated and untreated complicated DM, and in treated uncomplicated DM significantly correlated with 8-OHdG while glucose in untreated uncomplicated DM significantly correlated with SBP, DBP, and 8-OHdG. Also, glucose significantly correlating with 8-OHdG goes in line with the global notion that DM is associated with increased production of reactive oxygen species, which results in oxidative stress and oxidative damage to DNA [23].

Haptoglobins (Hp) are acute-phase α-glycoprotein which binds to free circulating haemoglobin, mediating its removal, thus preventing oxidative tissue damage. HP gene codes for two common alleles Hp 1 and Hp 2, yielding three phenotypes (Hp 1-1, Hp 2-1 and Hp 2-2). These Hp polymers differ in their biological properties and functions [24]. In this study, Hp 2-1 (51.6%) and Hp 2-2

(35.5%) were higher in complicated DM than in uncomplicated DM, while Hp1-1 (23.1%) was higher in uncomplicated DM than in complicated DM. These findings goes along with the works of Olaniyan *et al.* [2] where the relationship between haptoglobin, DM and the development of hypertension was recorded. The reasons for the higher incidence of Hp 2 allele in DM and much more in complication of DM with hypertension could be as a result of the molecular size hence deficient ROS scavenging capability of this allele, paving way for the development of hypertension, ROS accumulation and hypertension being correlated in DM [2]. As a result of the above findings, any question of whether some haptoglobin phenotypes will predispose T2DM to vascular complications can be answered by stressing that Hp phenotype 2 (Hp 2-1 and Hp 2-2) can be said to be associated with a higher incidence both of DM and also for the development a complication of hypertension in DM.

It has been recognized that oxidative DNA damage can result in cytotoxicity and is implicated in the pathogenesis and progression of numerous diseases including neurodegenerative diseases, cardiovascular diseases, chronic inflammatory diseases and cancers. 8-OHdG derived from hydroxyl radical attack of deoxyguanosine residues has been commonly chosen as a biomarker of oxidative damage to DNA, assessing both antioxidants status and DNA damage [8]. Reactive Oxygen Species (ROS) are generated during normal cellular metabolic processes and after exposure to harmful environmental factors, such as ionizing radiation and chemical carcinogens. An imbalance between the production and scavenging of ROS, known as oxidative stress, will lead to the damage of cellular proteins, lipids and nucleic acids. Among these cellular biomolecules that may be attacked and modified by ROS, major efforts have been devoted to DNA, as ROS pile up, antioxidants status decreases [7]. In this study, 8-OHdG in treated and untreated complicated diabetics ($p<0.0001$) and uncomplicated diabetics ($p<0.0001$), was significantly higher when compared to controls. 8-OHdG was significantly higher in treated and untreated complicated diabetics when compared to treated and untreated uncomplicated DM, a finding similar with the work of Aubaidi *et al.* [25] where 8-OHdG was higher in DM relative to controls. The reasons for the higher tendency of 8-OHdG to accumulate in DM and much more in complication of DM with hypertension could be as a result of the antioxidant system being overwhelmed. An increased level of blood glucose is usually associated with the generation of free radicals, this together with increased BMI aggravates the diabetic condition [25], making the development of hypertension in DM more probable. As a result of the findings above, this research therefore answers any question of whether there will be a significant increase in antioxidant parameters in T2DM mellitus over that of control.

Astroglial proteins (S100B) was the first member of the S100 protein family to be identified. It belongs to a multigene family of -binding proteins of the EF-hand type and is highly abundant in the brain where it localizes to astrocytes [26]. In this study, S100B in complicated treated and untreated and uncomplicated untreated DM were significantly higher ($p<0.0001$) when compared to controls while uncomplicated treated DM ($p=0.0085$) was insignificantly higher when

compared to controls. Astroglial proteins in treated complicated DM when compared with treated uncomplicated DM and also untreated complicated compared to untreated uncomplicated DM was significantly higher. These findings are similar to Parpura *et al*. [27] where changes in glucose levels can disrupt the normal function of neurons and trigger adaptive responses in both physiological and pathological states. S100B is significantly higher in complicated untreated DM when compared to complicated treated and also when uncomplicated untreated is compared to uncomplicated treated DM, this indicates that the complication of DM with hypertension poses a threat to the nervous system thereby increasing levels of S100B. The deleterious effect of DM and hypertension on the neurons of the CNS could be in terms of shortage of blood supply through remodelled capillaries. Cellular deterioration and apoptosis due to nuclear and mitochondrial oxidative DNA damage is another explanation.

A sustained elevation of blood pressure values are a common finding in patients with T2DM and are thought to reflect, at least in part, the impact of the underlying insulin resistance on the vasculature. The development of hypertension in diabetic individuals not only makes a mockery of treatment plan and increases healthcare costs but also increases the risk for macrovascular and microvascular complications [2]. Therefore this research would not be complete without discussing the effects of complication on assessed parameters. 8-OHdG and protein S100B were seen to be higher in DM complicated with hypertension than when DM is uncomplicated. As a matter of fact, there seems to be a link between Hp 2 and all estimated parameters. The diabetic condition is one in which when glucose has flooded the bloodstream, there is virtually any pathway through which it can be expended, the polyol pathway being the most important viable channel. The body, in the quest for alternative means of energy then breaks down compounds such as NADH resulting in a flood of ROS [28] which ends up damaging macromolecular complexes e.g. DNA and, lowering the antioxidant status. This is coupled with the fact that the possession of the Hp 2 gene (in the homozygote or heterozygote form) may bring about some form of aggravation consequent to deficient ROS scavenging, hence about a 3-fold increased risk of developing vascular complications [29], this would imply that possessing the Hp 2-1 phenotype alone increases the risk of DM complicated with hypertension, while those homozygous for Hp 2 (Hp 2-2) even have an increased likelihood [30]. As Hp2 has also been reported to have reduced antioxidant properties, possibly as a result of its reduced ROS scavenging capability, the overall effect of ROS build up on the walls of the blood vessels can not only be imagined but could be seen to result in a poorer glucose tolerance/glycaemic indices and an elevation of blood pressure. The elevation of blood pressure would in turn indicate a reduced blood supply to the central nervous system, causing damage to neurones.

**Conclusion**

DNA and neuronal damage are characteristic of DM. This damage could be much higher when DM is complicated with hypertension and/or in the event of being of haptoglobin 2 phenotype. Response to treatment may also be ineffective or slower in the presence of these confounding factors. These findings could also help in

determining better management of complicated and uncomplicated diabetic cases.

**Recommendation**

Patients who have complicated DM should be given a different medication from those with uncomplicated DM, so as not to be undertreated as this would help make treatment more effective. Patients with uncomplicated DM should be given wholesome treatment so that the likelihood of complication will not set in. Haptoglobin characterization testing should be made a routine testing in the laboratory for all DM patients so that those who possess the Hp 2 phenotype will get wholesome and adequate care. Assessment of neuronal damage markers may also be a predictor of stroke.

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