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Glyceamic and Oxidative Stress Markers in Different Haptoglobin Phenotypes of A Type-2 Diabetics' Population in Nigeria

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Abstract

Diabetes has long been known to be synonymous with body oxidative stress and development of vascular complications. Different clinical functions including oxidative capabilities have been credited to the different haptoglobin polymorphism. This study is aimed at finding possible association between haptoglobin gene polymorphism, glyceamic control indices and antioxidants parameters in Nigerian type 2 diabetics. The study included 170 subjects divided into three groups of 60 type 2 diabetics without any vascular complications, 60 type 2 diabetics with various vascular complications, and 50 age and sex matched apparently healthy subjects. All participants were subjected to full history taking, complete clinical examination, and routine laboratory investigations. Haptoglobin phenotypes, glyceamic control index and antioxidant parameters were measured in all subjects. Hp1-1 polymorphic type 2 diabetics show good glyceamic control indices than Hp2-1 and Hp 2-2 individuals. Likewise, Hp2-2 polymorphic type 2 diabetics show significantly reduced antioxidant potentials than Hp2-1 and Hp1-1 polymorphic individuals. Hp 2-2 polymorphism is a likely genetic predisposing factor contributing to development of diabetes vascular complications via low antioxidant level. Awareness of this gene susceptibility should raise future research for proper treatment and prevention of vascular complications in diabetics.

Keywords: Haptoglobin polymorphism, Antioxidant, type 2 diabetes, Vascular complications, Glyceamic control

1. Introduction

Diabetes mellitus (DM) is a major health challenge worldwide and a rising epidemics in Nigeria, with long-term diabetes vascular complications as the leading cause of morbidity and mortality in these individuals [1]. Type 2 diabetes, the commonest form of diabetes disease in Nigeria [2], resulted from continuous or gradual development of insulin resistance accompanied with or without a relative deficiency in insulin secretion resulting from β -cell dysfunction, to cause hyperglycaemia [3]. Chronic hyperglycaemia, a primary feature of diabetes mellitus leads to the overproduction of mitochondrial reactive oxygen species (ROS) and subsequent formation of sustained cellular oxidative stress, due to increased oxidant production (accumulation of free electrons) and/or decreased antioxidant activity (such as glutathione and superoxide dismutase) [4,5]. Hyperglycaemia also increases the lipid peroxidation and non enzymatic protein glycosylation [6,7], causing changes that stimulate the production of inflammatory cytokines which has been implicated in morphological and pathological changes found in macro and micro vascular diabetes complications [8].

Haptoglobin (Hp), a major positive acute phase glycoprotein [9], that binds free oxygenated haemoglobin released from erythrocytes in response to inflammation or infection. It also undergoes glycation and it is expressed as a genetic polymorphism comprising of two alleles denoted as 1 and 2 to give rise to three major polymorphism / phenotypes commonly referred to as Hp1-1, Hp2-1, and Hp2-2 in humans [10-12]. Circulating Hp complexes is an aggregate (polymer) of stoichiometrically dependent Hp monomers. The protein products of homozygote Hp1 allele cross-link only with one other Hp1 monomer to form a dimeric protein product whereas, the Hp2 allele protein can cross link with two distinct Hp monomers to form either a homozygous protein product which can be cyclic trimers, quatermers and pentamers or heterozygote (2 haptoglobin and 1 monomers) protein product of liner trmers, quatermers and pentamers of mixtures of Hp1 and Hp2 products [13]. Hp is involved in preventing the generation of hemoglobin-driven hydroxyl and lipid peroxides radicals [14], the protective ability of haptoglobin against haemoglobin driven oxidative injury is evident when haemoglobin becomes glycated, (a process that is markedly accelerated in the diabetics state) forming glycohemoglobin-haptoglobin complexes which are catalytically redox active [15], and therefore the rate at which haptoglobin-haemoglobin complexes are cleared from the serum and extra-vascular space is of believed to be haptoglobin dependent and of heightened importance in the diabetic state.

Several prospective and cross-sectional population studies have demonstrated that haemoglobin scavenger receptor, CD163 (monocyte-macrophage scavenger receptor) provides a mechanism by which Hp-Hb complexes are being cleared in the body system [16]. This clearance however, has been attributed to affect oxidative capacity among different Hp polymorphic individuals with an observation that Hp1-1-Hb complexes are being cleared more rapidly than Hp2-1Hb and Hp2-2Hb complexes and subsequently reduces the burden of oxidative vascular damage among Hp1-1individuals than Hp2-1 and Hp2-2 individuals [13,17-19]. Despite an extensive research in the field of diabetes, a limited number of these researches have linked Hp genetic polymorphism to development of diabetes and its vascular complications. Also to our knowledge, the Hp gene polymorphism has not been linked to glyceamic and oxidative parameters. The aim of this study was to find an association between Hp gene polymorphism, glyceamic control indices and antioxidants parameters among Nigerian type 2 diabetics.

2. Materials and Methods

2.1 Research design and subjects

The study protocol was approved by the Osun State University Health Research Ethics Committee. Consents were sought and obtained from all participants and all procedures were in accordance to Helenski declaration and guidelines. 120 confirmed and known type 2 diabetes mellitus patients attending Diabetes clinic at a tertiary health institution in Osogbo, Nigeria were studied and divided into 2 groups of 60 diabetics without vascular complications (DM-VC, 25males and 35 females) and another 60 diabetics with vascular

complications (DM+VC, 20 males and 40 females). Apparently healthy 50 volunteers were recruited among the staff of Osun State University to serves as normal controls (NC, 20 males and 30 females). Exclusion criteria included subjects who had a diagnosis of type 1 diabetes, or any other disease other than type 2 diabetes, abnormal liver functions, chronic inflammatory diseases, acute infections, hematologic disorders, acutely ill patients, malignancy, and pregnant women.

Each patient was subjected to full physical examination aside their clinical history including their demographic data and major traditional diabetes factors tracing (age, sex, hypertension, dyslipidemia, family history of diabetes), educational and treatment history were recorded using a structured questionnaire. Participants were also weighed with a weighing scale to the nearest 0.5kg in light clothing without shoes and their heights measured using a stadiometer to the nearest 0.5cm. Body mass index (BMI) was calculated by the Quetelet's index, as weight (kg)/height2 (m2). The mean of two blood pressure (BP) readings, measured on the left arm after participants had rested for 5 min on a sitting position was recorded using mercury sphygmomanometers (Accoson). Hypertension was defined as systolic blood pressure ⩾ 140 mmHg or diastolic blood pressure ⩾ 90 mmHg or self reported use of antihypertensive medication [20]. Dyslipidemia, was defined according to NCEP expert panel report [21], type 2 diabetes diagnosis was based on the World Health Organization criteria [22], and vascular disease was defined using standard clinical protocol as complications (retinopathy, diabetic foot, neuropathy, nephropathy or cardiovascular disease) which resulted after development of type 2 diabetes in an individual.

2.2 Blood Sampling and biochemical analysis

After overnight fasting of 10 hours blood samples were collected from patients and control subjects into both heparinized vacutainer and a fluoride oxalate bottle for biochemical analysis and centrifuged at 3000 rpm for 15 min, aliquots of the plasma were stored at -80℃ until analysis though, glycated haemoglobin (HbA1c) and haptoglobin (Hp) gene polymorphism was done sane day. All spectrophotometric assays were done on JenwayR. UV- 6302 spectrophotometer, while chemicals for the analysis were sourced from Sigma-Aldrich, UK.

Haptoglobin polymorphism: Haptoglobin gene polymorphism was demonstrated using polyacrylamide gel electrophoresis-PAGE as previously described [23]. Briefly, 6 μ L of plasma sample was incubated with 1 μ L of standard erythrocyte haemolysate of washed human red blood cells. 6 μ l of loading buffer was then added to the mixture and 8 μ L from the resultant mixture were loaded onto the gel for electrophoresis, after migration, proteins were fixed using 10% trichloroacetic acid solution and stained with benzidine solution. The bands were then observed for Hp phenotype fractions.

Glyceamic controls: Glycated haemoglobin (HbA1c) in whole blood and plasma glucose (FPG) were estimated using ion exchange resin [Spectrum Diagnostics, Egypt, (Ref: 254 003)] and enzymatic glucose oxidase hydrolysis [Randox, UK, (Cat No: GL364)] kits respectively.

Oxidative stress parameters: Plasma total antioxidant concentration (pTAC) was measured using the modified method of Benzie and Strain [24], as reported by Kaushik et al [25], the method is based on the ability of plasma antioxidants to reduces Fe3+-TPTZ to Fe2+-TPTZ.

Erythrocyte Glucose-6-Phosphate dehydrogenase (eG6PD) activity was determined using a commercially available kit [Randox, UK, (Cat No: PD 410)], where eG6PD reduces NADP to NADPH and the rate of reduction of NADP+ was measured at 340nm.

Erythrocyte glutathione (eGSH) level was determined using modified method of Chakrabarty et al [26], where glutathione in the protein-free red cell lysates react with DTNB solution to form a colour complex measured at 412nm.

Plasma malondialdehyde (MDA) level was measured spectrophotometrically at 523nm using the method of Song et al [27], where MDA in protein free plasma precipitate form a pink colour chromogen with thiobarbituric acid (TBA).

Erythrocyte superoxide dismutase (eSOD) activity was however determined using the protocols of Engwa et al [28], based on the ability of SOD to inhibit pyrogallol autoxidation.

2.3 Statistical analysis

Qualitative and quantitative data collected from diabetics with or without complications, and control subjects were presented as frequencies/percentages and the mean ± SD (standard deviation) respectively. The distributions of Hp phenotypes among the groups were represented using bar graphs. Comparison of quantitative normally distributed data between the polymorphism groups were analyzed using student 't' and one way analysis of Variance (ANOVA) tests, p-value was considered statistically significant when it is less than 0.05. GraphPad 8.0 statistical software was used for the analysis.

3. Results

3.1 Population Characteristics

This study comprised 120 type 2 diabetes mellitus patients and 50 apparently healthy individuals. 60 of the diabetics had various documented vascular complications [Diabetes eye complications of retinopathy and cataract in 31 (25.83%) and 8 (6.67%) patients respectively, 14 (11.67%) had diabetes neuropathy, nephropathy was present in 2 (1.67%) individuals while, diabetes coronary heart disease and diabetic foot were indicated in 3 (2.50%) and 2 (1.67%) patients respectively] while the remaining 60 diabetics have no vascular complication (Figure 1).

Table 1, shows a not significantly ($p>0.05$) reduced number of diabetics with vascular complications having less number of subjects with family history of diabetes and also a not significantly ($p>0.05$) higher number of individuals suffering from hypertension, and dyslipidaemia than diabetics without vascular complications. BMI data and duration of diabetes among type 2 diabetics with vascular complications were significantly higher ($p<0.05$) than those without vascular complications. There was no significant difference in terms of mean age of the study groups. Diabetes groups showed significantly higher systolic and diastolic blood pressures of both SBP and DBP ($p<0.001$) compared to controls. Diabetics with vascular complications have significantly ($p<0.05$) and not significantly ($p>0.05$) higher DBP and SBP values respectively when compared to those without vascular complications.

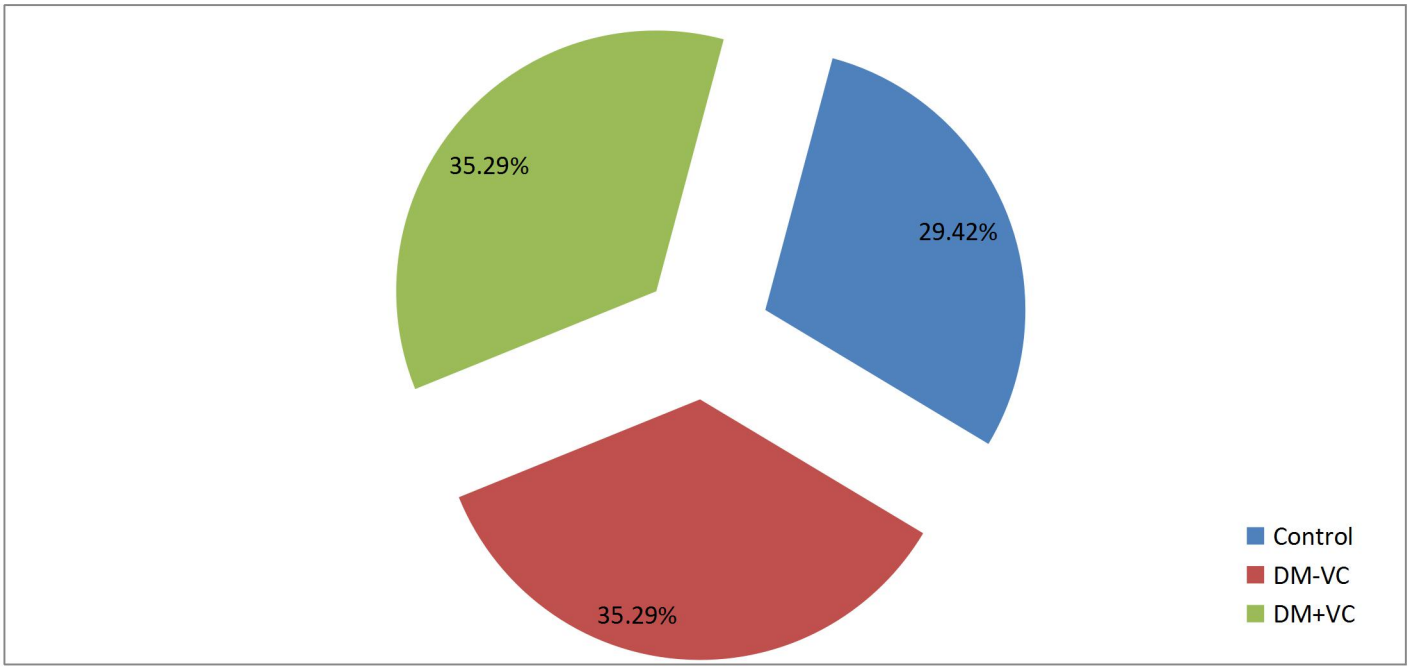

Figure 1: Distribution of subjects in the study population

Table 1: Risk factors, demographic and laboratory data of the study population

Parameters	Control n = 50	DM-VC n = 60	DM+VC n = 60
Sex (male/female)	$20/30_a$	$25/35_a$	$20/40_a$
Diabetes duration (yrs)	NA	$4.43\pm2.55_a$	$6.67\pm2.84_b$
BMI (kg/m^2)	$20.91\pm0.85_a$	$24.86\pm3.43_b$	$26.56\pm4.61_c$
Family history of DM	NA	$35\ (58.33)_a$	$32\ (53.33)_a$
Hypertension [n (%)]	NA	$4\ (6.67)_a$	$9\ (15)_a$
Dyslipidemia [n (%)]	NA	$8\ (13.3)_a$	$12\ (20)_a$
Age (years)	$54.16\pm9.61_a$	$53.97\pm9.71_a$	$53.35\pm8.41_a$
SBP (mm/Hg)	$117.00\pm13.48_a$	$130.65\pm8.71_b$	$130.58\pm8.38_b$
DBP (mm/Hg)	$83.70\pm4.77_a$	$87.73\pm5.16_b$	$90.13\pm5.05_c$
Haptoglobin Polymorphism			
1-1 [n (%)]	$23\ (46)_a$	$8\ (13.33)_b$	$3\ (5)_b$
2-1 [n (%)]	$21\ (42)_a$	$33\ (55)_a$	$24\ (40)_a$
2-2 [n (%)]	$6\ (12)_a$	$19\ (31.76)_b$	$33\ (55)_c$

Result expressed as mean ± SD, n= number of subjects per group, (%) = percentage of subjects per group

BMI = body mass index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure
NA = Not applicable
$p< 0.05$ is statistically significant (values with different subscripts a, b, c along a row are significantly different from each other)

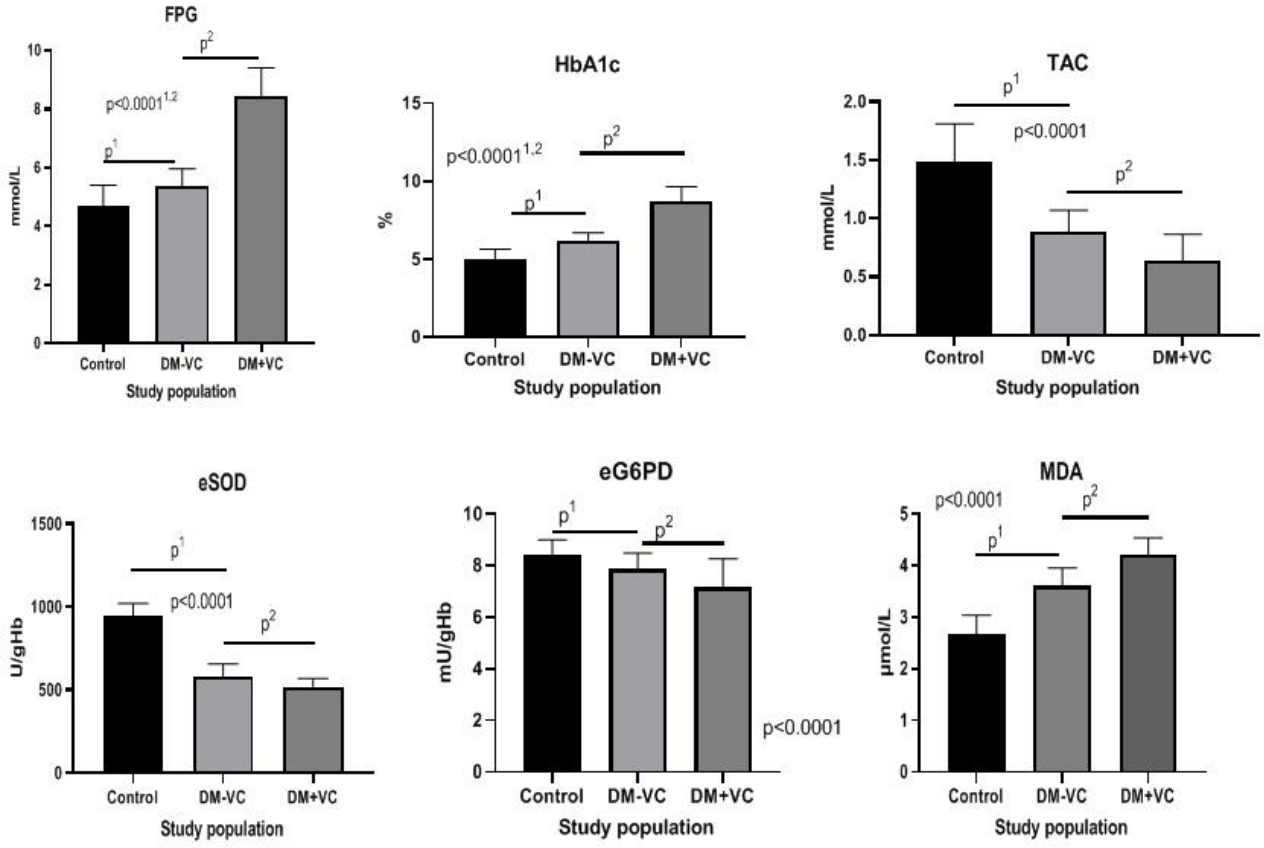

Figure 2: Glyceamic and antioxidants parameters in different study population

Table 2: Measured parameters in different haptoglobin phenotypes of type 2 diabetics

Parameters	Total patients (n = 120)	Haptoglobin phenotypes		
		Hp 1-1 (n = 11)	Hp 2-1 (n = 57)	Hp 2-2 (n = 52)
Age (years)	53.38±9.38	$52.36±10.16_a$	$53.40±9.66_a$	$54.39±8.20_a$
BMI (kg/m^2)	25.71±4.13	$23.56±3.82_a$	$24.91±3.16_a$	$27.11±4.76_b$
Diabetes duration (yrs)	4.55 ± 2.91	$3.59±1.66_a$	$4.93±2.70_b$	$6.67±2.98_c$
Hypertension n (%)	13(10.83)	$2(18.18)_a$	$3(5.27)_a$	$8(15.38)_b$
Dyslipidaemia n (%)	20(33.33)	$3(15)_a$	$4(20)_a$	$13(65)_b$
FPG (mmol/L)	6.92±1.74	$5.90±1.21_a$	$6.63±1.71_a$	$7.45±1.72_b$
HbA1c (%)	7.43±1.49	$6.26±1.08_a$	$7.14±1.44_b$	$7.92±1.48_c$
pTAC (mmol/L)	0.76±0.24	$1.03±0.13_a$	$0.78±0.24_b$	$0.68±0.21_c$
eSOD (u/gHb)	551.09±69.33	$614.27±70.64_a$	$562.12±68.70_b$	$525.63±59.35_c$
eGSH (mg/gHb)	64.67±3.54	$68.20±2.86_a$	$66.43±3.40_a$	$64.76±3.50_b$
MDA (µmol/L)	3.92±0.44	$3.63±0.35_a$	$3.81±0.47_a$	$4.09±0.36_b$
eG-6-PD (mU/gHb)	6.18±1.85	$7.91±0.32_a$	$7.96±0.54_a$	$8.08±0.73_a$

Result expressed as mean ± SD, n= number of subjects per group and (%) = percentage of subjects per group
BMI = body mass index eSOD = erythrocyte superoxide dismutase
FPG = Fasting plasma glucose HbA1c = Glycated haemoglobin
SBP = Systolic blood pressure TAC = Total antioxidant concentration
DBP = Diastolic blood pressure eGSH = erythrocyte glutathione
eG-6-PDH: erythrocyte Glucose-6-phosphate dehydrogenase
$p< 0.05$ is statistically significant (values with different subscripts a, b, c along a row are significantly different from each other)

3.2 Relationship between haptoglobin phenotypes, risk factors and measured parameters in type 2 diabetics

From figure 2 FPG, HbA1c and eG-6-PD were significantly higher in diabetics with vascular complications compared to diabetics without vascular complications ($p<0.001$) and control subjects ($p<0.001$). On the other hand, TAC, eSOD and eGSH levels were significantly decreased in diabetics with vascular complications compared to controls (<0.001) and diabetics without vascular complications ($p<0.001$). However, the number of subjects with Hp 1-1 polymorphism was significantly higher in control subjects as compared to diabetics with ($p<0.001$) and without vascular complications ($p<0.001$). Likewise, Hp 2-2 polymorph was significantly higher among type 2 diabetics with vascular complications than those without vascular complications ($p<0.001$) and control subjects ($p<0.05$) (Table 2).

4. Discussion

Despite significant advances in preventive efforts and medical care geared towards diabetes mellitus treatment / management, vascular disease (either micro or macro) continues to disproportionately affect individuals living with this disease thus, signalling genetic predisposition as a key factor in the development of vascular complications [29]. This study was an extension of our previous study [23] which provides data on Hp phenotypes and type 2 diabetes mellitus in Nigerians with a view of determining if the glyceamic control indices and oxidative stress parameters in type 2 diabetics were Hp polymorphism/phenotype driven.

The glyceamic control (FPG and HbA1c) parameters measured in this study were significantly high in Hp2-2 diabetics than Hp1-1 individuals. Another finding from this study was that Hp1-1 has good antioxidant properties than Hp2-1 and Hp2-2 in decreasing order. Haptoglobin is likely to be exerting its oxidative protective role via the differences in the phenotypic molecular size and shape of the protein products encoded by the two different Hp alleles. Smaller Hp complexes are believed to quickly mobilise into the extracellular space and undergo glomerular sieving better than the larger Hp [13].

The mechanism of Hp1-1 exhibiting better good glyceamic and oxidative properties than other Hp polymorphs is not fully known but can be partially explained on the basis that hyperglycaemia (which is more pronounced in Hp2-2) caused increased body oxidation leading to generation of more reactive oxygen species (ROS) thereby causing depletion of total body antioxidant system. The generated ROS however, initiate the steps involved in pro-inflamatory vascular damage and consequential increase in tissue/vascular damage within the body system among diabetics.

In order words, Hp 2-2 polymorphism role in development of vascular complications in diabetes can be attributed to inefficient scavenging mechanism exhibited by the Hp 2-2 Hb complex which binds the free haemoglobin through Fenton reaction at a lesser extent (to Hp 1-1 Hb complex through CD163) with less clearance by the haemoglobin scavenger receptor [15,16] thereby promoting LDL damage within the blood vessels (a characteristics of early stage endothelia dysfunction in type 2 diabetes) [30].

5. Conclusion

This study, to our knowledge, was among the first to attempt the association between haptoglobin phenotypes and antioxidant parameters among diabetics in Nigeria population thereby advocating Hp phenotyping among diabetics. Thus, we suggest further study with large data base as to further elucidate our findings. Summarily, screening of type 2 diabetes for Hp phenotypes would be an essential therapeutic monitoring tool in patient focus treatment as Hp 2 allele and Hp 2-2 polymorphism is a marker to development of type 2 diabetes and vascular complications.

Conflicts of Interest

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors report no conflict of interest. The data from this research is available to be shared upon request.

Authors Contributions

Conception or design: Olaniyan OO

Acquisition, analysis, or interpretation of data: Olaniyan OO, Odewusi OO, Osadolor HB

Drafting the work or revising: Olaniyan OO, Odewusi OO

Critical revision of the manuscript for important intellectual content: Olaniyan OO, Osadolor, HB

Final approval of the manuscript: Olaniyan OO, Odewusi OO, Osadolor HB

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