American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Haptoglobin Phenotypes and Association with Vascular Complications in Nigerian Type 2 Diabetes Patients

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Received: 01 July 2018; | Revised:23 July 2018; | Accepted: 10 September 2018

Abstract

Haptoglobin phenotypes were determined in 120 type 2 diabetes mellitus (including 60 diabetics with vascular complications and 60 diabetics without any vascular complications) and were compared to 50 apparently healthy individuals. A higher frequency of Hp 2 allele gene and Hp 2-2 phenotype was observed in diabetics and diabetics with vascular complications respectively. We found an association between Hp 2 allele in type 2 diabetes mellitus and Hp 2-2 phenotypes in diabetics with vascular complications. Hence, we conclude that screening diabetics for Hp 2-2 phenotypes would be an added advantage in patient focus management.

Keywords: Haptoglobin phenotypes, Type 2 Diabetes, Vascular complications, Nigerian

1. Introduction

Haptoglobin (Hp) an α2-sialoglycoprotein [1] that is synthesized majorly by the hepatic and also by kidney, skin and lung tissues [2], is a positive acute phase reactant haemoglobin binding protein that binds free oxygenated haemoglobin produced in response to infection or inflammation through induction by interleukins (IL-6, IL-1) and tumour necrosis factor-α (TNF-α) [3]. In man Hp is expressed by a genetic polymorphism of the α-chain to form three major phenotypes commonly referred to as Hp1-1, Hp2-1, and Hp2-2 [4]. Hp 2-1M phenotype is more frequent in African population [5] and has been shown to have greater number of Hp1 bands [6] generated by the polymorphism of a single nucleotide in the promoter region of the Hp2 gene [4].

The occurrence of these polymorphism at the haptoglobin locus has make it a potential genetic

marker and motivated many investigations directed at the determination of possible associations between haptoglobin (Hp) and different disorders [6]. The significance of this polymorphism in diabetes mellitus has also been investigated. Several prospective and cross-sectional population studies have demonstrated an association of the Hp 2 gene and Hp 2-2 phenotype as an independent risk factor for diabetes and development of vascular disease in diabetes mellitus [7-10]. Therefore, the aim of this present study was to investigate the association between Hp gene phenotype and incidence of type 2 diabetes mellitus in a Nigerian population.

2. Materials and Methods

Blood samples (plasma) were obtained from 120 adult patients with type 2 diabetes mellitus at a tertiary health facility in Osogbo, Osun State, and Southwest Nigeria. Diabetes patients were diagnosed according to WHO criteria [11] and classified into two main groups of those without vascular complications (DM–VC, n =60), and those with vascular complications (DM+VC, n=60) based on the presence or absence of any complications due to diabetes mellitus. The control group (CG) comprises 50 apparently healthy non-diabetes individuals with no personal or family history of either diabetes or dyslipidemia selected from among the Osun State University staff. Neither DM patients nor healthy controls were smokers and were not receiving vitamin or mineral supplements for the past 1 month. Control groups were not taking any drugs known to affect carbohydrate or lipid metabolism. All of the subjects enrolled in the present study were of Yoruba ethnicity in southwest Nigeria (to avoid mixed population and ancestral differences). Written informed consent was taken from all the participants and all the experiments strictly adhered to the tenets of the Helsinki declaration. The study protocol was approved by the Ethical Committee of the Osun State University.

Hp phenotypes were determined by acidic urea polyacrylamide gel electrophoresis (PAGE) as previously described [12-13], in brief, 6µl of plasma samples were incubated with 1µl of erythrocyte haemolysate of washed human red blood cells. 6µl of loading buffer was then added to the mixture and 8µl 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.

Hardy-Weinberg equilibrium (HWE) was used to calculate Hp phenotypic distribution and allelic frequencies. Groups were compared with each other using chi square and odd ratio of Vassar Stats (http://vassarstats.net/index.html) online software (assessed on 2nd of June, 2018).

3. Results

Table 1 demonstrates that the frequency of the haptoglobin phenotype and allele distribution while table 2 shows the odd ratio existing in the study population respectively. Hp 1-1 in type 2 diabetics which was 9.17% was significantly lower ($p<0.0001$) than the control subjects 46.00%, while, the frequency of Hp 2-2 in type 2 diabetics (42.33%) was significantly higher ($p<0.0001$) than in control subjects (12.00%). Likewise, Hp 2-2 frequency of 55% in diabetics with vascular complications was significantly greater ($p<0.001$) than in diabetics without vascular complications (31.67%). On the other hand, there was no significant difference ($p>0.05$) between in the frequency of Hp 2-1 in diabetics (42.333%) and control subjects (42.00), and also between diabetics with vascular complications (55.00%) and diabetics without vascular complications (31.67%). respectively.

Table 1:Haptoglobin phenotype distribution and allele frequency among the study Population

	Haptoglobin phenotypes distribution			Hp Allele frequency	
Groups	**Hp 1-1**	**Hp 2-1**	**Hp 2-2**	**Hp1**	**Hp 2**
Control group	23 (46.00%)	21(42.00%)	6 (12.00%)	0.654	0.346
Type 2 Diabetes	11 (9.17%)	57 (47.50%)	52 (42.33%)	0.349	0.651
DM – VC	8 (13.33%)	33 (55.00%)	19 (31.67%)	0.437	0.563
DM + VC	3 (5.00%)	24 (40.00%)	33 (55.00%)	0.258	0.742

Result presented as no (%)
no = number of subjects
% = percentage of subjects
DM-VC = diabetics without vascular complications
DM+VC = diabetics with vascular complications
Hp = Haptoglobin

Table 2:Odd ratio table for developing type 2 diabetes and vascular complications in type 2 diabetes

	Haptoglobin phenotypes		
Groups	**Hp 1-1**	**Hp 2-1**	**Hp 2-2**
Type 2 diabetics/non diabetes			
ODD Ratio (95% CI)	0.12 (0.52-.27)	1.25 (0.64-1.48)	5.61 (2.22-14.16)
p-value	<0.00	0.51	<0.00
DM–VC/non diabetes			
ODD Ratio (95% CI)	0.18 (0.07-0.46)	1.69 (0.79-3.60)	3.40 (1.24-9.35)
p-value	0.00	0.17	0.01
DM+VC/non diabetes			
ODD Ratio (95% CI)	0.06 (0.02-0.22)	0.92 (0.43-1.97)	8.96 (3.32-24.20)
P-value	<0.00	0.82	<0.00
DM–VC/DM+VC			
ODD Ratio (95% CI)	0.34 (0.09-1.36)	0.55 (0.26-1.13)	2.66 (1.25-5.55)
p-value	0.11	0.10	0.01

Values presented in 2 decimal places
Hp = Haptoglobin
DM+VC = diabetics with vascular complications
DM-VC = diabetics without vascular complications

4. Discussion and Conclusion

The key to reducing the burden (morbidity and mortality) of diabetes mellitus disease is early detection and timely therapeutic interventions. Nigeria is known to have the highest number of individuals living with diabetes mellitus in sub-Saharan Africa [14], and despite, reporting contrast opinions linking haptoglobin polymorphism with DM by various studies worldwide, Nigeria data on haptoglobin and diabetes mellitus still remain scanty.

Haptoglobin, an acute phase protein acting as an antioxidant by virtue of its ability to bind free haemoglobin and prevent hae.me-iron mediated oxidation [15] is found to be phenotypically associated with type 2 diabetes from our study. The result of this study showed that Hp 2 allele is a dependent factor in type 2 DM in Nigerians therefore, suggesting a strong association between the haptoglobin allele and particularly Hp 2-2 phenotypes in diabetics with vascular complications. This study therefore agrees with Quaye et al [16] , Adinortey, et al., [17] and Amiri et al., [18] who demonstrated association between diabetes mellitus and Hp 2-2 phenotype in their study populations where as, the result is in contrast to the report of Awadallah and Hamad [19] who reported no association between haptoglobin and diabetes in the Jordanian population.

Free haemoglobin (produced during Fenton reaction) increases the rate of oxidized LDL which can cause endothelia dysfunction and consequently vascular complications in diabetes [20]. It has also been proven that CD163/Hp1-1-Hb complex scavenge free haemoglobin efficiently and effectively than CD163/Hp 2-2Hb, with the haemoglobin binding capacity and antioxidant function of Hp 1 more than that of Hp 2 [16]. Thus, linking the role of haptoglobin phenotypes in diabetes risk directly to its function as an antioxidant would not be out of place.

None of the subjects from our study population was of Hp 2-1M phenotypes which was claimed to be common haptoglobin polymorphism in Africa [5,12] though, the reason for non-occurrence of this phenotype is unknown, but might not be unconnected to our study population which are homogenous in nature (only one ethnic group), and the potential limitation posed by the small sample size which may confer bias on the phenotype distribution and allele frequency of haptoglobin. Thus, we advocate further study with large sample size among different Nigerian ethnic groups.

Summarily from our study, we conclude that Hp 2 allele is a risk factor for type 2 diabetes and that Hp 2-2 phenotype predisposes to vascular compilations in Nigeria type 2 diabetics. Thus, screening of type 2 diabetics for Hp phenotypes could be an essential therapeutic assessment tool in patient focus treatment.

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