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Association of Haptoglobin Phenotype and Lipid peroxidation among Type 2 Diabetic patients

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ABSTRACT

Type 2 diabetes is associated with increased oxidative stress, which causes a variety of damages of several molecules. Malondialdehyde, the marker of lipid peroxidation is increased among diabetic. Haptoglobin, the polymorphic protein has strong association with Diabetes. The Present study did not show any significant association between the Haptoglobin Polymorphisms and Lipid peroxidation marker in diabetic patients.

Key words: Haptoglobin Phenotype, Diabetes, Lipid peroxidation, Oxidative stress, malondialdehyde.

INTRODUCTION

Diabetes is associated with high risk of dyslipidemia, which is responsible for micro and macro vascular complications of disease [1]. Hyperglycemia, characteristic feature of diabetes, induces oxidative stress either by the direct generation of reactive oxygen species (ROS) or by altering the redox balance [2]. Moreover, increased serum levels of the products of oxidative damage to lipids, proteins and DNA have been detected in diabetics, and their presence is correlated with the development of diabetes-associated complications [3]. Malondialdehyde (MDA) is regarded as one of the most stable products of lipid peroxidation [4].

Haptoglobin (Hp) is a polymorphic protein which exists in three major phenotypes: Hp1-1, 2-1, and 2-2. These Hp phenotypes exhibit distinct structural and functional properties with significant biological and clinical implications [5, 6]. Hp binds with free hemoglobin (Hb) in circulation forming Hb–Hp complex that prevents the loss of iron and iron-driven oxidative damage. Hp 1-1 protein may have superior antioxidant protection than Hp 2-2 protein. [7]. Many reports have established a strong association between Hp phenotypes and diabetes and its complications [5]. Furthermore, diabetic patients as well as transgenic mice with the Hp 2-2 genotype showed increased oxidative stress markers and are at increased risk for cardiovascular disease [8, 9]. Recently, it is reported that type 2 diabetics with Hp 2-2 phenotype may benefit from vitamin E supplementation [10, 11]. Therefore, identifying patients with this genotype is more likely to benefit them by allowing an antioxidant treatment specific to their disease genotype. The present study is one the first study to find out association between Hp polymorphisms and Lipid peroxidation among Saudi Diabetic population.

Study Design:
This case- control study was conducted in the research laboratories, Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University.

Ethical approval was obtained as per the rules of the Deanship of Research of Qassim University. Informed consent was obtained from each participant.

The study was funded by the directorate of Scientific Research of Qassim University.

Study population:
80 Saudi type 2 diabetics were recruited From Diabetes clinic, King Fahad Specialist Hospital, Buraidah, Al-Qassim Region, Kingdom of Saudi Arabia. While healthy "non-diabetic subjects" (n= 60) recruited from public places, i.e. Estarahes (party lounges).

Inclusion criteria for diabetic subjects included: Saudi male/female citizen; age between 15 to 60 years old; diagnosed with type 2 diabetes, the usage of anti-diabetic agents, or both.

Inclusion criteria for healthy (non-diabetic) control subjects included: Saudi male/female citizen; age between 15 to 60 years old; neither had been diagnosed by a physician as having diabetes nor use hypoglycaemic medication; not suffering from any chronic disease such as rheumatic diseases or any history of known Disease.

Confidentiality of all subjects was maintained as no names were recorded

Measurement of BMI:
Body weight and height were recorded for each subject. Weight was measured using calibrated electronic weighing scales (Proton Digital Scale, Model PHC 309 MD) and height was measured using a Portable Height Scale (Mentone Educational, Model PE087, Australia). BMI was calculated as weight (in kilograms) divided by height (in meters) squared.

The WHO classification for BMI was used to estimate the degree of obesity. Subjects were categorized as normal if BMI was less than 25 kg/m^2, overweight if BMI was between 25-29.9 kg/m^2, and obese if BMI was greater than or equal to 30 kg/m^2.

Laboratory investigation:
Subject preparation:
No special preparation or instruction to the subjects as not required by any laboratory tests.

Sample collection & Transportation:
Blood samples were collected as per the CLSI document (H3-A6—Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition).Samples collected in one heparanised vacutainer (4 ml) and one plain vacutainer (4 ml) for each subjects at King Fahad specialist Hospital, Buraidah. Healthy "non diabetic subjects" (n= 60) recruited from public places i.e. Estarahes (party lounges). Samples were transported to Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University in a temperature controlled container.

Laboratory analysis:
Hp phenotyping:
Hp-Hb complex solution was prepared by adding 2 -3µL Hb solution to 10µL Plasma and mixing for 5 minutes at room temperature. Followed by addition of 10 µL sample buffer (50% v/v glycerol and 0.001 w/v bromophenol blue) to each sample prior to running on the gel. Native polyacrylamide gel electrophoresis (native-PAGE) was performed according to the Laemmli's method. The electrophoresis was performed using a protein vertical mini-gel electrophoresis system (Bio-Rad Mini protean III apparatus; USA) with a thickness of 0.75 mm. Total polyacrylamide concentrations of 7.0% and 4% were used respectively for separation and stacking gels of native-PAGE. On completion of electrophoresis, after 2 hours under 140 V constant voltage condition, the gels were stained with peroxidase stain to visualize the different bands; Hp phenotypes were evaluated and documented by Photography .The Hp phenotype was determined from the relative migration position of slow and fast migrating Hp–Hb bands .

Measurement of Random Blood Glucose:
Glucose level was measured on the plasma samples using Human Diagnostics GOD/PAP reagents by Hospitex Eos Bravo Plus clinical chemistry analyzer

Measurement of MDA:
Plasma malondialdehyde (MDA) levels were determined spectrophotometrically using a NWLSSTM MDA Assay kit (NWLSS, WA, U.S.A.). The MDA concentration was calculated by third derivative analysis for the reaction mixture using the Math Application supplied in Cary Win UV 50 conc, Varian. Briefly, the NWK-MDA assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.Butylated hydroxytoluene (BHT) and EDTA are added to the sample and reaction mixture to minimize oxidation of lipids that contribute artifactually during sample processing and the TBA reaction.

Statistical analysis:
The data collected and analyzed using the statistical package for social sciences (SPSS) software (version 13). Results expressed as mean ±SD. Comparison of variables between two groups performed with student t-test for continuous. The p values ≤ 0.05 were considered significant.

RESULTS

The experiments were performed on a total of 140 subjects (80 type 2 diabetics & 60 healthy non-diabetic donors). The diabetics were significantly older in age compared to non-diabetic healthy subjects (51.5±10.7 vs. 40.75±7.9 years, p= .000). Although the mean BMI in diabetics was significantly higher than healthy subjects (30.18±5.2 vs. 27.3±3.6 kg/m^2, p=.029), the majority of the participants in both groups were overweight or obese (BMI ≥ 25). Table1.

Table 1. Demographic and clinical characteristics of the study participants; comparing type 2 diabetic patients to healthy donors

Variable	T2DM (n=80)	HD (n=60)	*p*-value
Age (yr)	51.5±10.7	40.75±7.9	.000*
Weight (kg)	77.13±11.3	73.08±12.4	.245
Height (cm)	158.58±14.0	164.83±12.2	.106
BMI (kg/m^2)	30.18±5.2	27.3±3.6	.029*
Blood Glucose (mmol/l)	12.9±5.7	6.01±1.8	.000*
Duration of T2DM (yr)	8.54±6.3	-----	---

Abbreviations: BMI, body mass index; HbA1c, glycosylated haemoglobin; T2DM, type 2 diabetes; HD, healthy donors
*Data presented as mean ±SD for all variables; * p-value <0.05*

This study data documented significant increase (p=.005) in concentration of MDA in type 2 diabetic patients compared to healthy non-diabetic subjects. In spite of this significant increase on MDA in diabetics, this study couldn`t find significant association of MDA and Hp phenotypes in diabetics. However, there was an apparent increase in MDA concentration based on Hp phenotype, i.e., Hp2-2>Hp2-1>Hp1-1. Table 2.

Table-2. Levels of MDA (expressed as mean ± SD) in type 2 diabetic patients compared to healthy donors

Hp phenotype	MDA (µmol/l) DM	P value	MDA (µmol/l) HD	P value
All subjects	2.871± .47		2.626± .49	.005[a]*
Hp 1-1	2.600 ± .49		2.620±. 52	
Hp 2-1	2.806 ± .39	.164[b]	2.585±.45	.819[c]
Hp 2-2	2.922 ± .44	.145[d]	2.927±.51	.237[e]

P ≤ 0.05 i.e., significantly different.*
a: p- value compared type 2 diabetic patients to healthy non-diabetic subjects; b: p- value compared diabetics with Hp 2-1 to diabetics with Hp 1-1; d: p- value compared diabetics with Hp 2-2 to diabetics with Hp 1-1; c: p- value compared healthy subjects with Hp 2-1 to healthy subjects with Hp 1-1; e: p- value compared healthy subjects with Hp 2-2 to healthy donors with Hp 1-1

DISCUSSION

T2DM is a common multi-factorial genetic syndrome, which is determined by several different genes and environmental factors. There is considerable evidence that hyperglycemia causes the generation of ROS, which ultimately leading to increase oxidative stress in a variety of tissues [12, 13]. In consequence, may suffer from an increased risk of oxidative stress-related diseases, including atherosclerosis, which is the most significant complication in diabetes and the most common reason for premature death [14,15]. Moreover, oxidative stress leads to protein, lipid, and DNA modifications that cause cellular dysfunction and contribute to the pathogenesis of macro- and micro-vascular complications of diabetes, including diabetic nephropathy, Retinopathy etc [16, 17, 18,19,20,21]. Oxidation of polyunsaturated fatty acids by redox-active metals (e.g., iron) and reactive oxygen species is considered to be the initiating step in the modification of LDL [22]. Pro-oxidative forms of iron (Fe^{2+}) and haem, derived from haemoglobin (Hb), are able to generate aggressive hydroxyl radicals in the presence of H_2O_2 (Fenton reaction) which can initiate lipid peroxidation by hydrogen abstraction from the fatty acids [22,23]. This causes the formation of reactive lipid decomposition products such as MDA. In this study higher level of MDA was denoted in diabetic patients than in the control group. The similar finding was reported by Dierckx *et al* [24] and Hanchi et al [25]. The Hp polymorphism has also been proposed as one of the candidate genes in the multigenic model of atherogenesis [26]. Men with Hp 2-2 phenotype are more at risk for developing premature coronary artery disease [27], peripheral vascular disease [28], and vascular diabetic complications [29]. In the present study, MDA concentrations were not different between Hp phenotypes, which similar to the study conducted by Annelies Brouwers et al [30].

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