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Polymorphism in Coronary artery disease in Indian Punjabi Population

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Abstract

Introduction: Glutathione S- transferase is an antioxidant enzyme which helps in regulating oxidative stress, and variation in GSTM1 genotype is associated with coronary artery disease (CAD)**Aim**: The present study was aimed to study the role of GSTM1 polymorphism in coronary artery disease in Indian Punjabi Population.**Material and Methods**: In this study we recruited 100 CAD patients and genotyped for GSTM1 null and GSTM1 active gene by taking albumin as internal control. 80 healthy individuals were taken as controls. All these subjects were studied for their Fasting Lipid profile, Lipid peroxidation levels (MDA), Glutathione S – transferase (GST), Reduced Glutathione (GSH) and Serum Total antioxidant capacity (TAC). Data was analyzed by adopting student's unpaired t- test. A p-value less than 0.05 were considered statistically significant.**Results**: It has been observed that 62 out of 100 CAD patients (62%) had GSTM1 null genotype and 38 out of 100 CAD patients (38%) had GSTM1 active genotype. Serum Triglyceride levels (p=0.01) and VLDL Cholesterol levels (p=0.05) were significantly raised in GSTM1null as compared to GSTM1 active CAD patients. Serum MDA levels(p=0.004) and GST(p=0.005) activity was found to be significantly raised in CAD patients with GSTM1 null genotype as compared to GSTM1 active genotype , where as Total antioxidant capacity was found to be low (p=0.02) in GSTM1 null genotype CAD patients. Difference in plasma GSH levels in CAD patients with null and active GSTM1 genotype was insignificant (p=0.10).**Conclusion**: Genetic absence of GSTM1 is form of the enzyme GST increases the susceptibility to coronary artery disease.

Key words: 1.Coronary artery disease, 2.Glutathione S -transferase, 3.GSTM1 genotype, 4.Polymorphism.

Introduction

Coronary artery disease is a vascular disease and a major cause of concern worldwide. The external factors such as exposure to pollution or passive smoking may serve as a strong trigger to stimulate the mechanisms at the genetic level which are involved in the pathogenesis of coronary artery disease. Among various risk factors, oxidative stress is one of the most important factors in which amount of reactive oxygen species increases as compared to antioxidants available in the body. Hence alteration in endogenous ROS scavenging system leads to oxidative damage (1). In order to reduce oxidative stress body has its own detoxifying system. One of the enzymes involved in Phase II detoxification mechanism is Glutathione S-transferase (GST). Detoxification role played by GST involves conjugation of reduced glutathione with exogenous or endogenous substrates and to detoxify them and facilitate their excretion (2).GSTs are present in cytosol, mitochondria and microsomes. Cytosolic GST are further divided in to several major classes such as Alpha(α), Zeta(ζ), Theta(θ) ,Mu(μ), Pi(π), Sigma(σ) and Omega (ω) (3). GSTM1 (μ) is localized on chromosome 1 in region 1p13.3 . Null polymorphism in this gene is due to a partial deletion of the gene (4) which affects the activity of GST. GSTM1 null polymorphism is associated with various diseases such as CAD (5), chronic kidney disease (6), Diabetes mellitus (7) and some cancer (8). The frequency of GSTM1

homozygous null genotype has been reported to be 66% in Malaysian population (9), 30.4% in south Indians (10) and 41% in Kashmiri population respectively (11). Presence of null alleles affects the enzyme activity and therefore the development of CAD. Few studies regarding the role of Glutathione S-transferase M1 polymorphism in CAD are available in the Indian population . Bhatt et al 2016 (12) reported a positive association of GSTM1 null genotype with increase risk of coronary artery disease in north Indian Punjabi population where as Ritambara et al 2017 (13) showed that GSTM1 null genotype plays an important role in hypertension in north Indian population from Allahabad. Therefore the association of GSTM1 polymorphism with the development of coronary artery disease is worth studying. Hence, the present study was aimed to investigate the relationship of GSTM1 polymorphism in relation to oxidative stress in CAD patients belonging to Indian Punjabi Population .

Material and Methods

Study Subjects (CAD patients and Healthy Controls)

Present study was case control study carried out in the Department of Biochemistry Government Medical College and Hospital Amritsar in association with Department of Biotechnology Guru Nanak Dev University Amritsar during the period from May 2017 to May 2018s. The study comprised of diagnosed cases of CAD (n=100) . These patients were visiting the Out patients department (OPD) of a Private Hospital and their duration from the onset of cardiac event varied from 3 months to 6 months. The written permission from the lab incharge was obtained to withdraw the blood samples of the CAD patients(Copy attached) .80 healthy individuals free from any present / past history of coronary artery disease or any other diseased condition were recruited from the general population . Controls were also subjected to routine investigations including ECG, to confirm their clinical condition. All the subjects were in the age group of 38-68 years . CAD patients were recruited from medicine department of private hospital in Amritsar. A Performa was designed to record the necessary details of patients and controls regarding age, gender, occupation, present/ past history of any disease , smoking , alcohol status and medication.

Exclusion criteria:-

Patients with following conditions were excluded from the present study: Lung Disease, Liver Disease, Major Renal Complication, Thyroid Disease, Patients on hypolipidemic drugs, Hormone replacement therapy (HRT) having acute infections, chronic alcoholics and smokers.

Venous blood (10ml) sample of CAD patients as well as of healthy control subjects was collected under the aseptic conditions after obtaining written informed consent from all the subjects. The blood sample was divided into two halves one in EDTA vial to obtain plasma and another in plain vial to obtain serum.

Serum Glutathione S-transferase assay was done using colorimetric method (14). Serum glutathione S-transferase activity was based upon the GST-catalyzed reaction between GSH and the GST substrate, 1-chloro-2, 4-dinitrobenzene (CDNB). The formation of dinitrophenyl thioether was directly proportional to the activity of Glutahione S-transferase. The assay was done at 340nm. Plasma GSH levels were assayed in haemolysate using colorimetric method (15) Serum Lipid Profile and Total Antioxidant Capacity (TAC) assayed by commercially available kits from Randox India Pvt Ltd. Briefly, 2, 2 Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) incubated with peroxidase (methmyoglobin) and hydrogen peroxide produced the radical cation $ABTS^{+}$. This has a relatively stable blue-green color, which was measured at 600nm. The suppression of the color production with

the addition of the antioxidant was proportional to the concentration of total antioxidant capacity in the sample. Serum Lipid oxidation product (MDA) was estimated by the method of Beuge and Aust (16).

Genotyping of GSTM1 gene

Genotyping of GSTM1 gene was studied as described by Syed et al 2009 . DNA was extracted from whole fresh blood by adopting the protocol and kit from Genei Pvt Ltd Banglore. PCR was carried out using purified DNA on a Sure Cycler 8800 (Agilent Technologies) .

The conditions for the PCR were Denaturation at 94 degree Celsius for 45s, Annealing at 51 degree Celsius for 30s and elongation at 72 degree Celsius for 30s for 35 cycles. PCR product was run on 2% agrose gel, stained with ethidium bromide and visualized in a gel documentation system. Amplicons corresponding to 218bp, and 350bp confirmed the presence of GSTM1 and Albumin respectively. Albumin was included as internal control to confirm the success of the PCR.

The data were analyzed by Statistical Package for the Social Sciences [SPSS] 16.0 software. Data were expressed as mean±SD standard deviation. The difference between the groups and sub groups were interpreted using independent and unpaired student's t- test. A p-value less than 0.05 was considered statistically significant.

Results

In the present study, CAD patients n=100 and healthy controls n=80 were taken and the average age of patient was 57±13 years and that of control was 46 ±7.6years. Out of 100 CAD patients, 70 were males and 30 were females, in control group 60 males and 20 females were taken . 75% of CAD patients were vegetarian and 25% were non –vegetarian. In control group 70% were vegetarian and 30% were non vegetarian.

Triglycerides (TG), High Density Lipid Cholesterol (HDL-C), Low Density Lipid Cholesterol (LDL-C) Very low density lipid Cholesterol (VLDL-C) Glutathione S-transferase (GST), Total antioxidant capacity (TAC), Reduced Glutathione (GSH), Malondialdehyde (MDA). Statistical test used was unpaired student's t- test.

Represents the variations in lipid profile and parameters of oxidative stress in CAD patients and controls . Serum Triglyceride levels and VLDL Cholesterol levels were found to be significantly raised in CAD patients as compared to controls (p-0.04 and p=0.03) whereas variation in serum HDL Cholesterol(p=0.2) and LDL Cholesterol(p=0.1) were insignificant., GST activity (p=0.01) and MDA levels (p=0.018) were found to be significantly raised in CAD patients where as TAC levels (p=0.025)were low as compare to controls. Variation in plasma GSH levels were found to be insignificant (p=0.2).

Triglycerides (TG), High Density Lipid Cholesterol (HDL-C), Low Density Lipid Cholesterol (LDL-C) Very low density lipid Cholesterol (VLDL-C) Glutathione S-transferase (GST), Total antioxidant capacity (TAC), Reduced Glutathione (GSH),Malondialdehyde (MDA). Statistical test used was unpaired student's t- test.

Variations in Lipid profile and antioxidant status of CAD patients with GSTM1 null and active genotype.GSTM1 null genotype CAD patients were having significantly raised serum Triglycerides and VLDL Cholesterol levels (p=0.01 and p=0.05) respectively as compared to patients having active

genotype . GSTM1 null genotype patients had significantly increased serum GST activity (p=0.005) , MDA levels (p=0.004) and significantly low total antioxidant capacity (p=0.02) as compared patients having GSTM1active genotype, whereas the difference in GSH concentration was insignificant (p=0.10).

Discussion

Oxidative stress due to internal and external factors plays a major role in aggravating the pathogenesis as well as incidence of coronary artery disease (CAD). It has been observed that with each passing day, more and more people are either visiting OPD or are being admitted in the wards of hospital with increased incidence of CAD and other allied conditions. Hence it is very important to focus on parameters which bear an important association with the presence of oxidative stress. Glutathione S- transferase enzyme is an important molecule whose main function is to detoxify the body from harmful free radicals. Any variation in its activity or changes at the molecular level may compromise the ability of an individual to combat the oxidative stress. GSTM1 gene exist in two forms i.e.GSTM1 active genotype and GSTM1 null genotype (inactive) (18) . The relationship between GSTM1 null genotype and risk of CAD is variable in different ethnic population as some studies showed positive association where as other studies reported no association between risk of CAD and GSTM1 null genotype (19,20) . The data regarding the association of GSTM1 null genotype with coronary artery disease in North Indian population is not extensive. Bhatt et al 2016 (12) reported gene polymorphism of both GSTM1 and GSTT1 in relation to CAD in North Indian Punjabi population and reported that GSTT1 null genotype protected against CAD. They observed increased frequency of GSTM1 null genotype in CAD patients as compared to controls thereby showed a positive association of this genotype with increased risk of CAD . Ritambara et al 2017 (13) reported that GSTT1 null genotype had a protective role towards hypertension whereas GSTM1 null genotype may play an independent role in the development of hypertension in North Indian population from the city of Allahabad. Mir et al 2017 (21) reported that GSTM1 null genotype increases the risk of CAD in smokers, diabetics and in hypertension. Lower detoxifying capacity could be due to the predominance of inactive form of GSTM1 i.e. null genotype . Therefore the present study was aimed to study the role of Glutathione S-transferase and its GSTM1 polymorphism in coronary artery disease patients belongining to North Indian Punjabi population. In the present study 62% of the CAD patients were observed to have GSTM1 null genotype, which is little higher as compared to other populations. Bhatti et al 2018 (22) reported increased frequency of GSTM1 null genotype in CAD patients as compared to controls in Asian Indians. They further showed a positive association of GSTM1 null genotype with increased risk of CAD. There are substantial difference in the frequencies of null genotype for GSTM1 in different ethnic groups, 31% in North Indian Punjabi population(12),36% in South African Indians (23),52% in Serbian Population(24). Since majority of CAD patients in the present study were GSTM1 null positive, it may affect the Lipid levels and parameters of oxidative stress. Serum Triglycerides and VLDL Cholesterol was found to be significantly raised in GSTM1 null as compared to GSTM1 active CAD patients. However the difference in total cholesterol , HDL cholesterol and LDL cholesterol between GSTM1 active and null genotype CAD patients was insignificant. Al-Barqaawee and Al-Nahi 2018 (25) reported raised triglyceride levels in Type 2 Diabetic patients having GSTM1 null genotype as compared to those with active GSTM1 genotype. They also observed insignificant difference in serum total cholesterol levels and LDL cholesterol levels in this respect . However the difference in HDL cholesterol was significant. Type 2 Diabetes is well established risk factor for coronary artery disease. In the present study the difference in serum triglycerides and VLDL cholesterol was significant between CAD patients (null and active genotype) as compared to healthy controls.

Glutathione S-transferase plays a key role in the defense mechanism against oxidative stress . It may be possible that polymorphism associated with this gene (GSTM1 under study) could affect lipid levels and parameters of oxidative stress. Significantly increased serum GST activity and MDA levels were observed in GSTM1 null genotype patients as compared to those having GSTM1 active genotype. Levels of total antioxidant capacity was significantly depressed in GSTM1 null genotype patient . However the difference in plasma GSH levels was insignificant between GSTM1 null and active genotype CAD patients. Tang et al 2010 (26) reported lower total antioxidant status and higher CRP levels in CAD patients with GSTM1 null genotype in Chinese population. Bhatti et al 2018 (22) reported increased lipid peroxidation and reduced antioxidant capacity in CAD patients with GSTM1/T1 null genotype in Asian Indian population. In the present study serum GST activity was observed to be increased in GSTM1 null genotype in CAD patients as compare to those having active genotype . However the relationship between GST activity and GSTM1 null genotype is inconsistent in the existing studies . Bhattacharjee et al 2013 (27) reported insignificant difference in serum GST activity between GSTM1 active and GSTM1 null healthy individuals . The variation of the results observed in the present study from the study of Bhattacharjee et al further strengthen the relationship between GSTM1 null genotype and increased risk of CAD . As in the present study GSTM1 null genotype was observed in majority of the CAD patients only. It is important to mention here that healthy individuals were not analyzed for the status of GSTM1 polymorphism in the present study.R Sireesha et al 2012 (28) reported increased GST activity in individuals with age related cataract having GSTM1 and GSTT1 null genotype. Overall CAD patients whether with active or null genotype were having significant oxidative stress as indicated by the raised GST activity and MDA levels and low total antioxidant capacity . Our results are in agreement with previous studies which showed the presence of increased oxidative stress in CAD patients as compared to controls (29, 30).

Limitiations

The major limitation in the present study is small sample size with respect to the data regarding GSTM1 gene polymorphism which could further strengthen the association of null polymorphism with CAD. Another limitation was the lack of data of healthy controls regarding the status of GSTM1 null or active genotype. We are in the process of planning this study which could be more conclusive. Despite these limitations the results of the present study concluded a robust relationship between GSTM1 null genotype and oxidative stress.

Conclusion

In the present study, GSTM1 null genotype has been observed to be a predominant genotype in CAD patients. This genotype may increase the susceptibility for the development of the coronary artery disease because persistent oxidative stress may lead to altered gene expression of Glutathione S-trassnsferase enzyme. Increased GST activity may serve as an indicator of high oxidative stress in CAD patients.

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