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GGT (GAMMA-GLUTAMIL TRANSFERASE), GLUTATHIONE, LIPID PEROXIDATION, CHOLESTEROL AND HDL-CHOLESTEROL LEVELS IN THE PLATELETS OF NORMAL AND ATHEROSCLEROTIC SUBJECTS

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SUMMARY

Between various functional and biochemical alterations in the platelets of atherosclerotic cases, the membrane alterations occupy an important place. The platelet intrinsic membrane protein gamma glutamyltransferase (GGT) which is involved in glutathione metabolism had decreased activity in the cases of atherosclerosis. To add new insights to the pathogenesis of atherosclerosis, GGT is characterized and correlated with other alterations. Triton X-100 solubilized membrane fractions of frozen and thawed platelets of atherosclerotic and normal subjects had 18.66+2.86 mU/10^9plts and 35.67+3.01 mU/10^9 plts,respectively.Cholesterol, HDL-cholesterol in the membrane fractions and platelet glutathione levels were unaltered,lipid peroxidation (membrane MDA) level was increased .

Key words: Human platelets, Atherosclerosis, Gamma glutamyltransferase, Gamma glutamyl transpeptidase, Platelet membrane.

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INTRODUCTION

The most striking biochemical and functional alterations are detected in the platelets of atherosclerotic patients. Hyperagregation, hypersecretion, hyperadhesion detected in atherosclerotic cases are related to platelet membrane alterations /1,2/. Also resistance of platelets from atherosclerotic patients to cold osmotic shock, impairement of active transport systems together with absence of specific binding proteins in the cold osmotic shock fluid /3/, membrane glycoprotein distribution /4/ and phospholipid composition changes are directly involved with the platelet membrane alterations in atherosclerosis /5/.

Gamma glutamyl-transferase (GGT) (gamma glutamyl-transpeptidase, EC 2.3.2.2) is localized in the membrane of most cells especially in tissues involved in absorption and secretion like intestinal and colon epithelial tissues with its active site facing to extracellular space /6,7/. In this study, the membrane GGT level in platelets of normal and atherosclerotic subjects are studied together with platelet membrane cholesterol and HDL-cholesterol and platelet membrane MDA and platelet glutathione. Also the correlation between these parameters are determined.

MATERIALS AND METHODS

Chemicals: γ-glutamyl p-nitroanilide (GPNA), glycylglycine, reduced glutathione (GSH), Triton X-100 were from Sigma , 5.5'-dithiobis 2-nitrobenzoik acid (DTNB) and 2-thiobarbituric acid were from Merck and Cholesterol, HDL Cholesterol test Combination kits were from Roche. Other chemicals used were analytical grade.

Subjects: The atherosclerotic cases had either ischemic heart disease, or POVD, showed defective in fundoscopic examinations atherosclerotic changes had typical plasma lipids distribution, had some alterations of the hemostatic factors like elevated fibrinogen levels, low fibrinolytic activity with defective answer to veneous statis test, platelet hyperaggregation and hypersecretion, had high levels of VWF Normal and atherosclerotic subjects were receiving no medication.

Isolation of platelets: Veneous blood was taken into 0.077 mol/L EDTA (9:1 ratio) from normal and atherosclerotic cases after overnight starvation. Centrifuged at 1500 rpm (Fete clinical centrifuge) for 8 min to obtain platelet rich plasma (PRP). PRP was further centrifuged for 10 min at 10,000 rpm (Beckman-J 21B, rotor No JA 20) to precipitate platelet pellet. Platelet pellet was washed for 3 times

with Tris- NaCl (0.03 mmol/L Tris 0.12 mmol/L NaCl, pH 7.4). Platelets were counted according to Brecher-Croncite method /8/.

Gamma glutamyl transferase assay: The washed platelet pellet obtained from normal and atherosclerotic cases was lysed by 4 times freezing and thawing in distilled water. After centrifugation at 10,000 rpm. for 15 min, the precipitate was solubilized with 1% Triton X-100 and in the solubilized fraction gamma glutamyl transferase activity was determined according to the method of Szasz /9/ using gamma glutamyl p-nitroanilide as the substrate and glycylglycine as the acceptor. The results are expressed as mU per 10^9 platelets.

Glutathione assay: Platelet pellet was suspended in distilled water and 0.5 ml of 15% metaphosphoric acid was added. It was frozen and thawed for 4 times and after centrifugation, glutathione was assayed in the supernatant according to the method of Mergel using DTNB /10/. The results were expressed as μg glutathione per 10^9 platelets.

Lipid peroxide (MDA) determination:Malondialdehyde (MDA) was determined according to Okhawa et al /11/.

Total Cholesterol and HDL-cholesterol assays: Total cholesterol was determined by enzymatic colorimetric method in test combination kits from Roche using cholesterol oxidase and peroxidase (00546 nm) in Triton X-100 solubilized membrane fractions of platelets isolated from normal and atherosclerotic subjects. HDL-cholesterol was determined in the supernatant after precipitating the low density lipoproteins with phosphotungstic acid.

RESULTS

Gamma glutamyltransferase (GGT) activities in the platelets of normals and atherosclerotic cases: The distrubution of GGT activities in the Triton X-100 solubilized membrane fractions of platelets in normals and atherosclerotics are seen in Fig 1.The mean value for GGT in normals (n:10) per 10^9 platelets was 35.65±3.17 and the mean value for GGT in atherosclerotics (n:12) per 10^9 platelets was 18.67±3.00 mU .The difference was significant.

Platelet glutathione and platelet membran malondialdehyde,cholesterol, HDL cholesterol of normal and atherosclerotic subjects: No statistically significant difference was observed in platelet glutathione levels between normals and atherosclerotics Difference of MDA levels was significant (fig 2,3). Platelet membrane cholesterol and HDL-cholesterol levels in normals atherosclerotics are statistically insignificant (fig 4,5).

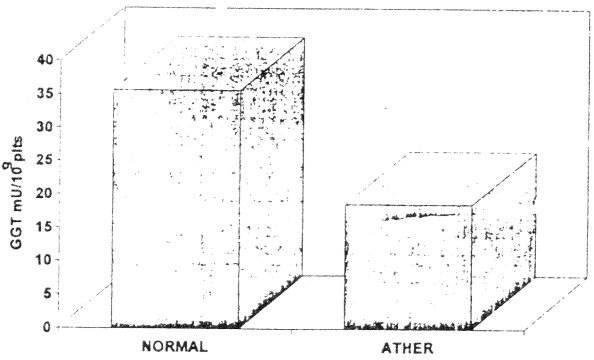

Fig 1.
The GGT levels in the Triton x-100 solubilized platelet membrane fractions from normals (Nor.) and atherosclerotics (Ather.)

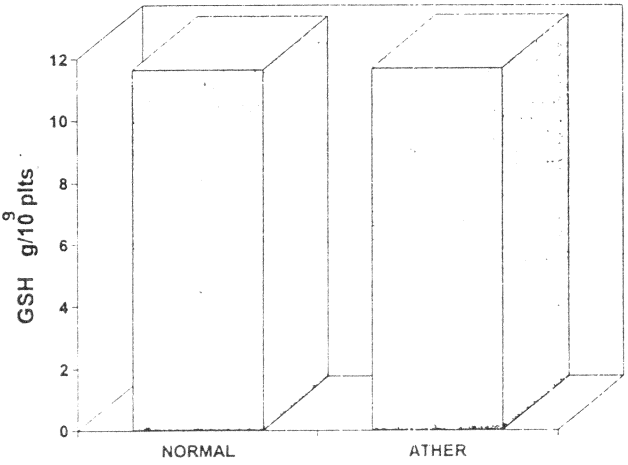

Fig 2.
The glutathione levels in the Triton x-100 solubilized platelet membrane fractions from normals (Nor.) and atherosclerotics (Ather.)

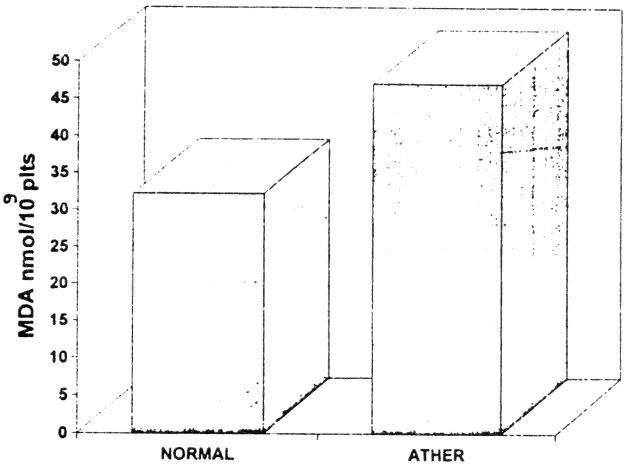

Fig 3.
The lipid peroxidation levels in the Triton x-100 solubilized platelet membrane fractions from normals (Nor.) and atherosclerotics (Ather.)

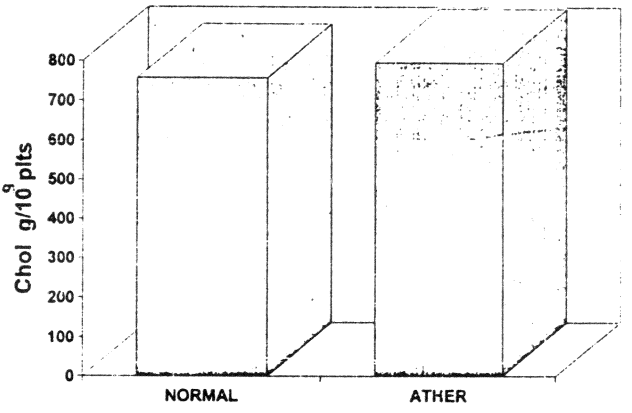

Fig 4.
The cholesterol levels in the Triton x-100 solubilized platelet membrane fractions from normals (Nor.) and atherosclerotics (Ather.)

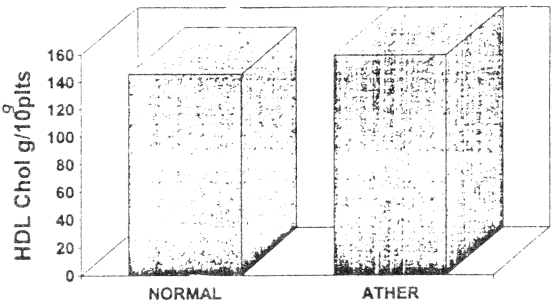

Fig 5.
The HDL-cholesterol in the Triton x-100 solubilized platelet membrane fractions from normals (Nor.) and atherosclerotics (Ather.)

Correlation

	normal	atherosclerotic
AGE/GGT	0.363736	0.194378
AGE/CHOL	0.563125	0.523576
AGE/HDL-CHOL	0.351960	0.088712
AGE/MDA	0.091249	-0.252730
GGT/CHOL	0.562593	-0.307830
CHOL/HDL-CHOL	0.671238	0.133545
HDL-CHOL/MDA	0.091245	0.366590
GGT/MDA	-0.147460	0.070841
CHOL/MDA	0.107710	-0.340690

No significant correlation existed between platelet glucose transport and platelet GGT in both subject groups. There was significant correlation ($p<0.05$) between age and membrane cholesterol both in normals and atherosclerotics. There was significant corelation between platelet GGT and platelet cholesterol in normals but not in atherosclerotics.Also between platelet cholesterol and HDL cholesterol there was significant correlation in normals but not in atherosclerotics.No correlation existed between platelet GGT and platelet membrane MDA.

DISCUSSION

We have observed statistically significant decrease in platelet GGT levels from 35.67+3.1 $mU/10^9$ platelets to 18.66 ±2.86$mU/10^9$ platelets, about 48% decrease, in the cases of atherosclerosis compared to normal subjects and no alteration in the level of platelet total cholesterol and platelet HDL-Cholesterol between normal and atherosclerotic cases, although these cases had high serum lipid levels. Also we could not find significant correlation between platelet membrane GGT and platelet membrane cholesterol and membrane HDL-cholesterol in atherosclerotics. But in the normal subjects there was significant correlation between platelet membrane GGT and platelet membrane cholesterol and between between platelet membrane cholesterol and platelet membrane HDL-Cholesterol. There was also correlation between age and cholesterol both in the platelets of atherosclerotics and normals.

In our studies on amino acid transport systems we showed indirectly that although GGT was not lost in the osmotically shocked platelets, the transport capacity of leucine and histidine was lost due to the release of specific binding proteins into the shock fluid .

Since GGT is not actively participate in amino acid transport, its main action in platelets could be in the degradation of glutathione. This could be the case since there was no alteration in platelet glutathione levels in atherosclerotics compared to that of normals /12/. It is well excepted that the free radicals and lipid peroxides are main causes of many pathological conditions. There is a close relationship between lipid peroxidation and aging /13,14/. Glutathione occupies a place in defence mechanisms againts peroxidation. We have observed in the cases of atherosclerosis increase in platelet membrane bound MDA showing increased levels of lipid peroxidation. We have found no alteration of platelet glutathione levels in atherosclerotics. This seems to be advantageous. The lowered levels of platelet GGT found in atherosclerosis might be important in the regulation of glutathione levels to protect platelets againts free radicals.

The lowering of platelet GGT in atherosclerosis is either due to its lowered synthesis in order to control the level of glutathione or a plasmatic factor or some other membrane alteration probably alteration in lipid structure inhibits the activity, or availability of GGT or there still is the possibility of the existance of an abnormal platelet GGT in atherosclerosis.

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