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γ-Glutamyl transferase as a marker of oxidative stress in pre and post-menopausal women

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Abstract
Introduction: Menopause is a physiologic process in women representing complete cessation of menstruation and gradual decrease of estrogen secretion. The antioxidant substances present in the body play a role in combating the oxidative stress in menopause. Since, the enzyme γ-glutamyl transferase plays an important role in the metabolism of the endogenous antioxidant reduced glutathione (GSH), it can reflect the antioxidant ability *in vivo*.
Aim: The present study was conducted to investigate γ-glutamyl transferase as a possible marker of total antioxidant capacity (TAC) of plasma.
Materials and Method: The study included 50 female volunteers, selected from medical & paramedical staff, and healthy attendants of patients as per inclusion and exclusion criteria and divided into 2 groups. Group I: Premenopausal women (n = 25); Group II: Postmenopausal women (n = 25). Venous blood samples were collected using aseptic techniques in plain vials for estimating estradiol hormone and Gamma GT and whole blood was collected in EDTA vials for the estimation of Ferric reducing ability of plasma (FRAP) which is a marker of antioxidant capacity. The data collected were analyzed using unpaired "t"-test for evaluating the level of oxidative stress in pre and post menopausal women and the variations in the levels of gamma GT in the two groups. The results were expressed as Mean ± Standard Deviation (SD).
Results and Conclusion: Depressed estrogen synthesis in postmenopausal women enhanced oxidative stress and lead to deficit of total antioxidant capacity of plasma. A significant increase in serum GGT in the post-menopausal group relative to the pre-menopausal group was found which was to compensate and correct the reduced glutathione levels by increasing its biosynthesis. Thus, GGT can be used as a marker of total antioxidant capacity of plasma and oxidative stress.

Keywords: Oxidative stress, γ-glutamyl transferase, total antioxidant capacity, menopause

Introduction
Menopause occurs in women in midlife, during their late 40s or early 50s, and it signals the end of the fertile phase of a woman's life [1, 2]. Estrogen acts as an immune-modulating hormone, associated with proper functioning of the immune system which in post-menopausal women may be compromised as its production decreases following menopause. The immune system encompasses an array of defences that help to guard against the development of a number of diseases, some of them age-related [3, 4]. Estradiol, apart from its main hormonal effect, probably displays two mechanisms of antioxidant action. One of them results from hydroxyphenolic structure of their molecule. Estradiol may donate hydrogen atoms from its phenolic hydroxyl group to lipid peroxyradicals what results in termination of the chain reactions of cell membrane phospholipids, which are the key reactions in cell damage [5]. Estradiol also inhibits oxidative modification of LDL lipoprotein which plays the main role in atherogenesis. The other mechanism of antioxidative action of estradiol is probably associated with its stimulatory effect on natural cellular antioxidant enzymes. An increase in oxidative stress and a decrease in estrogen place postmenopausal women at increased risk for several diseases [6, 7].
Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants, and their elimination by protective mechanisms, referred to as anti-oxidative systems [8]. Normally, antioxidants neutralize ROS and thus help to prevent over exposure from oxidative stress [9].

Three conditions that deal with the role of oxidative stress in aging include: 1) levels of molecular oxidative damage increase during aging; 2) a relatively longer life expectancy within and among species is associated with a correspondingly lower accrual of oxidative damage; 3) a prolongation of life-span by regimens such as caloric restriction in mammals is associated with the amelioration of oxidative damage [10].

Gamma-glutamyltransferase (GGT) which is an enzyme involved in the transfer of the γ-glutamyl residue from γ-glutamyl peptides to amino acids, H_2O, and other small peptides that can be donated by glutathione. In most biological systems, glutathione, an endogenous antioxidant serves as the γ-glutamyl donor [11]. On the other hand, GGT is also involved in the synthesis of glutathione [12]. The intracellular glutathione (GSH) level depends upon the equilibrium between processes during which it is consumed and its biosynthesis is limited by cysteine availability [13]. The importance of the γ-glutamyl cycle lies in recovering and delivering cysteine. The availability of cysteine, necessary for the biosynthesis of cellular glutathione, the most important cell antioxidant, depends upon gamma glutamyltranspeptidase (GGT) activity; hence this enzyme may play an important role in the anti-oxidative defence system of the cell [14]. Oxidative stress can be assessed as the antioxidant activity of all the substances present in plasma or total antioxidant capacity [15]. Several markers have been studied in menopausal women but no specific conclusion has been drawn so, the level of GGT can be considered an index or a marker of oxidative stress as it plays a role in regeneration of reduced glutathione [16].

The aim of the present study is to evaluate the oxidative stress as total antioxidant capacity of plasma in pre and post-menopausal conditions and to find the variation in level of γ-GT as a marker of oxidative stress associated with menopause in women.

Material and Methods

The present study was carried out in the Department of Biochemistry, NIMS Medical College and Hospital, Shobha Nagar, Jaipur, Rajasthan in collaboration with the Department of Obstetrics and Gynaecology. The protocol of the study was approved by the Institutional Ethical Committee, NIMS Medical College, Shobha Nagar, Jaipur, and informed signed consent was given by each subject.

It included 50 female volunteers, selected from medical & paramedical staff, and healthy attendants of patients. The personal and clinical history of all subjects was recorded with the help of a questionnaire. Clinical examination was carried out by a competent gynaecologist. The subjects were categorized into following groups:

Group I: Premenopausal women (n = 25)

The normally menstruating women were included in this group; but women experiencing amenorrhea were excluded. These selected subjects were considered as control group and their age range was 25-45 years (34.52±8.03 years).

Group II: Postmenopausal women (n = 25)

Those women with one year of amenorrhea and not receiving hormone replacement therapy were considered as study group and their age range was 46-70 years (55.67±5.34 years).

Inclusion Criteria

Non-smokers, non-alcoholics

Age 25 years or more

No vitamins/minerals taken as supplements since last 3 months

Healthy females in age group 25-45 years, for premenopausal group.

Healthy females in age group 46-70 years, for postmenopausal group.

Exclusion Criteria

Subjects with hypertension, cardiovascular diseases, diabetes, venereal diseases, any pathology (including carcinoma).

- Women taking oral contraceptives, antioxidants or any other drug.
- Subjects with any concurrent sickness.
- Pregnancy or lactation
- Females on hormone replacement therapy

Sample Collection

Venous blood sample was collected using aseptic techniques:-

1. 2.0 ml of fasting sample (8 – 12 hrs) was collected in a plain tube for estimating estradiol hormone and Gamma GT. It was centrifuged at 1500rpm for 15min. and serum separated was stored at 4^0C.
2. 3.0 ml of whole blood was collected in EDTA vial for the estimation of FRAP.

Biochemical Analysis

The serum levels of estradiol (E2) and GGT were measured using standard kits on an auto analyser and Total Antioxidant Capacity (TAC) was determined by FRAP (Ferric reducing ability of plasma) method, which is based on the reduction of a colourless ferric tripyridyl-triazine complex to a blue ferrous complex by the antioxidants in the plasma. The change in absorbance at 593nm is directly related to the total reducing power of electron donating antioxidants present in the plasma [17].

Statistical analysis

Statistical analysis was done, using the statistical package for social science (SPSS 20) for Windows software, Microsoft Excel 2007 and scientific calculator. The results were expressed as Mean ± Standard Deviation (SD).

The data collected for control and study group were analyzed using unpaired "t"-test for finding the level of oxidative stress in pre and post menopausal women and the variation in the levels of gamma GT in the two groups. Pearson's correlation was applied to determine the relationship between Oxidative Stress (as measure of ferric reducing ability of plasma, FRAP and levels of GGT. Statistical significance was defined at $p<0.001$.

Observation Table

Table 1: Comparison of FRAP and Estradiol (E2) in Control and Study group

Parameter	Control group (n=25)	Study group (n=25)
FRAP (Ferric Reducing Ability of Plasma) (µmoles of $FeSO_4$ equivalent/ L of plasma)	916.08±131.08	483.12±53.64 *
E2 (pg/ml)	152.95±60.18	18.32±5.27 *

Values in mean ± SD; ***p*< 0.001

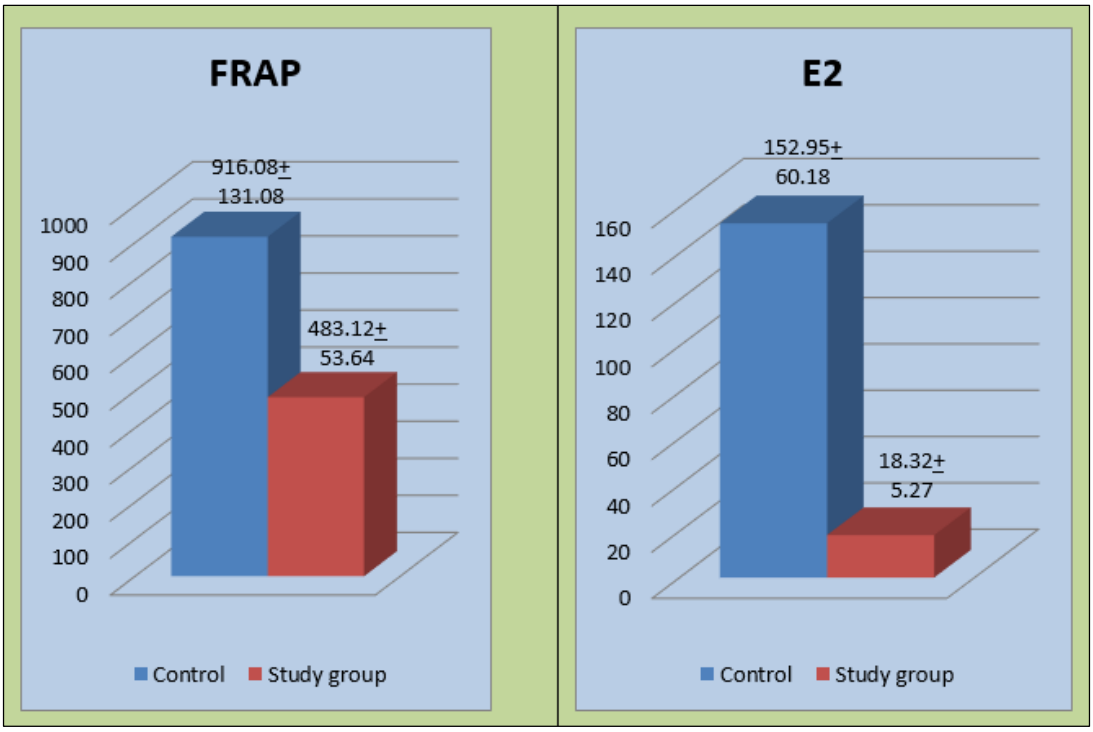

Fig 1: Comparison of FRAP and Estradiol (E2) in Control and Study group

Table 2: Comparison of FRAP and GammaGT in Control & Study group

Parameter	Control group (n=25)	Study group (n=25)
Ferric Reducing Ability of Plasma (FRAP) (µ moles of $FeSO_4$ equivalent/ L of plasma)	916.08±131.08	483.12±53.64 *
γ-G T (U/L)	29.86±7.73	59.87±6.35 *

Values in mean ± SD; *p< 0.001

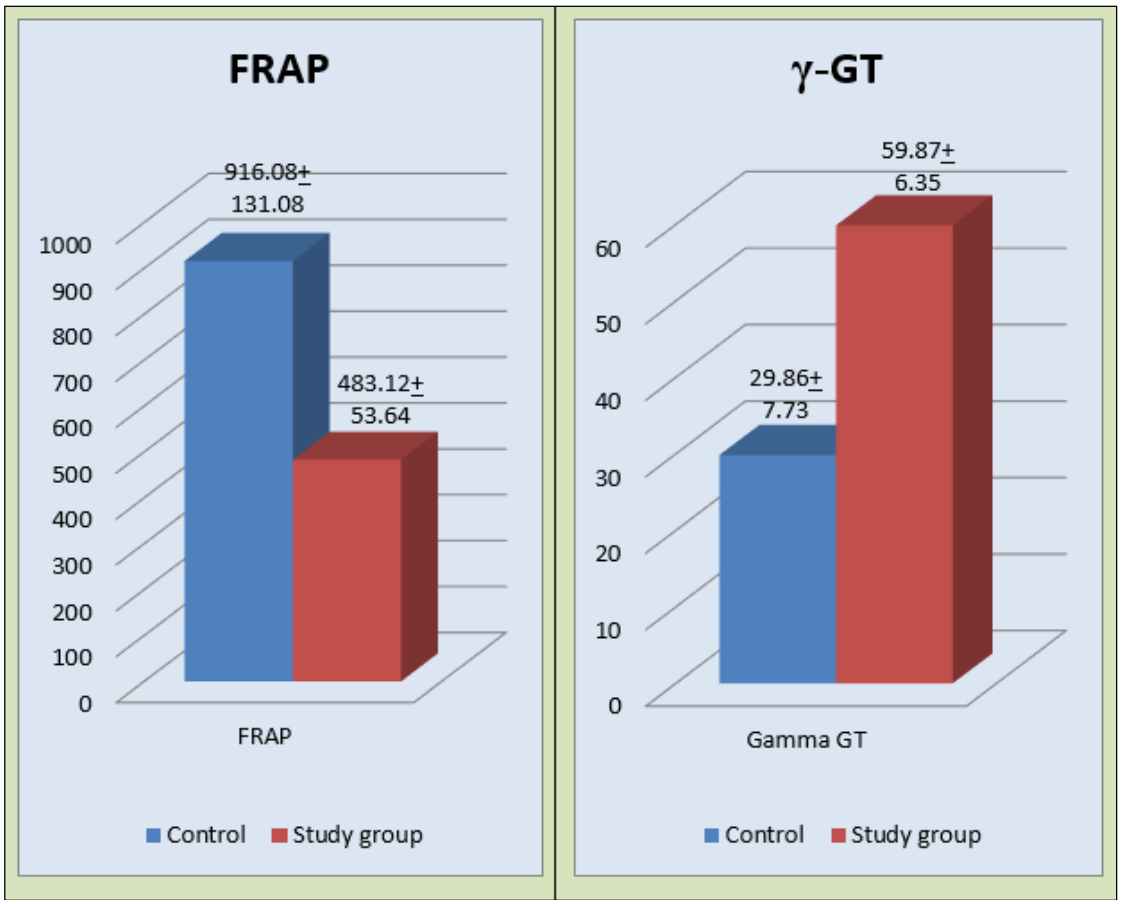

Fig 2: Comparison of FRAP and GammaGT in Control & Study group

Table 3: Comparison of activity of GammaGT and Estradiol (E2) in Control and Study group

Parameter	Control group (n=25)	Study group (n=25)
γ- GT (U/L)	29.86±7.73	59.87±6.35 *
E2 (pg/ml)	152.95±60.18	18.32 ± 5.27 *

Values in mean ± SD; *****p< 0.001

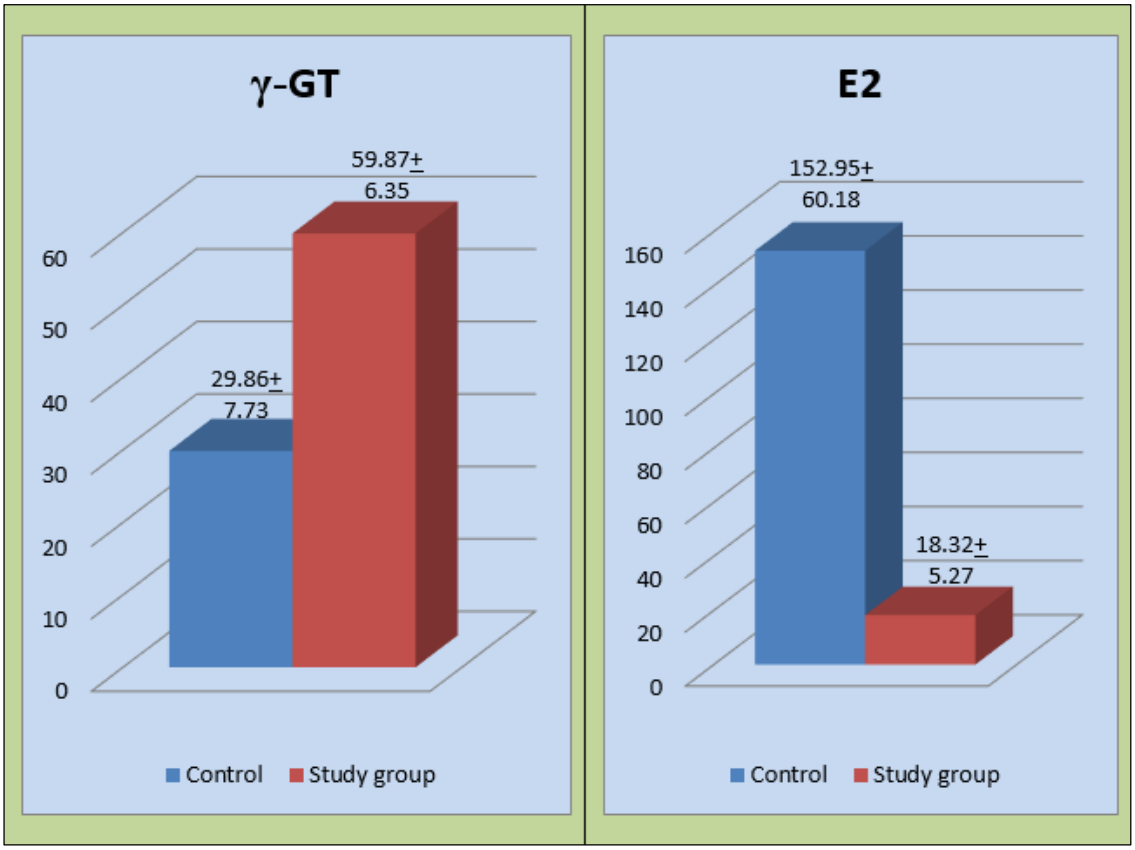

Fig 3: Comparison of activity of Gamma GT and Estradiol (E2) in Control and Study group

Discussion

Menopause is characterised by a gradual decrease in oestrogen levels. It is a natural step in the process of ageing and free oxygen radicals have been proposed as important causative agents of ageing [10, 11]. Studies documented by various authors, show ambiguity in the results with regard to the effect of menopause on oxidative stress levels. Some studies in recent years concluded that oxidative stress influences the entire reproductive lifespan of a woman and even thereafter i.e. menopause [10]. Estrogens have free radical scavenging structures and have been shown to have *in vitro* antioxidant effects on membrane phospholipid peroxidation [18, 19]. The process of ageing is enhanced due to the damage caused by free radicals; hence menopausal women are proposed to develop oxidative stress because of estrogen deficiency and advancing age [20, 21]. In the present study the total antioxidant capacity of plasma measured as ferric reducing ability of plasma (FRAP) was reduced from 916.08+131.08 µmol/L in controls to 483.12+53.64 µmol/L of plasma in postmenopausal group, a decrease of 50% which was statistically significant (p<0.001). This is in concordance with the levels of estradiol (E2) that reduced drastically in menopause (Table-1, Fig no-1). This observation is most likely the result of depressed estrogen synthesis in postmenopausal women that enhanced oxidative stress and lead to deficit of total antioxidant capacity of plasma [22].

The formation of excessive amounts of reactive oxygen species (ROS) is toxic for the cells and therefore metabolizing and scavenging systems to remove them are functionally critical and tightly controlled in the cells. The antioxidant enzyme Glutathione peroxidase (GSHPx) detoxifies peroxides with glutathione (GSH) acting as electron donor in the reduction reaction, producing oxidized glutathione (GSSG) as an end product [23].

Recently it has been proposed that Gamma-glutamyltransferase (GGT), which is an enzyme involved in the transfer of the gamma-glutamyl residue from gamma-glutamyl peptides to amino acids, H2O, and other small peptides can be donated by glutathione. On the other hand, GGT is also involved in the production of glutathione, which is limited by cysteine availability [11]. GGT participates in the pathway of extracellular GSH in consequence the biosynthesis of cellular glutathione, the most important cell antioxidant, depends on GGT activity; hence this enzyme may play an important role in the anti-oxidative defence system of the cell [16].

In the present study, the mean level of Gamma GT in premenopausal women (control group) was 29.86±7.73 U/L and the mean level of Gamma GT in postmenopausal women (study group) was 59.87±6.35 U/L. The value of Gamma glutamyl transferase (GGT) significantly increased in case of postmenopausal women as compared to control (p< 0.001) (Table-2, Fig N 2).

A significant correlation between decrease in Ferric reducing ability of plasma and increase in activity of Gamma glutamyl transferase was observed. This important finding indicated that a higher level than the normal serum GGT concentration is associated with the presence of oxidative stress [24].

A significant increase in activity of γ-glutamyl transferase (GGT) in case of postmenopausal women is concurrent with the significant decrease in levels of estradiol (E2) in postmenopausal women attributed to the loss of antioxidant defence systems and aging (Table-3, Fig.no.-3).

Conclusion

The significant increase in serum GGT in the post-menopausal group relative to the pre-menopausal group is to compensate and correct the reduced glutathione levels by increasing its biosynthesis. Thus, GGT can be used as a marker of total antioxidant capacity of plasma and oxidative stress.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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