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SERUM GAMMA GLUTAMYL TRANSFERASE, LEVEL IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE - A CROSS SECTIONAL COMPARATIVE STUDY

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Abstract

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of morbidity and mortality worldwide. Global Burden of disease is increasing and it would be the fourth leading cause of death in 2030. Gamma glutamyl transferase (GGT) has been regarded as a novel marker of oxidative stress. So, this study was done to assess whether the serum level of GGT might be used as a marker in monitoring level of oxidative stress in clinically stable COPD.

Objectives: (1) To estimate the serum levels of GGT, in patients with COPD and compare it with healthy individuals. (2) To evaluate the correlation between serum levels of GGT and severity of COPD.

Methods: A comparative study was conducted on patients who attended pulmonary medicine OPD and admitted in ward at Government Medical College, Kozhikode. Data was collected from 96 people of age > 40 years, of which 48 were patients with COPD (Group1) and 48 were age and sex matched healthy individuals (Group 2). Samples were collected from study subjects and estimation of GGT was done. Pulmonary function test reports were collected from case records to assess severity. Statistical analysis was performed using SPSS version 22.0 software.

Results: The mean serum GGT level was found to be significantly higher in Group 152.827±1.837 as compared to Group 2-15.752±3.341 and the difference in the mean serum GGT levels between 2 groups was statistically significant at $p<0.001$. The difference in the mean serum GGT levels between different GOLD Grades in Group 1 was statistically significant at $p<0.001$.

Conclusion: Serum GGT level has got a positive correlation in patients with COPD when compared to healthy individuals.

Keywords: Chronic Obstructive Pulmonary Disease, Serum Gamma Glutamyl Transferase

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major health problem and is one of the three most common causes of death worldwide. It affects 1 in 10 of the adult population and is rare before the age of 40[(1, 2)]. COPD can be due to the result of oxidative stress. Oxidative stress can be due to a decreased antioxidant capacities or increased exposure to oxidants. Oxidants from cigarette can cause toxic injury to lung structures like DNA, lipids, connective tissues, and proteins. It also impairs the cilia function and repair mechanisms. It

decreases surfactant activity and increase mucus production. Antioxidants helps to improve the defense mechanism. Examples for antioxidants are vitamin E, β-carotene, vitamin C, uric acid, flavonoids, bilirubin etc.[(1)]. Studies on an imbalance between oxidants and antioxidants in the pathogenesis of COPD are few and various biomarkers of oxidative stress have been evaluated in COPD[(3)].

Glutathione (GSH) is an antioxidant. It helps in the detoxification and inflammation process and is also in cell cycle regulation, apoptosis, and cell signaling. GGT is a membrane bound enzyme which can initiate and turnover of GSH. In many studies the GGT levels have been elevated in COPD patients and unregulated in oxidative stress and inflammation[(4)].

The key component of COPD is inflammation, which involves a cells and mediators. E xample is C-reactive protein (CRP), an acute phase reactant, its levels are elevated in inflammation[(5)]. The coexistence of airway and parenchymal inflammation in most patients with symptomatic chronic air flow limitation, underlines the role of inflammation in COPD. The airway and parenchymal inflammation were assessed by sputum analysis, bronchoscopy, biopsy, and lung lavage, which also revealed that inflammation contributes to the development of COPD[(1)].

Aim of the study

1. To estimate the serum level of Gamma glutamyl transferase (GGT)in patients with COPD and compare it with healthy individuals.
2. ToevaluatethecorrelationbetweenserumlevelofGammaglutamyltransferaseand the severity of COPD.

Background & review of literature

Chronic Obstructive Pulmonary Disease is a preventable and treatable disease that is characterized by air flow limitation and persistent respiratory symptoms due to air way and alveolar abnormalities because of the exposure to noxious particles or gases and abnormal lung development[(6-9)]. COPD is a leading cause of morbidity and mortality worldwide and the global prevalence is 11.7% and in India is 7.4%. The incidence of COPD is gradually increasing and resulting in an impaired the quality of life and loss of productivity[(7,8)]. COPD occurs due to the prolonged exposure to harmful gases or particles. (e.g. cigarette smoking - the damage in the lungs is mainly due to the tar present in the smoke, but the nicotine result in addiction.) Other causes of COPD include- asthma, infections, low birth weight, alpha-1-antitrypsin deficiency and exposure to dusts, fumes etc. [(9, 10, 11)].

The severity of COPD is classified as per the GOLD classification (Global Initiative for Chronic Obstructive Lung Disease) based on the percentage of predicted FEV1, the incorporated dyspnea severity and the history of exacerbation - into four “groups” A–D and this classification is the basis of recent treatment recommendations. Spirometry is used to assess the air flow obstruction in the conditions such as chronic bronchitis, emphysema and asthma[(12)].COPD is characterised by airflow obstruction and an inflammatory response leads to a decrease in the forced expiratory volume (FEV1) and impaired gas exchange in

the lungs. It is associated with chronic bronchitis, emphysema due to long term exposure to noxious particles, gases, cigarette smoke etc. This leads to decreased lung elastic recoil resulting in airway collapse during exhalation. The reduction in ventilation leads to CO_2 retention and pulmonary hypertension. The two processes involved in the pathogenesis of COPD are an imbalance between (1) proteases & antiproteases (2) oxidants and antioxidants (oxidative stress) **(1,13).**

Signs and symptoms of COPD include: - shortness of breath, wheezing, chest tightness, chronic cough with clear sputum, frequent respiratory infections etc.**(14)**. The diagnosis depends up on the pulmonary function test (spirometry) demonstration of persistent air flow limitation, as defined by post bronchodilator FEV1/FVC ratio<0.7, in patients with appropriate symptoms and the history of exposure to noxious stimuli**(15).**The GOLD criteria for categorizing COPD patients according to severity and airflow limitation is based up on the spirometric findings were assessed. Post bronchodilator ratio of forced expiratory volume (FEV1) to Forced Vital Capacity (FVC) less than 0.7 was considered to determine airflow limitation. Grading of severity as per GOLD criteria is as follows: GOLD 1) mild- (FEV1 ≥ 80% predicted), GOLD 2) moderate- (50% ≤ FEV1 < 80% predicted), GOLD 3) severe- (30% ≤ FEV1 < 50% predicted); and GOLD 4) very severe- (FEV1 < 30% predicted)**(12)**.

Gamma glutamyl transferase (GGT) is bound on the extracellular surface of the membranes of the secretory cells and is worldwide recognized as a diagnostic marker for many diseases in humans. The catalytic activities of GGT are basically of three types. 1. Transfer Reaction 2. Auto-transpeptidation 3. Hydrolysis**(16)**. It regulates the metabolism of the antioxidant glutathione (GSH, γ-glutamyl-cysteinyl-glycine) Glutathione is essential for oxidative stress regulation and detoxification of drugs, pollutants, carcinogens etc. GGT is significantly increased in several tumors, neurodegenerative and cardiovascular diseases. GGT value are predictive of many diseases like liver disease, chronic kidney disease, cardiovascular disease, type 2 diabetes, and cancer etc. Serum GGT levels can be used as a marker for oxidative stress. The Serum levels of gamma-GT may be helpful in grading the severity of COPD **(7,17).** In adults, the upper reference limit for serum GGT activity is 40 U/L for females and 70 U/L for males **(18, 19)**.

Though many studies have been done worldwide, studies done in Indian population are very few. In this study, our aim is to estimate the serum levels of GGT in COPD patients and compare it with healthy individuals and also to evaluate their correlation with severity of COPD.

METHODOLOGY

Data collection:

Study was conducted after the approval of Institutional Ethics Committee and getting written informed consent. Detailed history and demographic data were obtained from all study subjects.

Study design: Comparative study.

Study setting: Pulmonary medicine OPD and ward, Govt. Medical College, Kozhikode.

Study duration: 1 year.

Sample size: 96 study subjects.

Inclusion criteria: Group 1 included patients from both genders diagnosed with COPD of age above 40 years attending OPD and admitted in wards of Pulmonary medicine department, Government Medical College, Kozhikode. Group 2 included age and sex matched apparently healthy individuals.

Exclusion criteria:

1. Other respiratory diseases like Pneumonia, Tuberculosis.
2. Hepatic diseases like Cirrhosis, Hepatitis, Cholestasis.
3. Alcohol consumption.
4. Neoplastic pathologies.
5. Other acute and chronic infections.
6. Other inflammatory diseases.
7. Diabetes Mellitus, CAD, CKD, Gout, Hypothyroidism.
8. Drugs: Salicylates, Thiazide diuretics, Pyrazinamide, Allopurinol, Statins, Febuxostat, Anti-cancer drugs, Phenytoin, Phenobarbital, Valproicacid.

Sampling procedure:

Study subjects included patients diagnosed with COPD and age and sex matched apparently healthy individuals who were more than 40 years of age. They were divided into two groups:-

Group 1: 48 patients diagnosed with Chronic Obstructive Pulmonary disease.

Group 2: 48 age and sex matched apparently healthy individuals.

Method of data collection and outcome measurement:

After taking informed consent from all study subjects a detailed history was taken including duration of the illness, other significant medical illness and drugs. General and systemic examination was done. Pulmonary function test reports were collected from case records. Sample for Serum Gamma glutamyltransferase estimation was collected from study subjects in blood collection tubes without anti-coagulant under aseptic precautions and centrifuged to separate out the serum.

Laboratory parameters- were analysed in Semiautoanalyzer**.** Serum GGT using carboxy substrate method.

STATISTICAL ANALYSIS:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 was used to perform statistical analysis.

DESCRIPTIVE STATISTICS:

Descriptive analysis of all the explanatory and outcome parameters was done using frequency and proportions for categorical variables, whereas mean & SD for continuous variables.

INFERENTIAL STATISTICS:

Mann Whitney's test was used to compare the mean age between 2 groups. Chi square test was used to compare the gender distribution between 2 groups. Independent Student t test was used to compare the mean Serum GGT levels between Group 1 & Group 2.

RESULTS

Table: 1 Age and gender distribution among two groups

Variable	Category	Group1		Group2		p-Value
		Mean	SD	Mean	SD	
Age	Mean & SD	62.88	7.41	61.90	8.13	0.54[a]
	Range	48 -80		46 -85		
		n	%	n	%	
Sex	Males	36	75.0%	36	75.0%	1.00[b]
	Females	12	25.0%	12	25.0%	

Note: a. Mann Whitney Test; b. Chi Square Test

The mean age of the Group 1 was 62.88 ± 7.41 with a range of 48-80 years.
The mean age of the Group 2 was 61.90±8.13 with a range of 46-85 years.

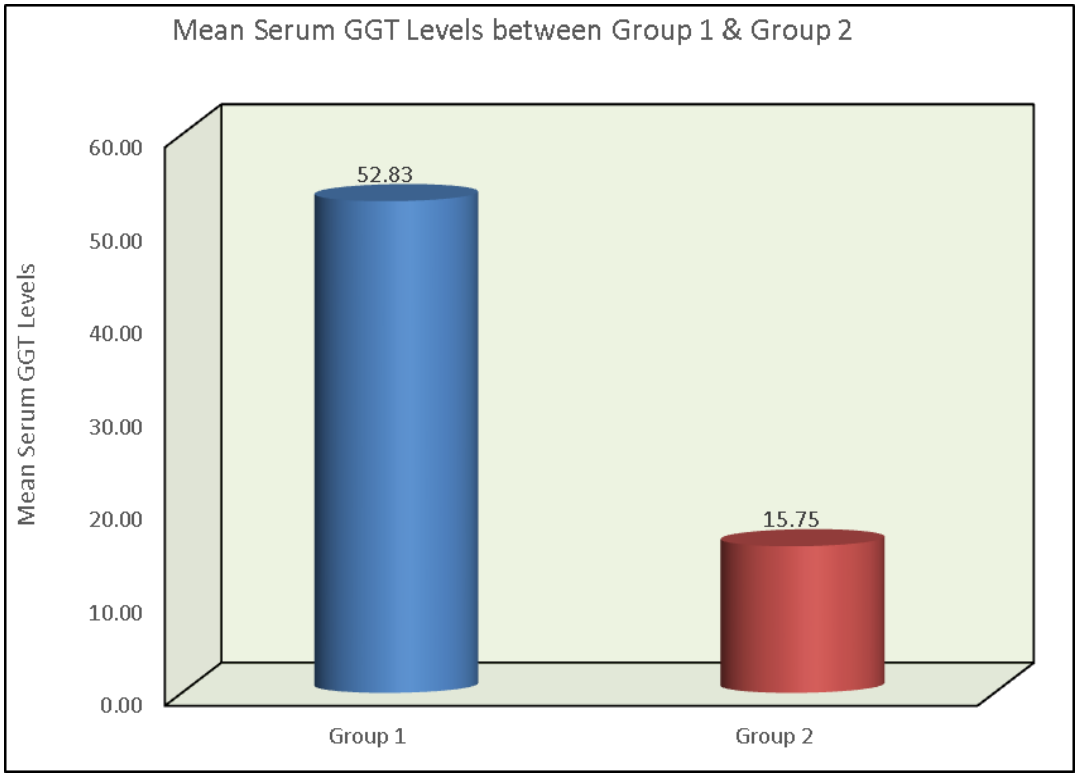
Gender wise distribution between Group1 & Group2-Among Group 1, N=48, males (n=36) 75% and females (n=12) 25%. Among Group2, N=48, males (n=36)75% and females (n=12)25%.

Table 2: Comparison of mean Serum GGT levels (in U/L) between Group 1 & Group 2 using Independent Student t Test

Parameter	Groups	N	Mean	SD	MeanDiff	p-Value
Serum GGT	Group1	48	52.827	1.837	37.075	<0.001*
	Group2	48	15.752	3.341		

* -Statistically Significant

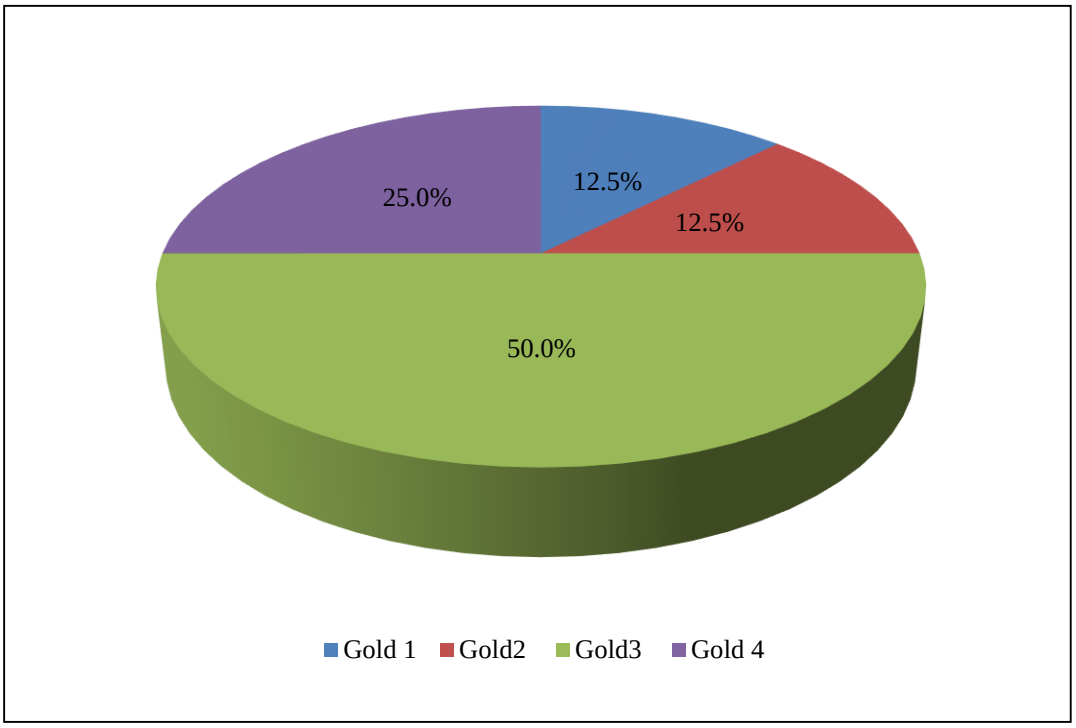
Graph 1: Mean Serum GGT levels (in U/L) between Group 1 & Group 2



The test results demonstrated that the mean Serum GGT levels in Group 1 was significantly higher [52.827 ± 1.837] as compared to Group 2 [15.752 ± 3.341] and the difference in the mean serum GGT levels between the 2 groups was statistically significant at p<0.001.

Table 3: Distribution of GOLD Grades among Group 1 patients

Variable	Category	n	%
GOLDGrade	GOLD1	6	12.5%
	GOLD2	6	12.5%
	GOLD3	24	50.0%
	GOLD4	12	25.0%



Graph 2: Distribution of GOLD Grades in Group 1

Among Group 1 patients, 12.5% were GOLD1, 12.5% were GOLD 2, 50% were GOLD3 and 25% were GOLD4. Majority of Group1 patients were in GOLD3.

Table 4: Comparison of mean Serum GGT levels (in U/L) based on GOLD Grades in Group1using One-way ANOVA Test

Grades	N	Mean	SD	Min	Max	p-value
GOLD1	6	50.732	1.317	48.67	52.34	<0.001*
GOLD2	6	51.330	0.999	50.01	52.2	
GOLD3	24	53.188	1.857	50.29	56.12	
GOLD4	12	53.903	0.895	52.18	55.28	

* -Statistically Significant

Table 5: Multiple comparison of mean difference in Serum GGT levels (in U/L) based on GOLD Grades in Group 1 using Tukey's Post hoc Test

(I)GOLD Grade	(J)GOLD Grade	Mean Diff.(I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
GOLD1	GOLD2	-0.598	-2.943	1.746	0.90
	GOLD3	-2.456	-4.310	-0.603	0.005*
	GOLD4	-3.171	-5.201	-1.140	0.001*
GOLD2	GOLD3	-1.858	-3.711	-0.004	0.04*
	GOLD4	-2.573	-4.603	-0.542	0.008*
GOLD3	GOLD4	-0.715	-2.150	0.721	0.55

* -Statistically Significant

The test results demonstrated that the mean Serum GGT levels in GOLD 1 was 50.732±1.317, GOLD 2 was 51.330±0.999, GOLD 3 was 53.188±1.857 and GOLD 4 was 53.903 ± 0.895. This difference in the mean Serum GGT levels between different GOLD Grades in Group1 was statistically significant at $p<0.001$.

Multiple comparison between GOLD Grades revealed that GOLD 4 showed significantly higher serum GGT levels as compared to GOLD 1 & 2 atp=0.001 & 0.008 respectively. This was then followed next by GOLD 3 showing significantly higher mean serum GGT levels as compared to GOLD1 & 2 atp=0.005 & 0.04 respectively. However, no significant difference was observed between GOLD 1&2 and also between GOLD 3&4.

DISCUSSION

Our comparative study revealed that COPD patients had significantly higher serum GGT level when compared to healthy individuals. We also found that the serum GGT level was found to be significantly higher in the GOLD Grade 4 as compared to other GOLD Grades. Radi et al. conducted a case control study which included 109 patients with clinically stable COPD and the control group of 51 healthy subjects at the Department for Pulmonology in the General Hospital, Croatia. The catalytic activity of GGT were measured and a statistically significant increase in GGT activity (for 63%) was found in all COPD patients when compared to healthy controls ($p<0.05$) **[20].**

In a comparative study conducted by Bozkus et al. with a total of 152 subjects, 70 (46.1%) with GOLD stages A and B (Group 1) and 82 (53.9%) with GOLD stages C and D (Group

2) at Chest Diseases Clinic of Necip Fazıl State Hospital, the level of GGT was found to be significantly ($p < 0.001$) higher in the GOLD stage C and D group than in the GOLD stage A and B group. Thus, he demonstrated that serum GGT may be helpful in grading the severity of COPD[21].

A comparative study conducted by Sun et al. included 117 patients with acute exacerbation of COPD, 107 patients with stable COPD, and 112 control subjects. Serum GGT, spirometry function, and other clinical parameters (anthropometric and biochemical measurements) were evaluated and compared among the subjects. A statistically significant increase of serum GGT activity was found in stable COPD patients when compared to healthy controls (24.0±7.2 vs.17.4±5.7; $p<0.001$), and serum GGT activity was found to be remarkably higher in acute exacerbation of COPD patients when compared to patients with stable COPD (32.9±9.9 vs. 24.0±7.2; $p<0.001$). Serum GGT level was inversely related to lung function and may serve as a biomarker during the progression of COPD [22].

Only few studies have investigated the relationship between serum GGT and COPD. However, it was found that serum GGT is increased in clinically stable COPD patients as compared to healthy controls. While some researchers have reported negative correlations between serum GGT levels and severity of COPD, others have reported positive correlations. Our study showed that the levels of serum GGT are significantly increased with increase in severity of COPD.

CONCLUSION

The mean Serum GGT level was found to be significantly higher in COPD patients when compared to healthy individuals. The difference in the mean Serum GGT levels between different GOLD Grades was statistically significant (positive correlation).

LIMITATIONS

- The major limitation of our study is that the sample size is small and the duration of study is limited.
- Since this is a Cross sectional comparative study, temporality of the association cannot be established. For that we need to conduct a longitudinal study.
- We did not assess Serum GGT levels in COPD patients suffering from an exacerbated episode.

Future Directions

More research is needed to understand better the pathophysiological role of GGT in development and progression of COPD.

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