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AN EXPLORATIVE STUDY OF GAMMA-GLUTAMYL TRANSFERASE AND MEAN CORPUSCULAR VOLUME AS BIOMARKERS IN PATIENTS OF ALCOHOL DEPENDENCE

Psychiatry

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ABSTRACT

Background: Biomarkers add credibility to research dealing with alcohol treatment efficacy and provide clinicians with an additional source of objective information on patients. Elevated GGT activity remains the most common marker of alcohol abuse. Mean Corpuscular Volume is also associated with heavy chronic drinking as the MCV in heavy drinkers tends to exceed the normal range.

Aim and objectives:

1. To measure and compare GGT and MCV in patients of alcohol dependence and controls
2. To compare GGT and MCV in uncomplicated alcohol withdrawal patients and alcohol withdrawal patients with convulsions
3. To measure the sensitivity and specificity of GGT and MCV as biomarkers in alcohol dependence

Methods and materials: This was a hospital based case control study conducted on 100 inpatients of alcohol dependence and equal number of age and sex matched controls. Blood samples were collected from the patients on the first day of admission and, GGT and MCV were measured using bichromatic technique and automated analyzer respectively. The results were analysed using SPSS Version 16.0 setting the significance threshold at p=0.05.

Results: There was significant elevation of GGT and MCV in patients of alcohol dependence when compared to controls. GGT was also significantly elevated in alcohol withdrawal cases with convulsions compared to cases with uncomplicated alcohol withdrawal. Overall GGT was found to be a more sensitive and specific marker than MCV.

Conclusion: Both GGT and MCV can be used effectively as alcohol markers, with GGT being more sensitive and specific between the two. Elevated GGT levels in alcohol withdrawal patients with convulsions indicate that it could be a risk factor for alcohol withdrawal seizures.

KEYWORDS

Gamma-glutamyl Transferase; Mean Corpuscular Volume; Alcohol Dependence; Alcohol Withdrawal; Sensitivity; Specificity.

INTRODUCTION:

Alcoholism is one among the major public health problems that the world is facing today.It is the leading risk factor for death in males aged between **15 and 59** years of age, mainly due to injuries, violence and cardiovascular diseases. [1] According to National Household Survey 2004 prevalence of "current use" of alcohol in India was **21%** and alcohol dependence rate was **16.5%.** Amongst treatment seekers prevalence was **43.9%** (Drug Abuse Monitoring System, 2004). [2]

Consumption of alcohol not only has an impact on the incidence of diseases, injuries and other health conditions, but also on the course of disorders and their outcomes in individuals. Apart from environmental factors **Alcohol-related harm** is determined by three related dimensions of drinking: the volume of alcohol consumed the pattern of drinking and, on rare occasions, also the quality of alcohol consumed. [3] There are three main direct mechanisms of harm caused by alcohol consumption in an individual **(Babor et al., 2003; WHO, 2004b; WHO, 2007).** These three mechanisms[3] are:

- Toxic effects on organs and tissues
- Intoxication, leading to impairment of physical coordination, consciousness, cognition, perception, affect or behaviour
- Dependence, whereby the drinker's self-control over his or her drinking behaviour is impaired.

In 1976 **Edwards and Gross** proposed the existence of alcohol dependence within a syndrome model. [4] **ALCOHOL DEPENDENCE (or ALCOHOL DEPENDENCE SYNDROME)** refers to certain physiological and psychological phenomenon that is induced by repeated taking of alcohol.[5] It is a cluster of physiological, behavioural and cognitive phenomena in which the use of alcohol takes on a much higher priority for a given individual than other behaviors that once had greater value. A central descriptive characteristic of the dependence syndrome is **the desire (often strong, sometimes overpowering) to take alcohol**. There may be evidence that return to alcohol use after a period of abstinence leads to a more rapid reappearance of other features of the syndrome than occurs with nondependent individuals.[6]

According to **ICD-10**[6] alcohol dependence can be diagnosed if 3 or more of the following are experienced or exhibited at some time during the last year –

1. A strong desire or sense of compulsion to take the substance.
2. Difficulties in controlling substance taking behavior in terms of its onset, termination or levels of use.
3. Physiological withdrawal state when alcohol use has ceased or been reduced.
4. Evidence of tolerance such that increased doses of the psychoactive substance are required in order to achieve effects originally produced by lower doses.
5. Progressive neglect of alternative pleasures or interests because of psychoactive substance use and increased amount of time necessary to obtain or take the substance or to recover from its effects.
6. Persisting with alcohol use despite clear evidence of overtly harmful consequences.

Narrowing of the personal repertoire of patterns of alcohol use is also a characteristic feature (e.g. a tendency to drink alcoholic drinks in the same way on weekdays or weekends, regardless of social constraints that determine appropriate drinking behaviour).[6]

Alcohol Dependence Syndrome includes Alcohol Withdrawal state and Delirium Tremens. Alcohol Withdrawal refers to a group of symptoms of variable clustering and severity occurring on absolute or relative withdrawal of alcohol after repeated and usually prolonged use of alcohol. Symptoms of Alcohol Withdrawal include insomnia, fatigue, tremor, mild anxiety, agitation, nausea and vomiting, headache, excessive sweating, palpitations, anorexia, hallucinations, delusions, withdrawal seizures and Delirium Tremens. [7]ICD-10 has sub-specified Alcohol Withdrawal State into Uncomplicated Alcohol Withdrawal State and Alcohol Withdrawal State with convulsions. [6]

Delirium Tremens is the most severe form of alcohol withdrawal manifested by altered mental status (global confusion), agitation, disorientation, hallucination, fluctuating levels of psychomotor activity ranging from lethargy to hyper excitability and autonomic hyperactivity such as tachycardia, fever, diaphoresis, anxiety, insomnia and hypertension which can progress to cardiovascular collapse. These symptoms may appear suddenly but can develop 2–3 days after cessation of drinking heavily with its highest intensity on the fourth or fifth day. Also, these "symptoms are characteristically worse

at night." [7] Less than **5 percent** of alcohol intoxications and withdrawals are accompanied by delirium tremens.[8]

Gamma-Glutamyl transferase (GGT) is a membrane bound glycoprotein which catalyses the transfer of gamma-glutamyl group to other peptides, amino acids and water. Large amounts are found in the kidneys, pancreas, liver, intestine and prostate. [9] Serum gamma-glutamyl transferase (GGT) activity is increased in the serum in hepatobiliary disorders and with fairly heavy consumption of alcohol. [10] GGT may elevate because of increased synthesis or accelerated release from damaged or dead liver cells. It seems to primarily indicate continuous, rather than episodic, heavy drinking, although a few moderate drinkers also produce elevated levels of GGT (Gjerde et al. 1988). [11] Serum levels of GGT have been found to be elevated in about 75% of individuals who are alcohol-dependent,[12-14] with a range in sensitivity of **60-90%.**[15-17] Normal Range (Reference Interval) is 5-85 Units/litre. The sensitivity is greatest when alcoholics and chronic heavy drinkers are compared to teetotallers and infrequent social drinkers.[18]

Mean Corpuscular Volume (**MCV**) is the average volume of a red blood corpuscle. MCV = Haematocrit/RBC. It is expressed in femtoliters. Normal range is **79-93.3** fl [19]. Elevated erythrocyte macrocytic volume (MCV) is common in alcoholic patients. This change results directly from the effect of alcohol on erythroblast development and persists as long as drinking continues (Buffet et al. 1975; Morgan et al. 1981; Whitehead et al. 1985). [11] The actual mechanism by which alcohol causes an increase in MCV appears to include a direct toxic effect of alcohol on red blood cells, folic acid deficiency secondary to alcohol abuse and hepatic damage. MCV has overall sensitivity of 40-50% both in hospital environments and particularly in primary health care, with an overall sensitivity of **40-50%,** but its specificity is high **(80-90%)** and very few teetotallers and social drinkers will have elevated MCV values. [20-22]

To treat people with alcoholism clinicians need tools that can properly assess not only the patient's past and recent drinking activity but also any history of drinking problems in the family that they may have. Biochemical substances in the body that can indicate the presence or progress of a condition, or any genetic predisposition toward it, are called **biomarkers.** Both Gamma-Glutamyl Transferase and Mean Corpuscular Volume are biomarkers of alcohol. [23] Despite the fact that biomarkers do not fully mirror the drinking behaviour, they can enhance the credibility of the research because they are not vulnerable to dissimulation by the subject. [11]

AIM AND OBJECTIVES:

1. To measure and compare GGT and MCV in patients of alcohol dependence and controls
2. To compare GGT and MCV in uncomplicated alcohol withdrawal patients and alcohol withdrawal patients with convulsions
3. To measure the sensitivity and specificity of GGT and MCV as biomarkers in alcohol dependence

METHODS AND MATERIALS:

This study was a hospital based case control study carried out in a tertiary medical institution located in the upper part of Assam, India. The study duration was one year (August 2016-July 2017). The study received the ethical approval from the institutional review board. An informed written consent was obtained from every participant and they were free to withdraw their consent at any point of time. The total sample size was 200 (100 cases and 100 controls). The cases were selected from inpatients, admitted in the institution between August 2016 and July 2017, who were diagnosed as **Alcohol Dependence Syndrome** or **Alcohol withdrawal state** with or without **Delirium Tremens** as per ICD-10, who fulfilled the inclusion and exclusion criteria and gave an informed written consent for participating in the study. In patients of Delirium Tremens written consent was taken from one adult family member (spouse/son/daughter) accompanying the patient. The diagnosis was confirmed by consultant Psychiatrist of the same institution. It was seen from previous admission registers of the institution that on an average around 100 patients of alcohol dependence were admitted in one year in the last 5 years (**2011-2016**). Hence the size of the study group (or case group) was taken to be **100**. An equal number of age and sex matched people from healthy population were selected as controls, fulfilling the inclusion and exclusion criteria. The control population comprised of adult family members accompanying the patient and staff members working in the same institution. They did not have any history of alcohol intake in their lifetime. Informed written consent was taken from each of the subjects and they were free to withdraw their consent at any point of time.

Inclusion Criteria:

Study Group -

1. Patients in the age group of 18 to 65 years.
2. Patients of both the sexes.
3. Cases of Alcohol dependence, Alcohol withdrawal state with or without delirium tremens diagnosed as per ICD-10 and confirmed by Consultant, Department of Psychiatry.
4. Patients giving informed written consent for the study.

Control Group –

1. Age and sex matched controls from healthy population who do not consume alcohol.
2. Persons giving informed written consent for the study.

Exclusion Criteria:

Study Group –

1. Those with co morbid systemic illness.
2. Those with co morbid mental illness.
3. Those with co morbid other substance abuse.

Control Group -

1. Those with history of hepatitis.
2. Those with any systemic illness or mental illness.
3. Those with history of any kind of substance abuse

Assessment Tools –

- Informed consent form
- The ICD-10 classification of Mental and Behavioural disorders
- Biochemical estimation of GGT by bio chromatic rate technique
- Estimation of MCV by automated haematology analyzer
- SPSS version 16.0 for statistical analysis of data

Procedure – Inpatients in the age group of **18 -65** years admitted within the time period of August 2016 to July 2017, and diagnosed as Alcohol dependence (or alcohol withdrawal state with or without delirium tremens) as per ICD-10, confirmed by the consultant and fulfilling the inclusion criteria and exclusion criteria were included in study or case group. Every consecutive case admitted in the study period was selected in the study group till the total sample size was reached. An equal sex and age matched control group was selected from normal healthy population who did not consume alcohol. Written informed consent was taken from each participant of both the study and control group. They were free to withdraw their consent at any given point of time. Gamma-glutamyl Transferase and Mean Corpuscular Volume were measured from all the participants of both the groups. From study group, blood samples were collected on the very first day of admission for the sake of uniformity. The blood investigations of both the groups were done in the Laboratories of Department of Biochemistry and Pathology of the same institution. Reference interval for GGT was used as followed in Laboratory of Department of Biochemistry but for MCV normal range (79-93.3 fl) was considered as per Harrison's Principles of Internal Medicine (17th Edition, Vol 2). This was because the Pathology Laboratory did not specify any normal range for MCV in their test results. Analysis of the observed data was done using tests like **Chi square test and unpaired sample t-test** in **SPSS** windows version 16.0. The significance threshold for the tests were set at **$p<0.05$**. Pie charts and bar diagram were used for graphical representation of the data.

RESULTS:

In both the study and control group most people were in the middle age group between **30 and 53** years. In both the study group and the control group **59** were in the age group of **30-41 years** and **26** were in the age group of **42-53** years out of the total sample size of 100 each. **Chi Square test** was applied to look for significant difference between the age distributions of the two groups. The test result showed a p-value of 0.910 which is statistically insignificant. The study group had a mean age of **40.47** whereas the control group had a mean age of **38.69**. **Unpaired sample t-test** was applied to look for any significant difference between the mean ages of the two groups The test result

showed a **p value of 0.1425** which denotes that that there was no statistical significant difference between the groups.

Table 1: Distribution of Case and Control on the basis of age

Age (in years)	Case		Control		X2	DF	p-value
	no	(%)	no	(%)			
18-29	7	7	9	9	**0.5357**	**3**	**.910**
30-41	59	59	59	59			
42-53	26	26	26	26			
54-65	8	8	6	6			

p-value significant at < 0.05, DF – Degree of Freedom, X2 – Pearson Coefficient

Table 2: Mean age distribution of case and control

	Case		Control		p-value
Age (in years)	Mean ± S.D	Range	Mean ± S.D	Range	**0.1425**
	40.47±8.456	20-60	38.69±8.640	22-60	

***p value significant at <0.05**

Both study and control group comprised of 98 males and 2 females respectively. On applying Chi Square no significant difference was found in the distribution of participants in both the groups on the basis of gender.

Table 3: Distribution of case and control according to Gender

Gender	Case		Control		X2	DF	p-value
	no	%	no	%	0.000	1	**1.000**
Male	98	98	98	98			
Female	2	2	2	2			

***p value significant at <0.05**

Figure 1: Pie Diagram depicting the diagnosis of cases as per ICD-10

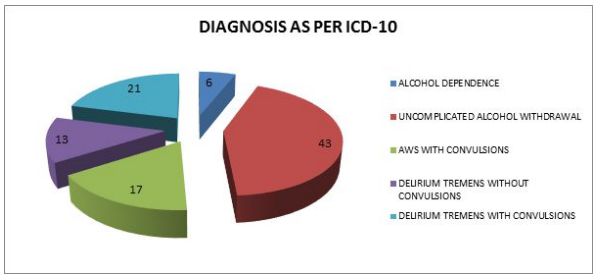


Table 4: Distribution of Case and Control according to Serum GGT level

Serum GGT (5-85 U/L)	Case		Control		p-value
	no	(%)	no	(%)	**<0.0001***
Normal	5	5	99	99	
Elevated	95	95	1	1	
Mean ± SD	555.33 ± 661.43	33.25 ± 1.93			

***p value significant at <0.05**

Table 4 shows that the mean GGT level in study group was 555.33 ± 661.43 (mean ± standard deviation) whereas the mean GGT level in the control group was 33.25 ± 1.93. On applying unpaired sample t-test p value came out to be <0.0001 which denotes that there is significant difference in GGT activities between the two groups.

Table 5: Distribution of Case and Control according to Mean Corpuscular Volume

MCV (79-93.3 fl)	Case		Control		p-value
	no	(%)	no	(%)	**<0.0001***
Decreased	7	7	48	48	
Normal	32	32	47	47	
Elevated	61	61	5	5	
Mean ± SD	94.22 ± 8.44	81.00 ± 8.25			

***p value significant at <0.05**

Table 5 shows that the mean MCV level in study group was 94.22 ± 8.44 (mean ± standard deviation) whereas the mean MCV level in the control group was 81.00 ± 8.25. On applying unpaired sample t-test, p value came out to be <0.0001 which denotes that there is significant difference in MCV between the two groups.

Table 6: Comparison of Hepatic Enzymes in Cases of uncomplicated AWS and AWS with convulsions

Hepatic Enzyme	Uncomplicated Alcohol Withdrawal State (AWS)		Alcohol withdrawal State (AWS) with convulsions		p-value
	Mean	SD	Mean	SD	
GGT	271.35	249.29	646.29	586.78	0.0009*

***p value significant at <0.05**

Table 6 shows the mean GGT levels in uncomplicated alcohol withdrawal cases and alcohol withdrawal cases with history of convulsions. A p value of < 0.05 indicates that there is significant difference in GGT activities between uncomplicated alcohol withdrawal state and alcohol withdrawal state with convulsions.

Table 7: Comparison of Mean Corpuscular Volume in Cases of uncomplicated AWS and AWS with convulsions

	Uncomplicated Alcohol withdrawal state		Alcohol withdrawal State (AWS) with convulsions		p-value
Mean Corpuscular Volume (79-93.3 fl)	Mean	SD	Mean	SD	**0.9487**
	93.281	8.089	93.435	8.909	

***p value significant at <0.05**

Table 7 shows that the mean MCV in uncomplicated alcohol withdrawal cases is 93.281 ± 8.089 whereas in alcohol withdrawal cases with convulsions mean MCV is 93.435 ± 8.909. Unpaired sample t test gives a p value of 0.9487 which indicates that there is no significant difference in MCV between these two groups.

Table 8: Sensitivity and specificity of GGT as a marker of alcohol

GGT	Case	Control	Total
Positive	95 (True Positive)	01 (False Positive)	96 (Test Positive)
Negative	05 (False Negative)	99 (True Negative)	104 (Test Negative)
	100 (Case)	100 (Control)	200 (Case + Control)
Sensitivity = True Positive/Total no of cases X 100 = 95% Specificity = true Negative/Total no of controls X 100 = 99%			

Table 9: Sensitivity and Specificity of MCV as state marker of alcohol

MCV	Case	Control	Total
Positive	61 (True Positive)	5 (False Positive)	66 (Test Positive)
Negative	39 (True Negative)	95 (True Negative)	134 (Test Negative)
	100 (Case)	100 (Control)	200 (Case + Control)
Sensitivity = True positive/total no of cases X 100 = 61% Specificity = True Negative/total no of controls X 100 = 95%			

Table 8 and **Table 9** show the sensitivity and specificity of GGT and MCV respectively as markers of alcohol. From Table 8, it is seen that GGT has a sensitivity of 95% and a specificity of 99% whereas from Table 9, it is seen that MCV has a sensitivity of 61% and a specificity of 95%. Sensitivity and specificity of these two tests have been measured considering clinical diagnosis of alcohol dependence as the gold standard.

Figure 2: Bar Diagram showing Mean GGT levels in case and control group

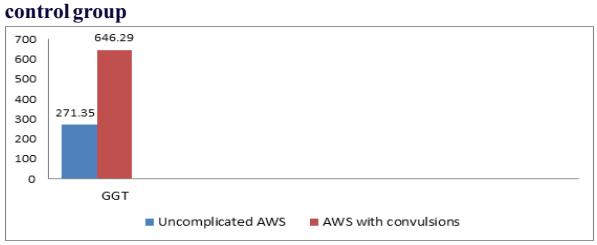


Figure 3: Bar Diagram showing Mean MCV levels in case and control group

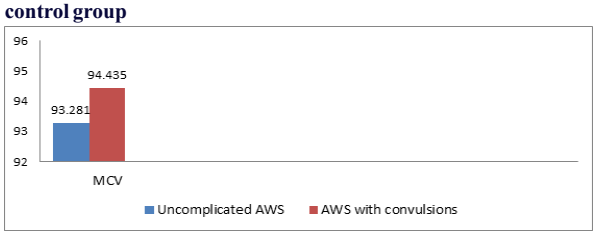
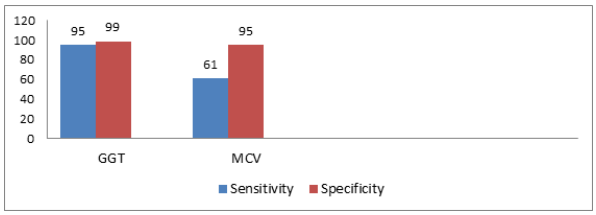


Figure 4: Sensitivity and Specificity of GGT and MCV as markers of alcohol



DISCUSSION:

Most of the subjects in both the study and control group belonged to the middle age group. The mean age for the study group was **40.47** years whereas the mean age for the control group was **38.69** years. There was no significant difference between the mean ages of the two groups. Majority of subjects in both the study and control groups were males (**98%** in both groups). There was no significant difference when it came to distribution of subjects in both the groups on the basis of gender. This was an expected finding as an age and sex matched control group was selected for the study sample. Our findings are in accordance with the findings of **Pitkänen et al**. [24] who found that level of alcohol use was significantly higher in men, **Jean H. Kim et al.** [25] who reported that prevalence of alcohol abuse and alcohol dependence were higher among men than women and **Juliana Gabrielle Martins-Oliveira et al.** [26] who found that male adolescents were more likely to develop alcohol dependence in comparison to females**.**

In the present study it was seen that **GGT** activity in the study group was significantly higher than in the control group. Our finding was in accordance with the findings of **Subir Kumar Das et al. 2005** [27], who reported significant increase in GGT activity in alcoholics in comparison to healthy controls; **Alatalo et al. 2008** [28] who reported that in heavy drinkers, serum GGT was significantly higher than in moderate drinkers or abstainers; **Honnamurthy et al**. [29] who found that activity of gamma glutamyl transferase (γ-GT) was significantly higher in alcohol dependence syndrome in comparison to healthy controls; **G.Skude et al. 1977** [30] who found that, among a total of 182 male chronic alcoholics 69% had increased level of GGT; **J.B. Gogoi et al.** [31] who reported that the enzyme activity of GGT in serum is increased in average by 527.31% in patients with alcoholic hepatitis compared to normal control subjects; **Salma Mahaboob R et al.** [32] who reported that GGT levels were raised in alcoholic liver disease; **Maria Franzini et al.** [33] who found that all fractions of GGT (obtained by chromatographic fractional analysis) were significantly increased in alcoholics and **Osaretin Albert Taiwo Ebuchi et al.** [34] who reported that the activities of AST, ALT, ALP and GGT in the non drinkers were significantly lower than in moderate or heavy drinkers of alcohol in both males and females.

In this study a comparison was also made between the activity of GGT in cases with uncomplicated alcohol withdrawal state and in alcohol withdrawal cases with convulsions (complicated). The result of this comparison showed that **GGT** activities were significantly higher in alcohol withdrawal cases with convulsions, than those with uncomplicated alcohol withdrawal state. Our finding was in line with the finding of **Carrie M. Goodson et al. 2014** [35] who reported that higher initial **GGT** was seen in patients with incident alcohol withdrawal seizures. This indicates that high GGT levels in alcohol dependence could be a risk factor for alcohol withdrawal seizures.

In the present study **MCV** of the study group was significantly higher than the control group. Our finding was in line with the findings of **R.J.L Davidson et al** [36] who reported raised MCV levels in alcoholics in the range 100-108 fl; **A.Wul Chanarin et al.** [37] who found that, among 63 patients regularly drinking more than 80g of ethanol, raised MCV was seen in **89%** of them generally unassociated with anemia; **Heidi Koivisto et al.** [38] who reported that highest MCV occurred in the alcoholics. There was however no significant difference in MCV between uncomplicated alcohol withdrawal cases and alcohol withdrawal cases with convulsions. It was contradictory to the finding of **Peter Metcalfe et al. 1995** [39] who reported that MCV was successful at predicting complicated withdrawals.

This study also measured the sensitivity and specificity of GGT and MCV as biomarkers of alcohol considering clinical diagnosis of alcohol dependence as the gold standard. Sensitivity of GGT and MCV were found to be 95% and 61% respectively whereas specificity of both GGT and MCV were on the higher side (99% and 95% respectively). This indicates that both markers are highly specific but in terms of sensitivity GGT has clear-cut superiority over MCV. Our findings were in line with **Raymond Schwan et al**. [40] who reported that **GGT** is a more sensitive marker of alcohol than **MCV**; **Scwarz et al.** [41] who reported that **GGT** is more sensitive than **MCV** (71.3% VS 64.4%); **Arumalla Virendra Kumar et al.** [42] **who** reported that GGT is a more sensitive marker of alcohol than MCV and **Reynaud et al.** [43] who reported that **GGT** is more sensitive than **MCV** as a marker of alcohol abuse. However our findings were contradictory to the findings of **Gotz Mundle et al.** [44] who reported that **MCV** was a more sensitive marker than **GGT** when patients were abstinent for **> 4 days** before the blood test and **Helen McDonald et al. 2013** [45] who found that sensitivity of GGT and MCV as alcohol biomarkers were below **60%**.

CONCLUSION: Both GGT and MCV are significantly elevated in patients of Alcohol Dependence. Higher GGT levels in alcohol withdrawal patients with convulsions indicate that it could be a risk factor of alcohol withdrawal seizures. However future research in this area is mandatory to validate our finding in this regard. Both GGT and MCV have high specificity which means that abstainers will seldom show elevated levels of these two parameters. But in terms of sensitivity GGT is clearly the superior marker between the two. Limitations of this study included its cross sectional design as the possibility of cohort effects could not be ruled out, last day of drink being not assessed which could have lead to variation of results and lack of investigations for screening the control population.

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