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Evaluation of Serum Immunoglobulins IgG, IgA, IgM and Total IgE in Chronic Alcoholics: A Community-based Study

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Abstract

Long-term intake of alcohol affects virtually every organ in the body including the immune system. The relation between alcohol abuse and immunoglobulin production has not been studied in Indian population. Serum levels of immunoglobulins (total IgE, IgG, IgM, and IgA) therefore, were analyzed in adult chronic alcoholics and were correlated with different epidemiological and alcohol-related parameters. The results showed that 98% of alcoholics had abnormal immunoglobulin levels and 92% showed high or very high total serum IgE levels compared to 24% of the control group. Long term and moderate consumption of alcohol were also associated with frequent infections. Serum immunoglobulin assay including total IgE may be helpful in screening and assessment of chronicity of alcohol abuse.

Keywords: Alcohol; Immunoglobulins; Total IgE; Total IgG; Total IgA; Total IgM

Introduction

Long-term intake of alcohol besides having psychosocial and financial impact imposes serious health problems [1]. It affects virtually every organ in the body including immune system [2]. Besides leading to development of liver disease, pancreatitis, heart and neurological problems, chronic alcohol intake is now recognized as common cause secondary immunodeficiency that in turn may cause frequent infections [3]. Alcohol abuse interferes with the host's immune surveillance system and leads to diminished humoral and cell-mediated immune responses against infections. It has been documented in the past that chronic alcohol consumption may alter B cell numbers and causes hypergammaglobulinemia possibly due to antigenic stimulation of B lymphocytes [4]. However, except total IgE, conflicting results have been shown regarding individual immunoglobulins [5-9].

Alcohol abuse is a growing problem of India with a prevalence of moderate to heavy alcohol consumption of 21.4% [10]. In addition more than half of all Indian drinkers fall into the criteria for hazardous drinking, which are characterized by bingeing and solitary consumption to the point of intoxication [11]. With such heavy consumption of alcohol there is a probability of high prevalence of alcohol-related problems like immune imbalance or immunodeficiency. Though literature is available on levels of immunoglobulins in alcoholics but still studies are limited and there is hardly any work comparing the levels of all immunoglobulins together (IgG, IgA, IgM and total IgE) in these individuals. Moreover, results of these immunoglobulins in Indian alcoholics have not been reported so far. This small preliminary study, therefore, makes an effort to assess the serum levels of immunoglobulins IgG, IgA, IgM and total IgE in alcoholics of the Northern region of India.

Methods

Selection of study group and sample collection

In a case-control study, adult chronic alcoholics, otherwise apparently healthy volunteers from general population of Chandigarh (UT) were offered a pre-designed questionnaire and interviewed by a medical/research officer to assess the severity of alcohol abuse and any associated morbidity. Information regarding age at first intake of alcohol, duration of alcohol consumption, type and amount of beverage, rate of infections and other illness were collected followed by an Alcohol Use Disorders Identification Test (AUDIT) score to assess the physical dependency.

Individuals were also enquired about any symptoms pertaining to allergic disorders and episodes of infections (bacterial, viral or parasitic). The information was documented in a separate proforma. Fifty alcoholics who agreed to participate in the study were recruited. Fifty non-alcoholic, age and sex matched volunteers from the same population were also included as control subjects. Volunteers with a history of previous episode(s) of allergy or symptoms related to infections particularly parasitic e.g., presences of worms in stools, were excluded from the study. After an informed written consent from the study participants, about 2-3 ml venous blood was collected in a plane vacutainer taking all aseptic precautions. The samples were immediately delivered to Immunopathology Lab where serum was separated after centrifugation and stored at -20°C till the time of analysis. Hemolyzed samples were excluded from the study.

IgG, IgM, IgA and total IgE assays

Nephelometry was done for determination of IgG, IgM and IgA using commercially available kits (MININEPH, Binding site, Birmingham, UK). Serum samples and reagents were brought to room temperature immediately before analysis. The samples were diluted in 1:11 ratio (40 µl of sample in 400 µl of diluent). Thereafter, samples (10 µl for IgG and 40 l for IgM and IgA) were mixed with 400 µl of buffer and 40 µl of anti-immunoglobulin in a cuvette already placed on cuvette holder on Nephelometer and readings were noted. Serum

levels of IgG, IgM, and IgA were taken as normal when they measured between 658-1837 mg/dl, 40-263 mg/dl and 71-360 mg/dl respectively.

Total IgE levels were measured using ELISA kit (IMMUNOLAB GmbH, Kassel, Germany) as per manufacturer's instructions. Optical densities were determined using an ELISA reader and levels of total IgE were calculated using standard curves performed for ELISA plate. A value of ≥ 100 IU/ml was considered as raised total serum IgE levels. Results obtained were correlated with various parameters including age, diet, infection episodes, amount and duration of alcohol consumption, type of beverage and AUDIT score.

Statistical analysis

Statistical analysis was performed with SPSS version 20 (SPSS, Chicago, IL). Association between alcohol consumption and continuous variables i.e., immunoglobulin levels was analyzed by Independent t-test and Mann-Whitney test. Mean, range and standard deviation were used for nonparametric variables. A p-value <0.05 was considered statistically significant.

Results

All alcoholics were males with mean age of 39 years (range 19-63 years) compared to 35 years (range 24-51 years) in controls (40 males, ten females). Five cases were young and unmarried, and 46 (92%) were non-vegetarian. Seventy-six percent (38/50) were chronic alcoholics consuming alcohol for >10 years. Sixty-eight percent of these (34/50) were heavy drinkers consuming >250 ml/day and frequently amounting to unstoppable drinking (Table 1). Sixty-two percent (31/50) were even unable to work after alcohol intake. AUDIT score among the alcoholics was >30 in 6, 16-30 in 32, and <16 in 12 cases indicating heavy, moderate and mild physical dependency among them respectively. Concern about alcohol abuse and guilt feeling was also found in 82% (41/50) of the cases.

Two of them had tuberculosis while 3 were hypertensive. Sickness-related to infections was seen in 28 (56%) cases with fever being the commonest symptom (32%) followed by a cough (25%), diarrhea (14%) and jaundice (7%). Of the symptomatic individuals, 28% developed one or more of the above symptoms almost every month while 39% had at least one episode every year. The other 33% fell sick after every six months. Forty-three percent of such individuals had either completed or were on treatment for infections or related symptoms. Thirty-six percent (18/50) also got injured at least once as a result of heavy drinking.

One or more immunoglobulins (IgG, IgA, IgM, and total IgE) were abnormal (low or high) in all except one alcoholic (98%) compared to controls (44%). A predominant increase was noted in all immunoglobulins except a decrease in IgM levels in 6% of cases. All four immunoglobulins (IgG, IgA, IgM, and IgE) were raised only in 8% of the cases while 2 or more immunoglobulins were increased in 70% (35/50) cases compared to 22% (11/50) in controls. Isolated immunoglobulin increase (IgG, IgM or IgA) was seen in 20% (10/50) of alcoholics compared to 18% (9/50) controls. High IgG levels were seen in 56% (versus 30% controls), IgA in 26% (versus 12% controls), and IgM in 16% cases (versus 4% controls). IgG, IgA and IgM elevation was more common in alcoholics compared to controls. However, the increase could not achieve statistically significant value (Table 2).

An imperative observation was a statistically significant rise in total IgE levels in 92% of cases (Table 2; Figure 1). In comparison only 24% of controls had high total IgE levels (p-value 0.0001; independent t-test). Besides this, the concentration of total IgE was very high in those consuming alcohol (>1000 IU/ml) compared to control subjects who had only borderline increase (<500 IU/ml) (Figure 2).

On correlating the immunoglobulins levels with nonparametric variables among alcoholics, high IgE levels were found to be associated with non-vegetarian diet, smoking, frequent infections, frequent and heavy drinking and high AUDIT score (Table 1). In control subjects also raised immunoglobulin levels were associated with smoking and non-vegetarian diet. IgG too showed a pattern similar to IgE except that those taking <80 ml/day had normal IgG levels. IgA levels were associated with non-vegetarian diet, smoking, and Desi Sharab (locally prepared) than another type of drinks. Raised IgM levels were seen in 16% cases only.

Discussion

The study provides important information on immunoglobulin status after prolonged and excessive intake of alcohol in Indian adult population. A positive association was found between smoking, non-vegetarian diet and heavy alcohol consumption. Immunoglobulin levels were abnormal in those with regular, moderate and heavy alcohol consumption and the majority of these alcoholics were consuming either whiskey or Desi Sharab both of which contain a high quantity of alcohol.

In the past studies, smoking has been proved to alter serum immunoglobulin levels [12,13]. It has been shown that serum IgG and IgA levels are lower in smokers as compared to non-smokers with minimal effect on IgM levels [14]. This is in contrast to alcoholics in our study who showed raised IgG and IgA levels. Hence it is unlikely that in the present study smoking influenced the serum IgG, IgA and IgM values. On the contrary, alcohol and smoking both increase total IgE levels. But the effect of smoking probably is minimal since 2/3rd of smokers in a study by Bahna et al. though had higher total IgE compared to non-smokers but the levels were within normal range (<100 IU/ml). On the contrary, heavy smokers showed a drop in IgE [15]. This could be an explanation for higher mean value of total IgE in non-smokers than smokers observed in our study.

Despite high total IgE, the mean value was lesser in non-vegetarians than in vegetarians. This could be because of the fact that non-vegetarian alcoholics were more in number (46/50 non-vegetarians versus 4/50 vegetarians) and among them few had only mild elevation in total IgE whereas, vegetarians were very few and had higher IgE levels. This could have resulted in wide variation and lower mean value in non-vegetarians. On the whole, the diet alone hardly has any impact on immunoglobulin levels [16]. In the present study whether the association between non-vegetarian food, alcohol and immunoglobulins is true or an effect of chance remains to be confirmed.

The mean value of total IgE was higher in heavy drinkers (>500 ml/day) compared to moderate drinkers (<500 ml). Another group which showed a marginal increase comprised of those with an intake between 100-250 ml/day. The reason for this may be the long term effect of alcohol as many of these were consuming 100-250 ml/day for 10-20 years.

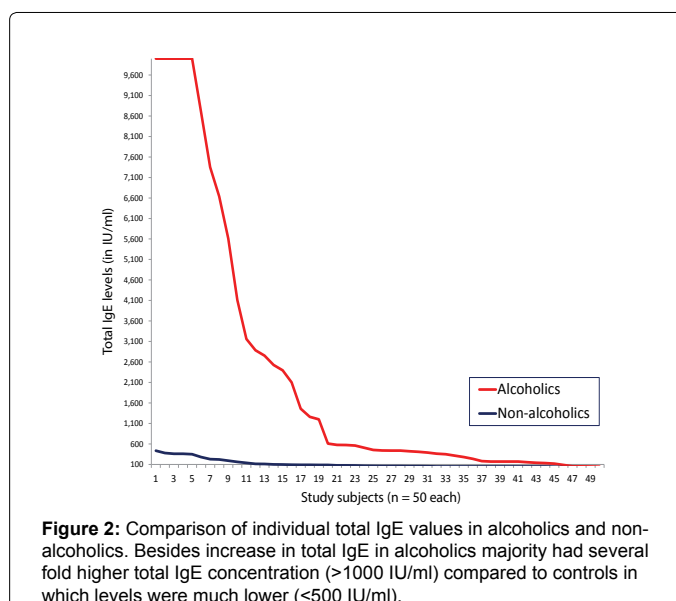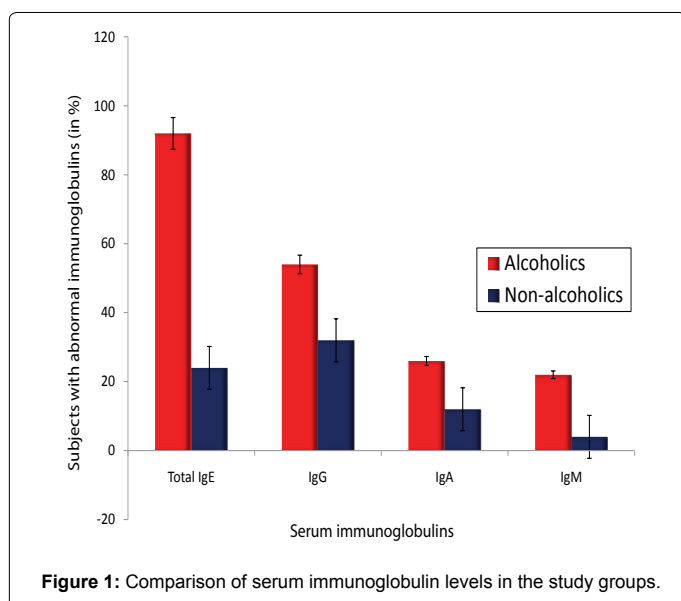Chronic alcoholics also had frequent episodes of infections in the form of fever, cough, and diarrhea, etc. Though the study subjects were enquired for different type of infections (e.g., presenting with fever, cough, respiratory distress, increased frequency and burning micturition, duration and type of diarrhea, passage of worms in stool etc), the exact type of infections could not be confirmed. Among

Variables	Alcoholics (n=50)	Total IgE Mean (range)	High IgE (In %)
Diet			
Non-vegetarian	46	1348 (<50->10000)	89.1
Vegetarian	4	1752 (70-5611)	50.0
Smoking			
Yes	24	1393 (56->10000)	94.3
No	11	1728 (45-5611)	81.8
No information provided	15	-	-
Frequent infections			
Yes	28	1722 (45->10000)	93.3
No	2	336 (56-280)	6.6
No information provided	20	-	-
AUDIT score			
<16	12	786 (56->10000)	83.3
>16	38	1591 (45-10000)	92.1
Frequency of drink			
Less than 4 times/week	3	2530 (86-7653)	66.6
More than 4 times/week	42	2468 (45->10000)	92.8
No information provided	5	-	-
Amount of drink/day			
<100 ml	3	3754 (170-8696)	100
100-250 ml	13	1339 (80->10000)	92.3
250-500 ml	13	1009 (56->10000)	84.6
>500 ml	21	1277 (<50->10000)	90.5
Duration of drinking			
<10 years	9	3177 (153-8696)	100
10-20 years	28	680 (53->10000)	89.2
>20 years	10	1767 (45-6639)	80.0
No information provided	3	-	-
Type of liquor			
Whisky	23	1212 (<50->10000)	95.6
Desi sharab	16	1805 (45->10000)	93.7
Other	6	1638 (56-8696)	66.6
All	5	2363 (53->10000)	80.0

Table 1: Correlation of total IgE with various parameters among the alcoholics.

Immuno-globulins	Alcoholics (n=50)			Non-alcoholics (n=50)			p value
	Abnormal (in %)	Mean (σ)	Range	Abnormal (in %)	Mean (σ)	Range	
IgE (IU/L)	92	1389 (3276)	45-8696	24	100 (94)	17-435	*<0.0001*
IgG (mg/dl)	54	1957 (624)	993-3606	15	1909 (613)	793-3936	0.697
IgA (mg/dl)	26	302 (114)	103-567	12	311 (183)	72-113	0.339
IgM (mg/dl)	22	170 (94)	36-373	4	144 (88)	28-332	0.789

Table 2: Comparison of serum immunoglobulin levels in alcoholics and non-alcoholics.

Figure 1: Comparison of serum immunoglobulin levels in the study groups.

Figure 2: Comparison of individual total IgE values in alcoholics and non-alcoholics. Besides increase in total IgE in alcoholics majority had several fold higher total IgE concentration (>1000 IU/ml) compared to controls in which levels were much lower (<500 IU/ml).

different infections, parasites have been shown to be associated with increased total IgE levels [17]. Though, a possibility of coexisting parasitic infestation in occasional cases cannot be ruled out, an increase of IgE due to parasite in all cases is unlikely. The increased frequency of microbial infection observed in our study could be because of development of secondary immunodeficiency state after prolonged and excessive intake of alcohol as suggested by some Western studies [18,19].

There was a statistically significant association (p-value 0.0001) between alcohol abuse and raised total IgE. Similar to our findings, few other studies have found a relation between alcohol abuse and raised total IgE [10,20-22]. A recent Korean study showed an increased prevalence of allergic sensitization to house dust mite in alcoholics [20]. There are few other studies also suggesting an association between increased IgE and allergic sensitization in alcoholics [22-24]. In control subjects who showed high total IgE levels (>500 IU/ml), specific IgE levels were measured against common allergens (house dust, tree, and food allergens) to detect an allergic component. Two out of 10 individuals were found to be positive for 1 or more allergens. Both of these subjects had very high total IgE levels (>10000 IU/ml) and were excluded from the study.

We in our study noted that besides high levels of total IgE, though statistically not significant, IgG, IgA and IgM levels were also increased except a decrease in IgM in a small number of cases. Gluud et al. in their study on patients with alcoholic cirrhosis have also shown occasionally increased levels of IgG, IgA, and IgM [6]. Gonzalez et al. reported deranged IgG and IgM levels and statistically significant rise in IgA levels in alcoholics [8]. McMillan et al. on the other hand, found a decrease in IgG and IgM and an increase in IgA levels in alcoholics and smokers [25]. There are other studies also in which statistically significant increase in IgA was associated with alcohol abuse [5,23,26]. In the present study though IgA levels were increased, however, to find out its statistical significance large number of cases need to be studied.

Immunoglobulins are raised once the liver damage has begun, and their values may be directly proportional to the degree of alcohol-induced liver damage. Abnormal immunoglobulin profile may be helpful in predicting the onset and degree of liver damage in alcoholics. Also, there are certain liver disorders, for example, autoimmune hepatitis that also show raised immunoglobulins, particularly IgG [27]. Alcohol abuse may sometimes be confused with such conditions. The results of biochemical tests to assess the degree of liver damage could have helped in better judgment of the association between alcohol abuse and immunoglobulins. Unfortunately liver function tests could not be performed which is a limitation of this study.

In conclusion, a complete panel including IgG, IgA, IgM and IgE may serve as a good laboratory parameter to screen and assess the chronicity of alcohol abuse. It may also be used to avoid confusion with other liver disorders and their misdiagnosis. Further studies, with addition of microbiological and biochemical parameters, if done on larger cohorts may provide additional information on utility of immunoglobulins panel in chronic alcoholics.

Acknowledgements

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