The Effect of Short Term Alcohol Abstinence on Neutrophil Phagocytic Properties

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Abstract

Background : Alcohol dysregulates the innate immunity in numerous ways, making alcohol dependants more susceptible to varied forms of infections and immunological assaults.

Objective : The current study on alcoholics throws light on the consequence of short term alcohol abstinence on neutrophil phagocytic power.

Method : 21 subjects meeting the inclusion and exclusion criteria were enrolled for the study.Their blood samples were collected before and after a period of abstinence and studied for neutrophil phagocytic index using *Candida* phagocytic assay test.

Result : An increase in the Mean Particle Number(MPN) of phagocytosis was observed in all the individuals after the alcohol-free period.

Conclusion : The improvement in phagocytic ability of neutrophils post-abstinence provides us an insight into the ways alcohol manipulates the defense mechanism of the human body .Understanding this might help us in exploring novel ideas to circumvent the mortality and morbidity in alcoholics.

Keywords : *Alcohol abstinence , Neutrophil phagocytosis, Candida albicans phagocytosis assay, Mean Particle Number*

Introduction

Alcoholism, or alcohol dependence is a chronic disease that interferes with physical and mental health, and with the family and social responsibilities. Alcoholism is incriminated as the leading risk factor in developing countries with high mortality rates, and ranks third in developed countries.[1]

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In India, alcohol abuse has emerged as a major public health problem, with 21% of the adult men indulging in the same. About 14 million people of this group are dependent drinkers requiring "help" .[2]

Several studies have asserted that alcohol suppresses several leukocyte functions like adhesion, chemotaxis, phagocytosis, superoxide anion production and oxygen metabolism .[3]

The pro inflammatory immune responses and the impaired anti-inflammatory cytokines caused by chronic alcohol, have a major role in the pathogenesis of alcoholic liver disease, pancreatitis, and numerous other organ and tissues injury. [4]

Factors that contribute to the high incidence of infections among alcoholics include breakdown of

local protective barriers, aspiration, exposure, and malnutrition .[5]

Animal experiments by Preheim LC et al [6] showed that the alcohol neutrophils phagocytosed bacteria efficiently, but did not effectively kill all strains of *Pneumococcus.*

In the present experimental study, an attempt is made to evaluate the effect of alcohol abstinence on neutrophil phagocytosis using the phagocytosis assay. [7,8]

We aim at contributing to the knowledge of the immune-modulatory role of chronic alcohol exposure on neutrophil related functions which may exert the pathophysiological assault, thereby allowing a better insight into the effect of alcohol on our body's defence mechanism.

Aims and Objectives

To assess the phagocytic activity of neutrophils, in alcohol dependents before and after abstinence

Materials and Methods

Study design: Before and After Comparison study.

Type of study: Non-randomized Interventional study

Study site: The study was conducted in the Department of Physiology, after recruiting alcoholic individuals from a Rehabilitation Centre , in Bengaluru.

Duration of study: May 2018 – June 2018.

Number of subjects: 21 alcohol dependent males

Ethical clearance and informed consent : Taken

Inclusion criteria:

1. Men in the age group between 25 and 50 years.

2. Men with a history of alcohol dependence.

Exclusion criteria:

1. History of diabetes and hypertension.

2. History of cardiac pathology.

3. History of neurological, psychiatric and endocrine disorder.

4. Subjects with hepatic cirrhosis.

5. Cases of any autoimmune disorder.

6. History of any acute or chronic infections.

7. Smokers.

8. History of any carcinoma

9. Hematological disorders.

Choice of subjects and control: 25 alcoholic males were enrolled for the study. Excluding four subjects as per the exclusion criteria, the study group contains 21 subjects.

Study Protocol:

Following the collection of baseline data and clinical examination, under strict aseptic precautions, 3 ml of venous blood samples was collected in a heparinized vial, twice from each subject, one at the time of enrollment and the other after 40 days of total alcohol abstinence..

Phagocytosis Assay:

A test tube with 1ml of the blood, 1 ml of gelatin and 1ml of Phosphate Buffered Saline (PBS) was allowed to stand for 45 min. The RBCs sediment at the bottom. The supernatant was centrifuged at 3000 rpm for 2 minutes. The upper layer was discarded. 2ml of PBS was added to the remaining cell pellet, mixed well and the solution was centrifuged at 3000 rpm for 2 min. The resulting supernatant was discarded.1ml of PBS was added to the residual cell pellet and mixed well to get a solution rich in neutrophils.

A suspension of *Candida albicans* prepared in 0.9 % saline was used as the indicator to determine the phagocytic function of neutrophils. [9]

For every subject, two test tubes labeled **'Control'(C)-** pre-abstinence and **'Test'(T)-** post abstinence- were taken. To both (C) and (T), 250 µL of the neutrophil enriched solution,250 µL of the *Candida* suspension, 250µL of Hank's balanced salt solution and 250 µL of serum were pipetted. The test tubes were incubated at 37°C for 30 minutes. Two thin smears (C and T) were prepared from the bottom part of the

mixture, dried, fixed with methanol and stained with Giemsa. After 10-15 minutes, the slides were washed under running water, air dried and then observed under 100x. The mean number of *Candida* cells phagocytosed per neutrophil was calculated and expressed as mean particle number (MPN) for both (C) and (T).

The characteristics of the study subjects were tabulated for description using frequencies. The Phagocytic MPN pre- and post-abstinence were compared.

Statistical Methods

The data was compiled in Microsoft Excel sheet. Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group.

Paired Proportion test has been used to find the significance of proportion in paired data. The statistically significant figures considered were:

+ Suggestive of significance (P value: $0.05<P<0.10$)

* Moderately significant (P value:$0.01<P \leq 0.05$)

** Strongly significant (P value: $P \leq 0.01$)

Results

Assessment of Phagocytic MPN -

The ability of the neutrophils to phagocytize *Candida* was assessed by MPN. The number of dead *Candida* cells was expressed as Mean Particle Number (MPN).

Table 1: Comparison of Study variables before and after rehabilitation

Variables	Before Rehab	After Rehab	Difference	t value	P value
Phagocytic MPN	1.63±0.47	2.39±0.69	-0.752	-4.863	<0.001**

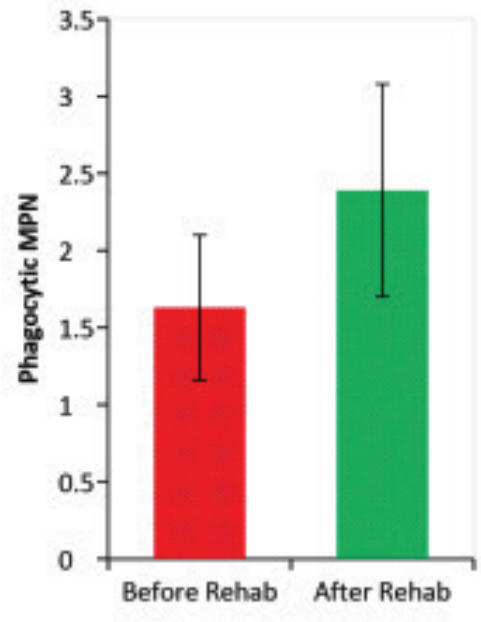

Figure-1

Statistically significant difference was observed in Mean Particle Number (MPN) of ingested *Candida,* by the phagocytizing neutrophils in alcohol dependent subjects, before and after rehabilitation (2.39±0.69 vs 1.63±0.47, t=-4.863, p= <0.001**). (Table- 1, Fig-1)

Discussion

The results of the present study indicate that there was a significant increase in Phagocytic MPN post abstinence, as compared to control samples. This perpetual increase in neutrophil activity may be due to the release of large amounts of various mediators, such as leukotriene B4, IL-8, and TNF, that occurs in the presence of chronic driving inflammatory process in the alcoholic subjects.[3]

Thus, the results hint at a possible pathogenic and immunomodulatory mechanism of alcohol on the neutrophil metabolic activity and their phagocytic functions. This is in consensus with a vivo human and animal study by Szabo et al [10] that alcohol itself is a potent modulator of the immune system at various levels.

Whether this neutrophil "transformation" is the result of alcohol dependence or an indicator of

pathogenesis of alcohol induced stress in stimulating the immune reactions towards both tissue injury and mortality is yet to be ascertained. A large number of studies on a wider spectrum of chronic alcoholics need to be performed to further elucidate this property.

Our results may have implications in the selection of patients for anti-inflammatory strategies and Immunosuppressive therapy. Targeting neutrophil related dysfunctions with molecular or biochemical techniques may reduce mortality due to alcohol induced assault at various levels.

CONCLUSION :

Our study demonstrates that the *Candida* –cidal arsenals could be impaired in alcohol dependents with altered immune functions, which is evidenced by a significant functional improvement after a short period of abstinence.This may form the basis for a strategy to improve the selection of alcohol dependents for current therapies and suggest new therapeutic approaches for rehabilitative management, aiming to identify persons in different levels of alcohol dependence, even before serious harm has been incurred.

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Conflict of Interest : Nil

Source of Funding : Self

Ethical Clearance : Taken

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