

The Study of Influence of Psychosocial Rehabilitation on the Immunomodulatory Role of Alcohol Dependence, on Neutrophil Oxidative Burst Mechanism

Sanjay M. Goudar¹, Sushma S.²

¹Fourth year MBBS Student, Bangalore Medical College and Research Institute, Fort, K.R. Road, Bengaluru-560002, ²Assistant Professor, Department of Physiology, Bangalore Medical College and Research Institute, Fort, K.R. Road, Bengaluru-560002

Abstract

Background: Alcohol is known to affect the immune system in diverse ways. Various studies have brought to light changes in both innate and acquired immunity in alcohol dependant subjects, making them more vulnerable to infections as compared to the general population.

Objective: The current study emphasizes on the impact of alcohol on neutrophil oxidative burst mechanism and the changes in the same after a period of abstinence.

Method: of the 25 alcohol dependant subjects, 21 subjects who met the inclusion and exclusion criteria were included in the study. SADQ questionnaire was administered to group them into mild, moderate and severely dependant. Their blood samples were collected before and after a psychosocial rehabilitation programme and studied for neutrophil oxidative burst phenomenon using nitro blue tetrazolium dye reduction test.

Result: There was an increase in oxidative burst in all the subjects after the rehabilitation, however the increase was significant in mild and moderately alcohol dependant subjects.

Conclusion: The impaired oxidative burst in alcohol dependant subjects may pave a way for infections. Hence an early detection of dysregulated immunity in alcohol dependant subjects may help decrease the morbidity and mortality.

Keywords: Alcohol dependance, Neutrophil Oxidative Burst, SADQ questionnaire, Nitroblue Tetrazolium Dye Reduction Test.

Introduction:

Neutrophils occupy a primal position in the non-specific immune response, more so in anti-bacterial defense mechanism as effectors, inducing and regulating cells.¹

Neutrophils adhere to vascular endothelial cells, migrate to inflammatory foci, recognize and phagocytose opsonized bacteria.² These processes are augmented by chemotactic factors which enhance the metabolic activity of neutrophils, their aggregation,³ and bactericidal abilities. The engulfed microorganisms are killed through both oxygen-dependent and independent mechanisms, simultaneously injuring the surrounding tissues.¹

It is reported that there is a causal relationship between alcohol consumption and the occurrence of lung abscess, empyema, spontaneous bacterial peritonitis, diphtheria, cellulitis, meningitis, hepatic cell injury, certain cancers, pulmonary tuberculosis, etc.

Corresponding Author:

Dr. Sushma S., MBBS, MD

Assistant Professor, Department of Physiology,
Bangalore Medical College and Research Institute,
Fort, K.R. Road, Bengaluru-560002

e-mail: suvina76@gmail.com

Phone: 9916915194

worldwide,^{4,5} stemming from an exaggeration of the oxidative stress mechanism⁶ and/or the remodeling of the body's normal immune systems at various levels.⁷

Several studies have asserted that alcohol suppresses several leukocyte functions like adhesion, chemotaxis, phagocytosis, superoxide anion production and oxygen metabolism.⁸

We hypothesized that neutrophil stimulation is associated with impaired immune responses to ongoing bacterial challenge, rendering alcohol dependent subjects more susceptible to infection. Furthermore, although neutrophil dysfunction is reported to contribute to immune paresis in alcohol intake, to date there is insufficient data available in connection with the changes of impaired neutrophil oxidative burst in psychosocially rehabilitated alcohol dependent subjects.

Hence, in the present experimental study, an attempt is made to assess the influence of psychosocial intervention on the neutrophil functions as estimated by superoxide ion release (oxidative burst) and to evaluate the possible correlation, in Indian alcohol dependent males, using the Nitro Blue Tetrazolium (NBT) reduction test.^{9,10}

Our data gives an insight into the conflicting results of clinical trials and may suggest a rationale to select an appropriate, evidence- based, non-pharmacological and psychosocial therapy tailored to the patient.

Aims and Objectives: To study the neutrophilic generation of Reactive Oxygen Intermediates (Oxidative Burst) in alcohol dependent subjects, before and after psychosocial rehabilitation.

Materials and Method

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 the subjects from a Psychosocial Rehabilitation center for Alcohol Dependents, 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 alcohol dependent males attending psychosocial rehabilitation programme were enrolled for the study. Excluding four subjects as per the exclusion criteria, the study group included 21 subjects.

Study Protocol: The experimental procedures were reviewed and approved by the Institutional Ethics Committee. The baseline data of the subjects was recorded by a questionnaire and clinical examination. The Severity of Alcohol Dependence Questionnaire (SADQ) was administered to measure the level of alcohol dependence and they were grouped into mild (score of below 16), moderate (score of 16-30) and severe alcohol dependence (score of 31 or higher).

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 recruitment into the center and the other after 40 days of rehabilitation programme.

NBT (Nitro Blue Tetrazolium) Test for the Detection of Generation of Reactive Oxygen Intermediates: This test utilized stimulated and unstimulated neutrophils for evaluation. *Escherichia coli* (*E. coli*) endotoxin (Hi Media Labs) was used to stimulate the cells. The unstimulated cells act as the control. Two test tubes labelled 'Control'(C) and 'Test'(T) were taken. To both the test tubes, 50µL of NBT dye 0.34% and 100 µL of heparinized blood of the

subject were added. Then, Hank’s balanced salt solution 250 µL in (C) and 200 µL in (T) was pipetted. Finally, 50 µL of the *E. coli* endotoxin was added to (T).

The assembly incubated at 37°C for 20 min was then allowed to cool for 20 min, at room temperature. Smears prepared from both test and control samples were air dried, fixed with methanol, and then stained with Giemsa. After 10-15 min, the slides were washed under running water and allowed to dry. 100 neutrophils were counted under 100x and the % of neutrophils exhibiting the dark blue formazan granules was estimated, for both (C) and (T), and the results were compared 9.

The above experiment was carried out with every subject’s blood sample, before and after the rehabilitation.

The characteristics of the study subjects were tabulated for description using frequencies. The NBT reduction, measuring the oxidative burst of neutrophils, before and after the psychosocial rehabilitation were compared.

Statistical Method: 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 ≤ 0.05)
- ** Strongly significant (P value: P≤0.01)

Results

Among the 21 alcohol dependent males in the age group of 25- 50 yrs, 7 had mild alcohol dependence (SADQ score ≤ 16), 10 had moderate (score 16-30) and 4 had severe alcohol dependence (>31), with Mean ± SD of 25.61 ± 9.8. (Table-1).

Table 1: Alcohol dependents as per SADQ score

SADQ score	No. of alcohol dependents	%
≤16	7	33.33%
16-30	10	47.61%
>31	4	19.04%
Total	21	100.0

Mean ± SD: 25.61 ± 9.

Assessment of Neutrophil Oxidative Burst: The Neutrophil Oxidative Burst was assessed by NBT reduction assay. The stimulated neutrophils sequester the NBT dye into the phagosomes and the intracellular reduction of dye converts it to insoluble blue crystals of formazan.

Table 2: % of Neutrophils showing oxidative burst according to SADQ score before and after rehabilitation

SADQ	% of neutrophils showing oxidative burst before rehabilitation		p-value b/w stimulated and unstimulated	% of neutrophils showing oxidative burst after rehabilitation		p-value b/w stimulated before and after rehabilitation
	Unstimulated*	Stimulated		Stimulated	p-value b/w stimulated and unstimulated	
≤16	24.28 ±	48.28 ±	0.005	64.14 ± 10.45	<0.001	0.018
Mild	14.84	11.31				
16-30	17.6 ± 6.82	46.2 ±	<0.001	58.8 ± 9.57	<0.001	0.1015
Moderate		12.25				
E						
>31	29.25 ±	58 ± 4	0.01582	61.75 ± 12.76	0.02163	0.5952
Severe	16.8					

*% of neutrophils showing oxidative burst in unstimulated state is taken as baseline control

After rehabilitation, the % of NBT positive stimulated cells was 64.14 ± 10.45 vs 48.28 ± 11.31 in mild dependence, 58.8 ± 9.57 vs 46.2 ± 12.25 in moderate, and 61.75 ± 12.76 vs 58 ± 4 in severe alcohol dependents, when compared to pre-rehabilitation. (Table-2, Fig-1) Ethanol resulted in an increase of neutrophil burst activity among all the dependents, after

rehabilitation. However, among the mild and moderate alcohol dependents, there was a statistically significant increase in oxidative burst activity after rehabilitation. There was no statistical difference in oxidative burst response to stimulation in severely alcohol dependents in pre and post rehabilitation.

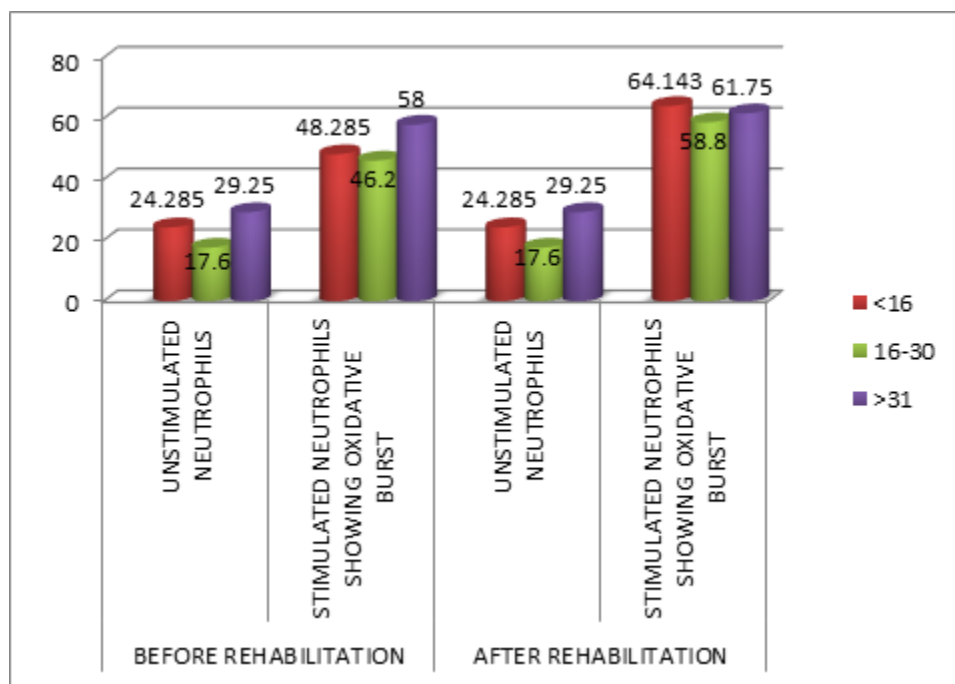


Figure 1: Percentage of neutrophils in different SADQ categories showing oxidative burst, in unstimulated and stimulated states, before and after rehabilitation

Discussion

The results of the present study indicate that there was a significant increase in the % of neutrophils showing Oxidative Burst, in alcoholic dependents post psychosocial rehabilitation, as compared to pre-rehabilitation.

However, the results were significantly more evident in mild and moderate alcohol dependents.

This signifies that neutrophils in alcoholics who are abstained from alcohol intake, tend to show an increased metabolic activity. The alcohol dependents in whom the % of neutrophils showing oxidative burst is high, (indicated by a resting burst greater than or equal to 20-30%) are likely to have a high risk of infection and strategies to remove endotoxin may be more beneficial.

Our findings corroborate with studies by Sato et al.¹¹ in male subjects, who showed a trend of increased ROS production.

Defective neutrophil Oxidative Burst can pave a way for the development of infection. Altogether, these observations suggest the cryptic role of ethanol-induced oxidative stress in stimulating the immune reactions against both allo- and self-antigens, causing tissue injury.

Conclusion

In conclusion our study demonstrated that the Neutrophil oxidative burst could be impaired in alcohol dependents with altered immune functions, Neutrophils in alcohol dependents seem to be much more effective in producing ROS, after a short term psychosocial

rehabilitation . The result of this study has demonstrated the importance of monitoring the neutrophil functions during rehabilitation.

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Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Taken

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