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# **Research Article**

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# Frequency of anti-nuclear antibody and anti dsDNA antibodies in subjects of oral addictive habits

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#### **ABSTRACT**

**Background:** People with addictive habits are prone to both infectious and non-infectious diseases. Conflicting results have been reported about propensity of these individuals for development of autoimmune diseases. Therefore, a study was planned to determine the frequency of anti-nuclear antibody (ANA) and anti-dsDNA (dsDNA) antibody in the serum of habitual smokers, paan (areca nut) chewers and other oral addictive habits as compared to subjects without such addictive habits.

**Methods:** Blood samples from 90 subjects (45 with addictive habits and 45 without any addiction) were taken by random sampling after getting written informed consent. Enzyme linked immunosorbent assay (ELISA) was used to test the sera for ANA and anti dsDNA.

**Results:** One subject in the addictive group had ANA and dsDNA antibodies, whereas in the control group, two subjects had anti dsDNA while none of them had ANA. No significant association of these antibodies was observed between the two groups.

**Conclusions:** Addictive habits do not predispose the subjects to develop autoimmune diseases.

Keywords: ANA, dsDNA, Autoantibodies, Autoimmunity, Smoking

# INTRODUCTION

In Pakistan, an alarming number of people indulge in oral addictive habits e.g. smoking, snuff and tobacco chewing, gutka, sheesha etc. A recent study from Karachi, Pakistan reported that the majority of males and females practice their addictive habits in public.

There are multiple reasons for the consumption of addictive substances that consequently leads to addiction of paan (Areca nut), chaliyah (betel nut), naswar, cigarette and gutka. About 9% males started their addictive habits as a fashion symbol, 41.1% males and

14.2% females started due to peer pressure, while 9.6% males equated it with pleasure. Another study reported that about 100 million people use smokeless tobacco in India and Pakistan. In Southeast Asia, tobacco is used in diverse forms, including cigarettes, sheesha and smokeless tobacco (Naswar, Paan, Gutka etc.).

Cigarette smoke contains hundreds of potentially toxic components which include tars, nicotine, carbon monoxide and polycyclic aromatic hydrocarbons among others. Owing to its nature as an impure mixture, cigarette smoke has multiple known and unknown effects on human body. Two phases of cigarette smoke exist: a

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tar or particulate phase and a gaseous phase, both of which contain extremely high concentrations of free radicals. Further, cigarette smoke activates endogenous sources of free radicals as well.<sup>4,5</sup>

These toxins and free radicals can interact with DNA, and could cause genetic mutations and gene activation responsible for the development of autoimmune diseases. Furthermore, cigarette smoke has been shown to increase the expression of Fas (CD95) on B and CD4 T lymphocyte surfaces. Furthermore, cigarette smoke has been shown to increase the expression of Fas (CD95) on B and CD4 T lymphocyte surfaces. Increasing the sensitivity of these cells to apoptotic signals could add towards the apoptotic material to be cleared by an inefficient clearance mechanism in patients at risk of autoimmunity.

There is plenty of evidence that environmental factors are noteworthy in the development of autoimmune diseases. The concordance rates for autoimmune diseases in monozygotic twins are well below 100% that indicates the interaction of environmental factors with genetics in determining disease susceptibility.<sup>8</sup>

Epstein–Barr virus, cigarette smoking and crystalline silica from agricultural and occupational sources have been associated with the development of multiple autoimmune diseases. Gigarette smoking has been incidentally linked to the development of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), Graves' hyperthyroidism, and primary biliary cirrhosis (PBC).

Cigarette smoking was associated with SLE in a Swedish study, but U.S.A. and Finland based studies reported quite opposite results and researchers were of the opinion that smoking has immunosuppressive effects for production of autoantibodies in patients with SLE. 11-13

Interactions between cigarette smoking, genetic and immunologic factors, such as human leukocyte antigen (HLA)- shared epitope, rheumatoid factor, anti-cyclic citrullinated peptide antibodies, and anti dsDNA antibodies, may point to the mechanisms in disease pathogenesis.<sup>8</sup>

Cigarette smoking has far-reaching and manifold effects on immunity. It can induce both pro-inflammatory as well as suppressive functions. Its influence on immunity depends on many variables, including the dose, route and type of tobacco used, chronicity of exposure, Toll like receptor ligands or other inflammatory mediators.

CS impairs innate defences against pathogens, modulates antigen presentation, and promotes autoimmunity. CS also depresses immunity in the oral cavity and leads to infections of gingival crevices, periodontal disease and oral cancer. The recognition of the specific mechanisms by which CS affects host immunity is an important step towards elucidating tobacco-induced diseases and may help in identification of novel therapeutic approaches for the management of such ailments. <sup>14,15</sup>

Considering the miserable situation of addictive habits among Pakistani population, a study was designed to determine the frequency of ANA and anti-dsDNA in the serum of subjects of smokers, paan and other oral addictive habits.

#### **METHODS**

#### Study subjects and antibody assays

This cross sectional study was carried out in the Department of Immunology, University of Health Sciences (UHS) Lahore Pakistan.

The study strictly followed the WMA Declaration of Helsinki and was approved by the Ethical Review Committee and Advanced Studies and research Board of UHS Lahore. Blood samples of 90 subjects (45 with addictive habits and 45 without such habits) were taken by random sampling after informed consent.

Three ml blood was collected and after centrifugation, serum was separated. Determination of ANA (Generic Assay, Germany) and anti dsDNA (INOVA Diagnostics, Inc. USA) antibodies was performed by Enzyme Linked Immunosorbent Assay (ELISA). Participants with established autoimmune diseases were excluded from this study. All the data were dealt with in an anonymous way.

#### Statistical analysis

SPSS version 20.0 (IBM-SPSS, Inc, Armonk, New York) was used for statistical calculations. An association of autoantibodies with addictive habits was calculated by  $\chi^2$  test. p $\leq$  0.05 was considered as statistically significant.

### **RESULTS**

All the controls, and study group with addictive habits were males and were from a small town/rural area, Khan Garh (Muzaffar Garh, an under developed district of Punjab, Pakistan). Most of the addictive habits group individuals were Urdu language speaking and of Indian origin because their forefathers were migrants from India after Indo-Pak partition in 1947. While only 3 individuals were of Saraiki language speaking, local origin.

Overall mean age of the addictive habits group was 28.88±9.50 years, whereas in the control group it was 26.95±4.21 years. In comparison, the difference in age between two groups was not statistically significant (p=0.215). Other common characteristics of the subjects with addictive habits are given below (Tables 1- 4).

After obtaining general characteristics of both control and addictive habits groups, detection of autoantibodies was carried out by ELISA technique. Only one individual in the addictive habits group had ANA and dsDNA antibodies.

Table 1: Common study variables of subjects with addictive habits.

Variables	Frequency (n, %)		
Marital Status			
Single	25 (55.5%)		
Married	20 (44.5%)		
Educational background			
No formal education	14 (31%0		
Primary	12 (27%)		
Middle	11 (24%)		
High	4 (9%)		
College	4 (9%)		
Oral Hygiene			
Good	17 (38%)		
Poor	28 (68%)		
White Mucosal Spots			
Yes	6 (11%)		
No	39 (89%)		
Oral Submucous Fibrosis			
Yes	7 (15.6%)		
No	38 (84.4%)		
Family history of addictive habits			
Yes	19 (42.2%)		
No	26 (57.8%)		

While in the control group, two individuals had anti dsDNA and none of the subject had ANA. The associations between these two groups were analyzed for ANA and anti dsDNA, a negative association was observed in both groups. Table 4 shows the detailed values in two groups (Table 5).

Table 2: Distribution of subjects with addictive habits on the basis of professions.

Professions	Frequency (n, %)
No job	4 (9%)
Shopkeeper	26 (57%)
Washer-man	3 (7%)
Wood Factory workers	3 (7%)
Laborers	3 (7%)
Tandoorchi	2 (4%)
Carpenter	1 (2%)
Ex-Army	1 (2%)
Farmer	1 (2%)
Driver	1 (2%)

Table 3: Frequency distribution of addictive habits among individuals in addictive habits group.

Addictive habits	Frequency (n, %)
Smoking	07 (15%)
Paan	11 (24%)
Naswar	02 (4%)
Gutka (alone)	0 (0%)
Mixed habits (combination of two or more habits)	25 (55%)

Table 4: Mean duration of different addictions.

Addictive habit	Mean±Duration (years)
Smoking	8.60±10.08
Paan chewing	9.10±7.95
Naswar use	6.78±7.05
Gutka chewing	7.25±7.95

Table 5: Comparison of autoantibodies between control and addictive habits groups.

Antibodies	Positive/Negative (Total controls = 45)	Positive/Negative (Total addictive habits subjects= 45)	Odds Ratio	95% CI (Lower-Upper)	P value
ANA	0/45	1/45	0.978	0.936-1.02	0.5
Anti dsDNA	2/45	1/45	2.04	0.179-23.40	1.000

#### **DISCUSSION**

It is the first study to compare the prevalence of ANA and anti dsDNA antibodies in Pakistani population with and without addictive habits. ANA was detected in 0% and 2.2% in people without and with addictive habits respectively. Other studies reported prevalence of ANA in US population at 13.8%, Japanese as 9.5%, whereas for Indians it was 12.3%. <sup>16-18</sup> The significantly higher prevalence of ANA in these studies might be due to larger study populations, genetic factors, environmental factors and most importantly, gender related findings because the current study comprised of male population only. This comparison of ANA among four different nations formulates the basis for the opinion that ANA

prevalence among adults could vary by demographic and geographic factors.

A study carried out by Azizah et al. reported ANA prevalence at 5.5%, while 0% prevalence of anti dsDNA antibodies in general public of Malaysia. A study conducted in Northern Sweden, reported that autoantibodies can be detected in the serum of individuals who develop systemic lupus erythematosus several years before the appearance of clinical signs and symptoms.

Considering the association of different oral addictive habits with the development of autoimmune diseases, some researchers have reported that smoking protects against production of antithyroid peroxidase antibodies.<sup>21</sup>

In a review article of "Nature Reviews Rheumatology", the authors suggested a link between smoking and autoimmune rheumatic diseases namely rheumatoid arthritis and systemic lupus erythematosus. The researchers had an opinion that smoking in combination with host genetic factors, increases the risk of autoimmunity and affects both progression and prognosis of disease. Muhammad Ammar et al. carried out meta-analysis and came out with an opinion that smoking predisposes to the synthesis of more autoantibodies i.e. ANA, anticitrulinated protein antibodies and rheumatoid factor. ANA

However, we are unable to discuss association of autoimmunity with oral addictive habits other than smoking because literature is scarce in this aspect. Therefore, this pilot study lays down the foundation for future studies about association of oral addictive habits and their predisposition to development of autoimmune diseases, especially in this part of the world where the oral addictive habits are quite strong. People indulge in oral addictive habits at a very young age, have a greater risk of malignant diseases of the head and neck region and may have risk of systemic diseases including autoimmune disorders.

We acknowledge the limitations of the present study as being a pilot study it was conducted on a small sample size without comprehensive consideration of other important factors such as genetic disposition and biochemical factors.

# **CONCLUSION**

Although it can be concluded from the findings of the present study that oral addictive habits do not lead to production of auto antibodies, but studies on a larger sample size should be carried out to investigate this aspect.

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