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SMOKING AND RHEUMATOID FACTOR LEVEL: ASSOCIATION WITH DEVELOPMENT OF RHEUMATOID ARTHRITIS IN KHARTOUM, SUDAN

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ABSTRACT

The incidence of rheumatoid arthritis (RA) has been associated with cigarettes smoking. The aim of our study was to demonstrate the influence of cigarettes smoking on the risk of developing rheumatoid arthritis disease. Rheumatoid factor (RF) analysis for 118 male students and laboratory assistance (73 smokers and 45 controls) in The National Ribat University, Faculty of Medical laboratory Sciences, Khartoum, Sudan was carried out using latex card (qualitative method). For comparison purpose, 25 random selected samples from smoker participants were further measured by Mindary technique (quantitative method). The results revealed that 3 (4.2%) of smokers showed a visible agglutination (positive RF results) by latex card and 3 (4.4%) controls were confirmed positive as well. This study was not sufficiently large enough to allow analysis of certain confounding variables in relation to disease onset and prognosis. Therefore, more studies with larger number of samples are much needed to strongly confirm the association between smoking and the onset of rheumatoid arthritis in Sudan.

KEYWORDS: Cigarettes smoking, Rheumatoid factor, Rheumatoid arthritis, latex method, Mindary technique.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease which typically involves the small joints of the hands and feet, often symmetrically. It affects approximately 1% of the population and is more common in women.[1] The course of RA is variable and unpredictable but for a significant number of patients it is a severe disease resulting in persistent pain and stiffness, progressive joint destruction, functional decline and premature mortality.[2]

RA is an autoimmune disease and until now the initial triggering mechanisms underlying disease development are not well understood.[3] There is abundant evidence that rheumatoid arthritis is immune mediated, but it is uncertain if the initiating agent is an infectious antigen, a self antigen, or both.[4] It is well established that genetic factors, such as human leukocyte antigen (HLA) and environmental factors, such as infection, ultraviolet, radiation and smoking, can affect the development of various autoimmune diseases. Among these factors, cigarettes smoking significantly increases the risk of not only various types of cancer, cardiopulmonary diseases and infections, but also autoimmune diseases, such as systemic lupus erythematosus and RA.

Smoking has been implicated as one of the most important extrinsic risk factors for its development and severity. Recent developments have shed light on the pathophysiology of RA in smokers, including oxidative stress, inflammation, autoantibody formation and epigenetic changes.[5]

Smoking is known to be a risk factor for RF-positive RA. Rheumatoid arthritis patients who smoke are more likely to be RF-positive and to have higher titers of RF than nonsmokers with RA. Indeed, cigarette smoking is also associated with the production of rheumatoid factor (RF) in people without RA. It seems that the amount and duration of cigarettes smoking, but not other tobacco use, is important for the development of RF-positive RA as well as for the development of cardiovascular disease.[6]

The cigarettes smoke contains more than 45,000 chemicals, which have various toxic, mutagenic and carcinogenic effects. The major components of smoke that lead to many of the deleterious and toxic effects include nicotine, tar, ammonia, carbon monooxide, carbon dioxide, formaldehyde, acrolein, acetone, benzopyrenes, hydroxyquinone, nitrogen oxides and cadmium.[7] Cigarettes smoke that contains high levels of tar and nicotine induce greater immunologic changes than cigarettes smoke that contains lower levels of these compounds.[8]

A previous research have shown in a well-characterized group of female patients with RA that a history of cigarettes smoking is associated with more severe disease, although the mechanism for this effect remains unclear.[9]

It has long been known that there is a connection between seropositive (RA) and smoking. The exact underlying mechanism, however, it has only been speculated.[10] Smoking is considered to have a crucial role in the pathogenesis of many diseases and, as a significant part of the population smokes, it is one of the most investigated and well-established environmental factors. Recently, an important gene–environment interaction has been revealed; that is, carrying specific HLA-DRB1 alleles encoding the shared epitope and smoking establish a significant risk for anti-citrullinated protein antibody-positive RA.[11]
RF seropositivity is most commonly determined by agglutination tests, which preferentially detect IgM RF, but several studies indicate that IgA RF may be a better prognostic indicator in RA patients.[12] Automated techniques such as nephelometry and enzyme-linked immunosorbent assays gradually replaced the other semiquantitative methods because of their simplicity and greater reproducibility.[13]

MATERIALS AND METHODS

Sample collection

A total number of 118 males participated in this study and they all attended The National Ribat University, Faculty of Medical Laboratory Sciences. Study subjects were 73 smokers (mean age 22.7 ± 5.6 years) and another 45 non smokers were included as control group. Blood was collected using universal safety precaution, in which 2 ml of blood was collected using standard vein puncture technique, transferred into plain containers and blood was separated by centrifugation at 1500 rpm for 5 minutes. RF testing was applied to all study subjects (smokers and controls) by latex technique, while only 25 random selected smoker's samples were subjected for RF analysis by Mindary method to compare between the two applications. Demographic data were collected in a data collection sheet and informed consent was obtained from each smoker participant.

Rheumatoid factor testing

Qualitative measurement (Latex technique)

One drop (approximately 50 µl) of each smoker serum was transferred into the test circle on the latex card and one drop of the latex reagent was added. Then the drops were mixed using disposable starrier and the test circle was covered by the mixture. Gently and evenly, the test

card was rotated for 5 minutes. After that, agglutination was examined under a strong light source after 2 minutes.

Quantitative method

Colorimetric (Mindary) method

Principle of the test

When an antigen-antibody reaction occurs between RF in a sample and denatured human IgG which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change (550 to 660 nm), with the magnitude of the change being proportional to the quantity of RF in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration and the reference intervals are ≤ 18 IU/ml.[14]

Table 1: Assay procedure

	Blank	**sample**
Reagent 1	240 µL	240 µL
Distilled water	8µL	---------
Sample	------	8 µL
Sample mixed, incubated at 37 ℃ for 30 min then reagent 2 was added:		
Reagent 2	80 µL	80 µL
Solution mixed thoroughly, incubated in 37 ℃ for 1.5 min, and then the absorbance change value was read after 1.5 min later.		
ΔA = [ΔA sample]- [ΔA blank]		

RESULTS

3 (4.2%) of smokers who age between 10-20 years had a positive RF results, although all of studied groups were not previously diagnosed with any type of autoimmune diseases, table 2. While among controls (non smokers) 2 (4.4%) showed positive results, table 3.

Table 2: Distribution of smoker's males according to their ages.

Age group	**Number of smokers**	**Positive RF result**
10-20	26	3 (11.5%)
21-30	41	0 (0%)
31-40	0	0 (0%)
41-50	5	0 (0%)
51-60	0	0 (0%)
61-70	0	0 (0%)
71-80	1	0 (0%)
Total	73	3 (4.2%)

Table 3: Comparative outlook between RF levels among cigarettes smokers and non smokers.

Result	Smokers	Non smokers
Positive	3 (4.2%)	2 (44.4%)
Negative	70 (95.8)	43 (95.5)
Total	73	45

Collected data indicated a relationship between the type cigarettes brand used and the elevation of rheumatoid factor, in which those who smoke Bringi brand were the only ones who showed positive RF results. The results also revealed that Bringe brand was the most brand consumed among smoker participants, representing (86.3%) of the total number, table 4.

Table 4: Association between RF positive results and cigarettes brand used.

Cigarettes brand	No. of smokers	RF Positive results
Bringi	63	3 (4.8%)
lord	3	0 (0%)
Benson	5	0 (0%)
Royal	1	0 (0%)
Aspine	1	0 (0%)
Total	73	3 (4.1%)

Among studied group 25 samples were randomly selected from cigarettes smokers to compare between qualitative technique (latex) and quantitative technique (Mindary) in RF measurement. The readings were identical in both methods showing the same results, table 5.

Table 5: Comparison between RF results of qualitative method (latex card) and quantitative method (Mindary).

Results	Latex Technique	Mindary Technique
Positive	2 (8%)	2 (8%)
Negative	23 (92)	23 (92%)
Total	25	25

DISCUSSION

Cigarettes smoking is one of the major environmental factors suggested playing a crucial role in the development of several diseases. More recently, it has been reported that smoking is involved in the pathogenesis of certain autoimmune diseases such as RA, systemic lupus erythematosus, systemic sclerosis, multiple sclerosis and Crohn's disease.[15, 16, 17]

However, the exclusion criteria for that study was subjects who were already diagnosed with any type of autoimmune disease, still the results revealed that 4.2% of smoker volunteers showed a positive RF level. This could reflect that fact that many previous studies have reported the effects of tobacco smoking on rheumatoid arthritis disease and revealed an obvious association.[6, 7, 15] Moreover, healthy individuals also showed positive rheumatoid factor, this finding agrees with previous report which also reported RF passivity in the healthy population and it is thought that genetic and environmental factors are responsible for the worldwide variability in distribution of RF.[18]

The study revealed that most of smoker students were addicted to Bringi cigarettes brand and only those whom were confirmed RF positive appeared to be Bringi smokers. This could be explained by the findings of a research article which searched the databases for relevant studies published from 1966 through 2013 to quantitatively summarize the accumulated evidence on the relation between lifelong exposure to cigarette smoking and risk of developing rheumatoid arthritis by performing a dose-response meta-analysis, the research article pointed to the fact that certain cigarette smoke components, such as nicotine, hydrocarbons and carbon monoxide, may enhance immune reactions.[19, 20,21]

This study was not sufficiently large to allow analysis of certain confounding variables or the timing of smoking in relation to disease onset and prognosis. Therefore, larger studies must be conducted in order to improve awareness of the links between smoking and rheumatoid arthritis; there is a need to develop preventive population strategies similar to those already in place for cancer and heart disease. Information about the health risks of smoking cessation and the exposure to second-hand smoke should be incorporated into guidelines for the management of rheumatoid arthritis and raised with patients and their families at every suitable opportunity in the care pathway.

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