



Research Article

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Assessment of inflammation and risk of bladder tumour in adult male smokers in Nnewi, Nigeria

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Abstract

Background: Cigarette smoking is a major risk factor in the development of several diseases. C-reactive protein is an acute phase protein of hepatic origin and the plasma concentration increases during inflammatory states while bladder tumour antigen is a complement factorH related protein (CFHrp) produced by bladder tumours. **Study design:** This is a case-control study designed to assess the levels of C-reactive protein and bladder tumor antigen in adult male smokers in Nnewi metropolis, Anambra state, Nigeria. **Methods:** A total of 100 subjects were recruited for the study. These comprised 50 adult male smokers and 50 adult male non-smokers. C-reactive protein and bladder tumour antigen levels were estimated by enzyme-linked immunosorbent assay technique. Ethical approval and informed consent were obtained before the commencement of the study. Statistical Package for Social Sciences version 20 was used for data analysis. **Result:** The result shows that there was a significant increase in the mean serum levels of bladder tumor antigen and C-reactive protein in adult male cigarette smokers compared with the control group ($p < 0.05$). A positive correlation existed between c-reactive protein level and age in adult male smokers ($r = 0.100, p = 0.517$). Similarly, a positive relationship was observed between c-reactive protein level and duration of smoking ($r = 0.082, p = 0.598$). Furthermore, a positive relationship was observed between bladder tumor antigen level in adult male smokers and age ($r = 0.044, p = 0.843$). Conversely, an inverse relationship existed between bladder tumor antigen levels and duration of smoking ($r = -0.180, p = 0.411$). **Conclusion:** Therefore smokers are predisposed to increased inflammation and heightened risk of bladder tumour.

Keywords: C-reactive protein, Bladder tumour antigen, Smokers.

INTRODUCTION

Smoking is one of the most common forms of recreational drug use. Tobacco smoking is, being practiced by over one billion people globally and majority are in the developing world^[1]. Statistics indicate that more than 700 million children are second-hand smokers^[2].

Smoking-causes lung cancer, other cancers, heart disease, and stroke. It has numerous immediate health effects on the brain and on the respiratory, cardiovascular, gastrointestinal, immune and metabolic systems^[3]. While these immediate effects do not all produce noticeable symptoms, most begin to damage the body with the first cigarette – sometimes irreversibly – and rapidly produce serious medical conditions and health consequences^[4]. Bladder cancer is any of several types of cancers arising from the epithelial lining (i.e., the urothelium) of the urinary bladder. It is a disease in which abnormal cells multiply without control in the bladder^[5]. The most common type of bladder cancer is known as transitional cell carcinoma or more properly urothelial cell carcinoma. Bladder cancer is the 9th leading cause of cancer with 430,000 new cases and 165,000 deaths occurring in 2012^[6].

Bladder tumor antigen has been identified as complement factorH related protein (CFHrp), a variant of human complement factor (FH). Factor H is a soluble protein that protects normal cells from being destroyed by complement. Production of this type of protein by the tumor may keep it from being destroyed even though the immune system recognizes it as foreign.

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C-reactive protein on the other hand is an acute phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T-cells [7]. Its plasma concentration increases during inflammatory states, a character that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. This study was therefore designed to assess inflammation and the risk of bladder tumour in smokers by measuring C-reactive protein and bladder tumour antigen.

MATERIALS AND METHODS

Study site

The study was carried out in Nnewi metropolis, Nnewi North Local Government area in Anambra state. Nnewi is the second largest city in Anambra State, South Eastern Nigeria. It has an estimated population of over 391, 277. It is made up of four quarters namely; Otolo, Uruagu, Umudim and Nnewichi. The inhabitants are majorly traders, health workers, students etc. It has hotels and drinking joints where people drink and smoke.

Study design

This is a case-control study designed to assess the levels of C-reactive protein and bladder tumor antigen in adult male smokers in Nnewi metropolis, Anambra state, Nigeria. A total of 100 subjects were recruited by simple random sampling for the study. These comprised 50 adult male smokers and 50 adult male non-smokers.

Inclusion criteria

Apparently healthy adult male cigarette smokers with smoking history of at least one cigarette a day for not less than one year and adult male non-smokers.

Exclusion criteria

Individuals with history of inflammatory and malignant diseases such as Tuberculosis and other chronic diseases.

Ethical consideration and informed consent

The ethical approval for this research was obtained from the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi. Informed consent of the subjects was sought and obtained from the subjects prior to this study.

Collection of samples

Five millilitres (5ml) of venous blood was collected aseptically from all the subjects and dispensed into a plain container. The samples were centrifuged at 5,000 rpm for 5 minutes and the serum separated into plain tubes and frozen until assayed for C-reactive protein and bladder tumor antigen. Questionnaires were used to obtain demographic data such as age and other information on their lifestyle.

C-reactive protein estimation

This was estimated by the method described by Scand [8]. This is essentially an Immunometric procedure.

Procedure

Both test and control serum were diluted 500 fold prior to use of Fifty microliter (50ul) of CRP standards, diluted specimens and diluted

controls were dispensed into appropriate wells. Fifty microliter (50ul) of CRP enzyme conjugate reagent was dispensed into each well. The wells were properly mixed for 30 seconds and incubated at 37°C for 15 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtitre wells were rinsed and flicked 5 times with buffer wash (minimum 350ul). An absorbent paper was used to remove all residual water droplets. Fifty microliter (50ul) of substrate A and fifty microliter (50ul) substrate B were dispensed into each well and gently mixed for 5 seconds. Incubation was done at 37°C for 15 minutes. The reaction was stopped by adding 50ul of stop solution to each well. The well was gently mixed for 30 seconds. Absorbance was then read at 450 nm with a microtitre well reader

Bladder tumor antigen estimation

Bladder tumor antigen was estimated using Immunometric method as described by Raitnem and Tammela [9].

Procedure:

To separate wells, 50µl, 5µl, and 50µl of standard, test and control samples were added respectively and 45µl of sample diluents added to each well. This was then incubated at 37°C for 30 minutes. Thereafter, 50µl of enzyme conjugate was added and incubation performed again for 30 minutes at 37°C. The wells were washed 5 times and blotted to remove water droplets. Into each well were dispensed 50µl of substrate A and 50µl of substrate B. This was gently mixed for 5 seconds and incubated at 37°C for 15 minutes. The reaction was stopped by adding 50µl of stop solution to each well. The solution was gently mixed for 30 seconds and read at 450nm.

Statistical analysis

The statistical package for Social Sciences (SPSS) version 20 was used for data analysis using the students' t-test and Pearson correlation coefficient. Values were deemed significant if $p < 0.05$.

RESULTS

There was a significant increase in the mean serum level of C-reactive protein and bladder tumor antigen in adult male cigarette smokers compared with non-smokers ($p < 0.05$) (Table 1).

Table 1: Comparison of C-reactive protein and Bladder tumor antigen levels in adult male cigarette smokers and nonsmokers

Groups	C-reactive protein (mg/l)	Bladder tumor Antigen (ng/ml)
Smokers N=50	9.64±2.34	33.87±12.86
Non-Smokers N= 50	1.83±0.56	26.93±4.29
t value	3.233	2.439
P value	0.002	0.021

A positive non-significant correlation existed between the serum levels of C-reactive protein in adult male smokers and Age ($r = 0.100$, $p = 0.517$) (fig 1). A positive non-significant correlation was observed between c-reactive protein levels and duration of smoking in years ($r = 0.082$, $p = 0.598$) (Fig 2).

A positive non-significant correlation existed between the serum levels of Bladder tumour antigen in cigarette smokers and Age ($r = 0.044$, $p = 0.843$) (Fig 3). An inverse non-significant correlation was observed

between Bladder antigen levels and duration of smoking in years ($r=0.180$, $p=0.411$) (Fig 4).

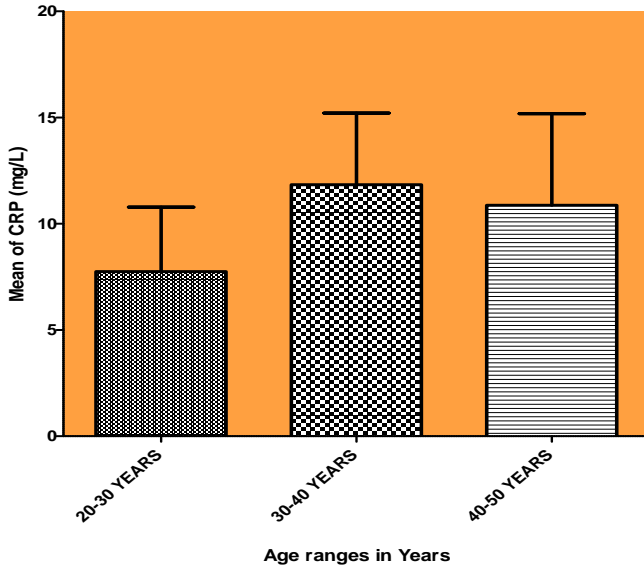


Figure 1: Correlation between age and C - reactive protein level in adult male smokers ($r= 0.100$).

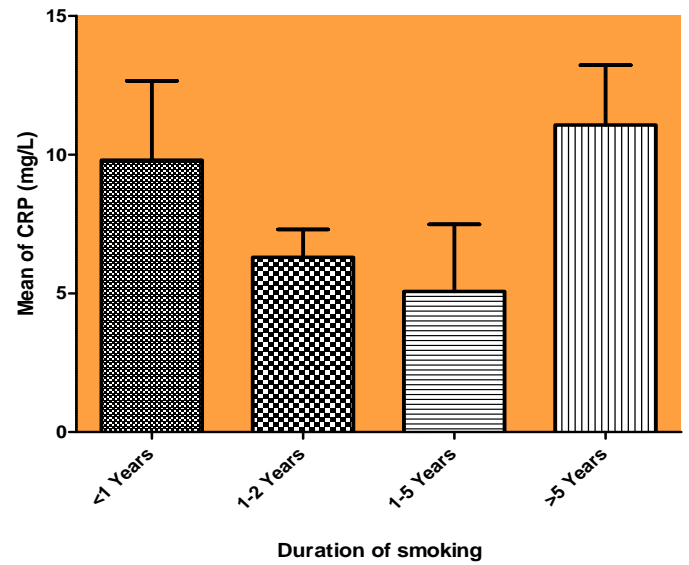


Figure 2: Correlation between duration and C-reactive Protein level in adult male smokers ($r= 0.082$).

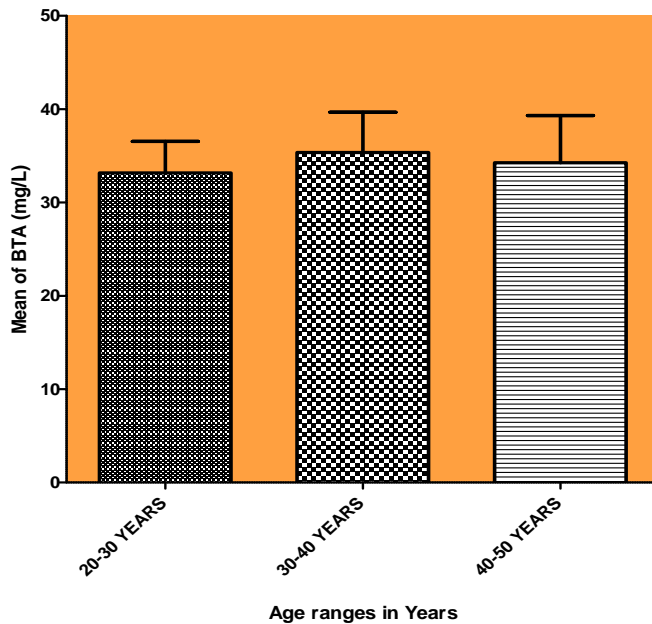


Figure 3: Correlation between age and Bladder tumour antigen level in adult male smokers ($r=0.044$).

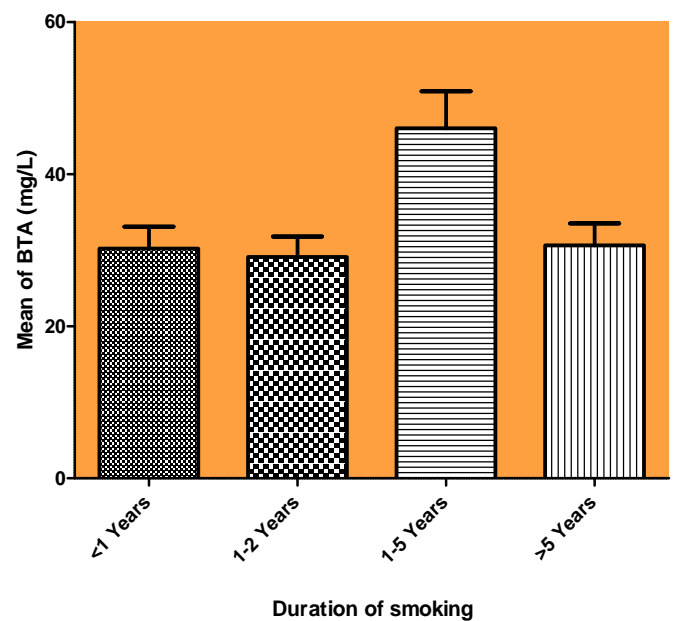


Figure 4: Correlation between duration of smoking and Bladder tumour antigen level in adult male smokers ($r= -0.180$).

DISCUSSION

Cigarette smoke contains a complex mixture of over 4000 chemicals. These chemicals cause gradual damage to the body systems on exposure to them leading to several kinds disease conditions. Smoking is known to predispose individuals to cancer and the degree of this risk is proportional to cigarette tobacco smoke exposure, other environmental exposures and behaviors. C-reactive protein and bladder tumour antigen are makers for disease states like bladder tumour and inflammatory conditions.

In this study, there was a significant increase in the serum C - reactive protein levels in adult male cigarette smokers compared with non-smokers. This suggests that smoking induces inflammation. This supports the assertion that smoking induces an inflammatory state and an elevated CRP levels have been associated with smoking-related diseases^[10]. Our result was also similar to that of Frolichet *al.*^[11] who observed that CRP concentrations were significantly higher in male

regular smokers than male never-smokers. Similarly, some researchers observed that when data from a study of adolescents were analysed, an increase in CRP levels was observed, with heavy smokers having twice the CRP concentration compared with non-smokers^[12]. Furthermore, CRP levels are significantly increased in children who are exposed to second hand smoke. Furthermore, Yanbaeva *et al.*^[13] examined CRP status in former smokers and discovered that levels did not fall immediately upon cessation, which reflects the fact that the underlying tissue damage caused by smoking takes some time to recover. We also observed a positive relationship between C - reactive protein level and duration of smoking. This suggests that inflammatory process in smokers increases with duration. However, this relationship was not significant. Our findings also showed that a non-significant positive correlation existed between C-reactive protein level and age of smokers. Some previous research reported that increased CRP has been found to be associated with increasing age, body mass index, weight and reduced fitness levels^{[14][15]}.

There was a significant increase in the mean serum levels of bladder tumor antigen in adult male cigarette smokers compared with non-smokers. This suggests that smokers are highly predisposed to bladder cancer. Freedman *et al*^[16] observed that 50% of bladder cancers attributable to smoking in men and 20% in women. Moore *et al*^[17] reported that increased bladder cancer risks were observed with all categories of smoking by duration and cigarettes/day. According to Cartwright *et al*^[18] and Vieniset *al.*^[19] the duration and intensity of cigarette smoking independently increased the risk of bladder cancer and smoking cessation reduces the risk of bladder cancer. Contrary to this, we found an inverse relationship between bladder tumour antigen level and duration of smoking, however this finding was statistically not significant. Furthermore a positive non-significant relationship was observed between bladder tumor antigen level and age. A study conducted by in China showed that the incidence of bladder cancer had an exact correlation with age. This means that with increasing age, the incidence of bladder cancer will increase^[20].

CONCLUSION

Cigarette smoking results in increased levels of C-reactive protein and bladder tumour antigen which are suggestive of inflammation and predisposition to bladder tumour.

Conflicts of interest

The authors declare that no conflict of interest exists in this research.

Support

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