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Research Article

EFFECT OF CIGARETTE SMOKING ON HAEMATOLOGICAL PARAMETERS: INFLUENCE OF *NQO1* GENE POLYMORPHISM

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ABSTRACT

Cigarette smoking carries higher risks for most of the chronic diseases. It also has chronic and acuteeffects on the haematologic system.NQO1 catalyzes highly toxic quinones derived from tobacco smoking to less toxic hydroquinone analogues, thus protects the cell from unwanted oxidative damage.This study aimed to determine the effect of cigarette smoking on haematological indices among Sudanese smokers with different *NQO1* genotypes. The study included 75 smokers (consuming minimum of 5 cigarettes per day for at least one year) that were selected randomly,there blood cell count (determined by Sysmex KX-21N) and NQO1 genotypes (detected by PCR-RFLP) were determined and compared with 75non-smokers as controls. Mean TWBC was significantly higher, with higher lymphocytes count, among smokers than non-smokers (*P* value=0.029 and 0.000 respectively).*NQO1*C609T genotype frequencies of cigarette smokers were as follows: Homozygous wild types(C/C) 85% (n 64/75) and heterozygous mutant type(C/T) 15% (n 11/75). No homozygous mutant (T/T) genotype was detected among the study group. No significant differences were observed in the haematological parameters between smokers with mutant type (*NQO1*609 C/T and those with wild type (*NQO1*609C/C). In conclusion, we found a significant increase in the TWBC count and lymphocyte count among cigarettes smokers group than the non-smokers group, with no modifying interaction by the different NQO1 genotypes.

Key words: Cigarette smoking, Haematologicalvalues, NQO1, Sudan.

INTRODUCTION

Cigarette smoking is a serious health problem and most important avoidable causes of death in world over (1). Some of the smoke constituents have been identified as carcinogens by International Agency for Research on Cancer (IARC) (2). Cigarette tobacco smoke contains over 4000 compounds, including at least 200 toxicants, 80 known or suspected carcinogens, large quantities of oxidants and free radicals that induce oxidative stress, oxidative lung injury and apoptosis (3-5). On the other hand cigarette smoking has a high risk for cardiovascular diseases, hypertension, inflammation, stroke, clotting disorder, and respiratory disease (6,7). Cigarette smoking includes pathogenesis in different type of cancers such as lung, pancreas, breast, liver and kidney (8-10).

NQO1 is primarily involved in the detoxification of potentially mutagenic and carcinogenic quinoneswhich derived from tobacco smoke, through their two electron reduction to hydroquinones (11).

NQO1 protects the cell from undesired oxidative damage by preventing redox cycling, which leads to the generation of free radicals. It is induced by oxidative stress and polycyclic aromatic hydrocarbons such as those resulting from combustion processes in cigarette smoking (12). *NQO1* gene is located in the long arm of chromosome 16 (16q22.1), it expands approximately 20 kb with 5 entron and 6 exons that code for NQO1 protein, a flavoenzyme mainly cytosolic enzyme formed of 273 amino acid residues, that plays necessary role in the protection against exogenous and endogenous quinone by catalyzing two and four electron

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reduction of these substrates, such as hydroquinone (13-15).

The polymorphism at position 609 in exon6 and (C-T) in the human *NQO1* gene results in a proline to serine substitution at position codon 187 in the amino acid structure of the NQO1 protein, resulting in loss of the enzyme activity(16). NQO1 enzyme activity is normal in individuals with 2 wild-type alleles (*NQO1* 609CC). It is variably reduced in individuals who are heterozygotes for the polymorphism (*NQO1* 609CT) (17). NQO1 enzyme activity is absent in those who are homozygous for the point mutation (*NQO1* 609TT) (18). NQO1 enzyme may play an important role in the protection against cigarette toxicity, so individual who have mutant alleles at postion609 in *NQO1* gene may be in a high risk of cigarette toxicity. This study aimed to determine the effect of cigarette smoking on haematological indices among Sudanese smokers with different *NQO1* genotypes.

MATERALS AND METHODS

One hundred and fifty healthy adult male subjects were enrolled in this cross-sectional study: 75 smokers (consuming minimum of 5 cigarettes per day for at least one year) that were selected randomly, and age and sex matched 75 non-smokers as controls. Subjects with gross anemia, known history of diabetes mellitus, cardiopulmonary disease, acute or chronic infection, autoimmune disease, malignancy were excluded from the study. Ethical approval was obtained from the ethical committee, Faculty of Medical laboratory Sciences, Alneelain University, Khartoum, Sudan. Informed consent was obtained from each subject before enrollment in the study.

Two ml of EDTA anticoagulated blood was collected from each individual for haematological and molecular analysis. Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. Blood cell count was performed by automated cell counter (Sysmex KX-21N). DNA was extracted by sodium chloride method, in brief, 300 µl of whole blood was lysed using red cell lysing buffer containing (8.3gm NH_4Cl, 1gm$KHCO_3$, 1.8ml 5% EDTA and 1liter of distilled water.), the pallet was lysed by white blood cell lysing buffer containing (1.576gm, Tris-HCL, 1.088gm, EDTA, 0.292gm NaCl, 2% SDS, and 100ml distilled water). High molecular concentration of sodium chloride was added (35gm to 1liter of distilled water) to separate the protein fraction. Finally, ice cold ethanol was added to get the DNA fiber which were separated and re-suspended in TE buffer (2.42 Tris base, 0.57ml acetic acid, 50µlEDTA (.01M), and 100ml distilled water) and stored until used. The quality of genomic DNA was determined by agarose gel electrophoresis. *NQO1* fragment Was Amplified using the forward primer: 5`-AGTGGCATTCTGCATTTCTGTG-3` and reverse primer: 5`-GATGGACTTGCCCAAGTGATG-3`.

The amplification was carried out in thermo-cycler (Techne) with initial denaturation step for 8 minutes at 95°C followed by 35 Cycles consisting of 3 steps: Denaturing step at 94 °C for 30 second, Annealing step at 56 °C For 1 minute and extension steps at72 °C for 40 seconds with final Extension step at 72 °C for 10 Minutes. The PCR reactions was performed in a final volume of 20 µl containing (4 µl premixed ready to use 5x FIREPol master mix (Solis BioDyne, Russian), 12.0µl DNAase free DW, 3 µl genomic DNA and 0.5 µl from each primer). The amplified fragment was digested with 10 U Hinf1 endonuclease (New England Bio lab, UK) over night and was visualized on agarose gel electrophoresis. Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of data was performed using the t-test and Pearson correlation test. Results with p value <0.05 were considered statistically significant.

RESULT

The median age of smokers was 31 years, with minimum age of 18 years and maximum age of 64 years. The median smoking duration was 8 years with minimum duration of 1years and maximum duration of 40 years, median number of cigarette smoked per day was 10 cigarettes with minimum of 5 cigarettes and maximum of 50 cigarettes smoked per day. All subjects were tested for blood count and NQO1 polymorphisms. Mean TWBC was significantly higher, with higher lymphocytes count, among smokers than non- smokers (*P* value=0.029 and 0.000 respectively). No significant differences were observed within the other haematological values (table 1).

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Table 1: comparison of haematological values between cigarette smokers and non- smokers.

Parameter	Smokers	non- smokers	P value
Hb (g/dl)	14.8 ±0.9	l4.7±1.2	0.795
RBC (X1012/L)	5.1±0.5	5.3±0.5	0. .076
PCV (%)	44.5±2.8	45.38	0.123
TWBC (X109/L)	6.2±1.7	5.6±1.2	0.029
Platelets (X109/L)	238.4 ±58.7	234±52.1	0. 669
Neutrophils (X109/L)	47.0±10.0	48.3±9.4	0. 605
Lymphocytes (X109/L)	44±10.0	38.2±8.6	0. 000

There was no significant correlation observed between number of cigarettes smoked per day and the haematological values (*p* value: TWBC 0.999, RBC 0.738, Hb 0.715, PCV 0.876, platelets 0.890, lymphocytes 0.737 and neutrophil 0.290). No significant correlation was observed between smoking duration and the haematological values (*p* value: TWBC 0.359, RBC 0.223, Hb 0.738, PCV 0.130, platelets 0.861, neutrophils 0.140 and lymphocytes 0.057).

Table 2: comparison of haematological characteristic between cigarette smokers with NQO1 wild type and those with mutant types.

Parameter	Wild type (609C/C)	Mutant type (609C/T)	P value
Hb (g/dl)	14.7±0.9	14.9±1.2	0.579
RBC (X1012/L)	5.1±0.5	5.4±0.5	0.129
PCV (%)	44.4±2.8	45.0±2.7	0.458
TWBC (X109/L)	6.3±1.7	6.1 ±1.6	0.729
Platelets (X109/L)	236.4±56.9	250.0±70.5	0.478
Neutrophils (X109/L)	47.6±10.5	46.4±10.2	0.712
Lymphocytes (X109/L)	44.3±9.9	42.2±10.5	0.519

*NQO1*C609T genotype frequencies of cigarette smokers were as follows: Homozygous wild types(C/C) 85% (n 64/75) and heterozygous mutant type(C/T) 15% (n 11/75). No homozygous mutant (T/T) genotype was detected among the study group. No significant differences were observed in the haematological parameters between smokers with mutant type (*NQO1*C609 C/T and those with wild type (*NQO1*C609 C/C) (table 2).

DISCUSSION

Smoking is the most important public health problem. Many studies performed have proved its deleterious effects on many organ systems, it exerts pharmacological, mutagenic, carcenogenic, toxic, and inflammatory effects (19). Effects of smoking on haemopoietic system have been also analyzed in many studies. In our study, we investigated the haematological changes among cigarette smokers with different NQO1 genotypes in Sudan. The study included 75 smokers, there blood cell count and NQO1 genotypes were

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determined and compared with 75 healthy (non-smokers) subjects as control. We found a significant increase in the TWBC count and lymphocyte count among cigarettes smokers group than non- smokers group.

An association of total leucocyte count with cigarette smoking has been reported by several researchers (20-23).

Increases in peripheral blood WBC counts, and alterations in WBC function can be the result of direct damage stemming from alterations in epithelial, and endothelial surfaces and/or cytokine levels (especially IL-6) caused by components of cigarette smoke (24).

While leukocytosis may simply be a marker of smoking-induced tissue damage, high WBC count can promote cardiovascular diseases through multiple pathologic mechanisms that mediate inflammation, plug the microvasculature, induce hypercoagulability and promote infarct expansion (8,25,26). In fact several studies have shown that WBC count is an independent predictor of atherosclerosis and cardiovascular disease (27,28). It has been suggested that nicotine may induce an increase in blood lymphocyte counts (25,26).

NQO1 catalyzes highly toxic quinones derived from tobacco smoking to less toxic hydroquinone analogues (11), thus protects the cell from unwanted oxidative damage. It seems likely that the lack of NQO1 activity might increase the risk of certain types of toxicity and cancer. When compared the haematological values between smokers with NQO1 C609T wild type (CC) and those with mutant types (CT), we observed no statistically significant differences, this finding indicated that different NQO1 C609T genotypes have no modifying interaction with harmful effects of smoking on haematological parameters.

CONCLUSION

In conclusion, we found a significant increase in the TWBC count and lymphocyte count among cigarettes smokers group than the non-smokers group, with no modifying interaction by the different NQO1 genotypes.

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