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Comparison of Anisocytosis between Active and Passive Smokers

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Abstract
Background: *Smoking is one of the most important public health problem, increasing the morbidity and mortality in the society. With prolonged exposure of RBCs to smoking, the deformability of RBCs is decreased, and its aggregation increases, and makes whole blood more viscous and causes microvascular complications. This decreased deformability is reflected as anisocytosis in peripheral smear. This study is aimed at comparing anisocytosis between Active and Passive smoking and educating the smoking population about the deleterious effects of active as well as passive smoking.*
Materials and Methods: *This study was done in 150 subjects in the age group 25 to 50 years (50 control , 50 male subjects with active smoking habit and 50 female subjects ,who were exposed to passive smoking. Peripheral smears were prepared and stained with leishman's stain. Smears were focused under oil immersion objective, and the images were captured using a digital camera. Images were transferred to the computer system and RBC diameter was measured using UTHSCA image tool software. Variation in size of the RBCs between control and active and passive smoking subjects were compared using Pearson's product moment correlation coefficient and Paired sample t test.*
Results: *Increase in the variation in the size of RBCs (anisocytosis) is observed in both active and passive smokers using Pearsons product moment correlation coefficient ,significant r value of 0.85 and0.8 are got in active and passive smoking. Using Paired sample t test, significant p value of < 0.01 is got in active and passive smokers.*
Conclusions: *Our study concluded that variation in the RBC size, is almost equal in active and passive smokers. This clearly shows the harmful effects of smoking is equal in family members of active smokers.*
Keywords: *Smoking, Red Blood Cell membrane deformability, Peripheral smear, Image tool, Anisocytosis.*

Introduction

Smoking is an important community based health problem which affects almost each and every organ system in the body. According to world Health Organisation (WHO), 12% of world's smoking population are in India[1]. Smoking is a major risk factor for heart attack, stroke, chronic obstructive pulmonary disease (COPD), emphysema, and cancer (particularly lung cancer, cancers of the larynx and mouth, esophageal cancer and pancreatic cancer. In a cigarette (which contains 0.49 to

0.89gram of tobacco), the nicotine content can vary between 13.79 and 22.68 milligrams per gram of dry tobacco [2,3] .

The family members of smokers who are exposed to passive smoking are equally prone for the deleterious effect of smoking. Smoking causes various changes in the RBC membrane. With prolonged exposure of RBCs to smoking, the deformability of RBCs is decreased, and its aggregation increases. This makes whole blood more viscous and responsible for microvascular complications. This decreased deformability is reflected as anisocytosis in peripheral smear. This study is aimed at comparing anisocytosis between active and passive smoking and educating the smoking population about the deleterious effects of active as well as passive smoking. Smoking characteristics such as quantity consumed and duration of smoking are included in finding the relationship between smoking and Anisocytosis. Thanks to the banning of cigarette smoking in public places the number of passive smokers is reduced.

Methods

This study was done in 150 subjects in the age group 25 to 50 years (50 control , 50 male subjects with active smoking habit and 50 female subjects, who were exposed to passive smoking, who attended the Annapoorana Medical College Hospital. This study was started after getting ethical clearance from the ethical committee of Annapoorana Medical College and Hospitals. 50 (25 male and 25 female) subjects without smoking habit were included in the control group. 50 male subjects with smoking habit who smoke 20 cigarettes per day for 3-5 years and 50 female subjects who were exposed to passive smoking (family members of active smokers) were included in the test group. Written consent was got from all the subjects who participated in the study. Ideal peripheral smears were prepared and stained with Leishman's staining. Smears were focused under oil immersion objective, and the images were captured using a camera. Images were transferred to the computer system and RBC diameter was measured using UTHSCA image tool software. The calibration image was taken from the Neubauer's counting chamber smallest square of RBC which is 50 micron. Magnification factor was kept constant for all the images. We compared the the variation in the size of erythrocytes between the control without smoking habit, and subjects with active and passive smoking .Since the details of morphology of RBCs cannot be obtained by automated analysers, manual method of measuring anisocytosis was preferred in this study. Best diagnostic support was given by Systematic examination of blood film and all the other test were either complimenting or confirming it.[4] Computer based image analysis method to determine red cell size, provides an accurate and reliable measurement, which is simple and cost effective[5]. The diameter of 25 RBCs were measured from each image. The variation in the size of RBCs (anisocytosis) (largest diameter_ smallest diameter), between the control and active and passive smokers were compared.

Results

We used Pearson's product moment correlation coefficient and Paired sample t test to find the correlation between smoking and anisocytosis. If the r value is near 1, there is significant correlation, if r value is positive there is positive correlation and if the r value is negative there is negative correlation. The variation in the size of RBCs (anisocytosis) was more in subjects with active and passive smoking. Smoking is positively and significantly correlated with anisocytosis. The r values got after comparing the smoking and anisocytosis in control and active and passive smokers are 0.85 and 0.8 respectively. (Table:1)

The normal variation in the rbc size is 1-2 microns. But, in subjects with active and passive smoking variation in the RBC size (anisocytosis) was 5.1 microns, which gives the significant r values. (Table2) By comparing the anisocytosis with smoking using Paired sample t-test, the significant p value of < 0.05 was got in active and passive smokers. (Table 1) (Figure1)

Table 1: Comparison of anisocytosis between active and passive smokers

Variable	Male active smokers	Female passive smokers
r value	0.85	0.8
p value	< 0.05	< 0.05

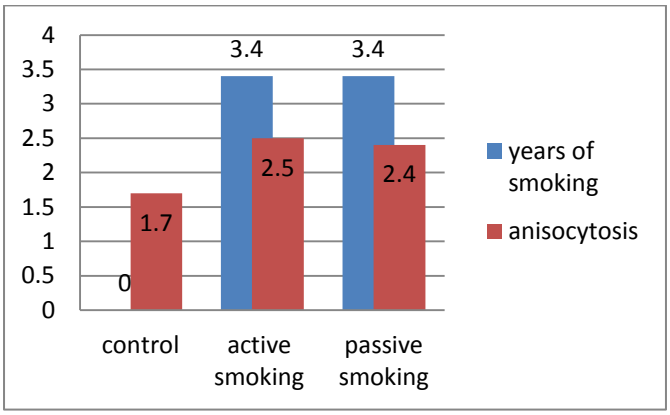

Figure 1: Comparison of anisocytosis between active and passive smoking

Table 2: Variation in RBC size in active and passive smokers

Study subjects.	Range of RBC size (microns)
controls (n= 50)	6.1 to 7.8
Male active smokers(n= 50)	5.17 to 10.15
Female passive smokers(n= 05)	5.1 to 10

Discussion

From our study, we came to know that, both active and passive smoking were positively and significantly correlated with anisocytosis. Let us discuss about the results got in our study. It has been shown that anisocytosis is present in cardiovascular and pulmonary diseases[6,7,8]. Nearly every organ and organ system in our body is affected by smoking and this affects a person's overall health. In the study done by Kurtoğlu E et al[9] they observed that that the mean RDW values were higher in smokers than in non-smokers (13.9±1.2vs.13.1±0.8, pvalue.0001). Significant positive correlations between RDW and number of cigarettes smoked per day and between RDW and duration of smoking were identified. Exposure to greater oxidative stress is the potential pathophysiologic mechanism linking higher RDW with smoking. A relationship between smoking and higher oxidative stress has been established[10]. Oxidative stress, causes oxidative damage to to the lipids and proteins in the RBC membrane[11]. Oxidative stress also causes, conformation changes in membrane cytoskeleton protein which alters fluidity of the membrane, erythrocyte shape, size and osmotic fragility[12]. It has been shown that oxidized RBCs lose their flexibility owing to a loss of lipid asymmetry and cytoskeleton rearrangement, causing them to be more rigid and thus develop anisocytosis[13]. Adrenergic activation caused by smoking may also affect bone marrow response, thus resulting anisocytosis[14]. Elevated RDW may also be a surrogate measure of the chronic inflammatory process in smokers, which results in ineffective erythropoiesis causing immature RBCs to enter the circulation and in turn results in heterogeneity in the size of RBCs causing anisocytosis[15]. The reason for the significant r and p values got in active and passive smokers was, Oxidative stress was equal in both.

Conclusions

Our study showed that anisocytosis was significantly higher in both active and passive smokers. Family members of active smokers are equally affected as active smokers. So they are equally prone for the deleterious effects of smoking such as, decreased lung functions, carcinoma etc. It is high time smokers have to be educated about passive smoking, and its deleterious effect to the innocent family members.

Early cessation of smoking habit is beneficial to the individual and prevents hazards of complication of smoking to the society.

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Declarations

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Conflict of interest: None declared.

Ethical approval: This study was approved by Institutional Ethics Committee.

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