EFFECT OF PASSIVE SMOKING ON THE LEVELS OF PREGNANCY ASSOCIATED PLASMA PROTEIN –A IN NORMAL RATS

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ABSTRACT

Objectives: To measure the levels of pregnancy associated plasma protein- A (PAPPA-A) in normal rats exposed to cigarette smoke.

Study Design: Experimental interventional study

Place and Duration of Study: Army Medical College, Rawalpindi, in collaboration with national institute of health (NIH), Islamabad. Total duration was of 4 weeks.

Material and Method: Sixty albino rats of Sprague- Dawley strain weighing 200-250 gm, divided into two groups. Both the groups were kept in identical chambers. One group of 30 rats was further exposed to passive cigarette smoke for 4 weeks.

Results: No increase was observed in the levels of serum PAPP-A of both the groups: Passive smokers and not exposed to passive smoking i .e. $P > 0.05$.

Conclusions: Smoking does not increase the levels of PAPP-A.

Keywords: Pregnancy associated plasma protein- A, Cigarette smoke, Sprague-Dawley strain.

INTRODUCTION

Cigarette smoking is one of the major classical risk factors for atherosclerosis and cardiovascular disease [1]. Smoking is one of the preventable causes of death, and it has increased considerably in most developing countries over the last quarter of the 20th century [2]. Tobacco use is widespread in both developed and developing countries and has been associated with mortality from multiple cardiovascular diseases [3, 4]. It is estimated that 930 million of the world's 1.1 billion smokers currently live in the developing countries [5]. Cigarette smoke is a heterogeneous aerosol. It contains more than 4000 chemicals. These compounds are capable of producing reactive oxygen species (ROS) like O_2.-, H_2O_2, OH; and ROO; These ROS produce oxidative damage. Therefore cigarette smoke is associated with an increase in the incidence of various diseases like cancer, chronic obstructive lung disease and atherosclerosis [6]. Atherosclerosis Risk in Communities (ARIC) studies demonstrated that both active and passive smoking are associated with accelerated atherosclerosis progression as assessed by intimal medial thickness [7]. Atherosclerosis and its complications constitute the most common causes of death in both western and eastern societies. Cardiovascular disease is a global health problem reaching epidemic proportions [8]. Pregnancy Associated Plasma Protein - A (PAPP-A) is a matrix metalloproteinase that cleaves specific insulin like growth factor binding proteins, which makes insulin like growth factor available at the site [9]. Insulin like growth factor -1 (IGF-1) is a mediator of atherosclerosis [10]. PAPP-A is found in both men and women [11]. It is a pro atherosclerotic molecule, and peripheral blood levels of PAPP-A have recently been proposed as a biological marker of acute coronary syndrome [12]. It has been proposed that elevated circulating PAPP-A, a marker of atherosclerotic plaque activity, might predict further clinical instability, infarction and cardiac death. PAPP-A is produced by the activated cells and is released into the extra cellular matrix and this might be due to the local inflammatory processes occurring in the arterial wall. This is indicated by the positive correlation which is observed between the levels of CRP and PAPP-A. Similar relationship was observed in the patients of ACS [13]. In non-ACS individuals, variable concentrations of PAPP-A can be measured but the exact source cannot be localized and it might be from

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seminal fluid, testes, corpus luteum, follicle fluid and other organs [14].

This study was designed to evaluate that whether serum PAPP-A levels are increased by passive smoking in rats. As serum PAPP-A levels predict future risk for cardiovascular disease that is independent of the classical risk factors.

MATERIAL AND METHODS

The study was conducted in the Department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Rats obtained were kept at the animal house of NIH. Total duration of study was four weeks. Total number of sixty rats was divided into two groups as follows:

Group 1: Thirty normal rats

Group 2: Thirty normal rats exposed to cigarette smoke

Rats were kept under standard conditions in the animal house of NIH for a week to acclimatize them and to rule out any abnormality regarding their health. Food and water was available to rats and 12 hour light and dark cycle was observed in a room at 22-24^{0}c. Pelleted form of diet prepared at NIH was given to them.

Plastic Chambers were specially built for the purpose of exposing the animals to cigarette smoke with separate inlets for smoke and fresh air, with two holes each 3 cm in diameter in two opposite walls. Lit Cigarette was kept on a cigarette stand. Cigarette smoke exposure was given to them for six hours per day i.e. from 8 o'clock in the morning to 2 o'clock in the afternoon for six days a week. Filter tipped cigarette were used (Gold Flake-Pakistan). Each cigarette would burn approximately for 10- 12 minutes, and during this time the chamber would completely fill with smoke. A break of 15 minutes was given before the start of next cigarette. Control animals were exposed only to fresh air in an identical chamber. Study design was experimental interventional study.

PAPP-A was measured by enzyme linked immunosorbent assay (ELISA) method. Kit used was Demeditc PAPP-A US (ultra sensitive) ELISA (DE 4512), Demeditec Diagnostics GmbH- Germany (17).

Statistical Analysis

Descriptive statistics were carried out by using software package SPSS version 15. Independent samples t- test was applied to compare the two groups.

RESULTS

Twelve weeks old, albino rats of Sprague Dawley strain; weighing 200-250 gm were obtained for this project. Total duration of study was four weeks and during this time three blood samples were withdrawn, at the start of the experiment, at 14 days and at the end of the experiment that is at 28 days. Results of the parameter are given in the tables 1 and 2, and comparison of the two groups for PAPP-A is shown in figure.

DISCUSSION

Evidence now supports the fact that atherosclerosis is an inflammatory process, and inflammation plays an important role in the pathogenesis of acute coronary syndrome

Table 1: Serum PAPP- A Levels of group-1 rats at various intervals not exposed to passive cigarette smoke in chamber

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
PAPP-A before (ng/ml)	30	5.33	8.95	7.2090	.96141
PAPP-A Mid (ng/ml)	30	5.34	8.53	7.0887	.79799
PAPP-A after (ng/ml)	30	5.46	8.53	7.0697	.84092

Table -2: Serum PAPP- A Levels of group-2 rats at various intervals after exposure to passive cigarette smoke in chamber

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
PAPP-A. before (ng/ml)	30	5.54	8.31	6.9423	.83537
PAPP-A.Mid (ng/ml)	30	5.54	8.31	7.0860	.63318
PAPP-A. after (ng/ml)	30	6.33	8.31	7.1687	.49634

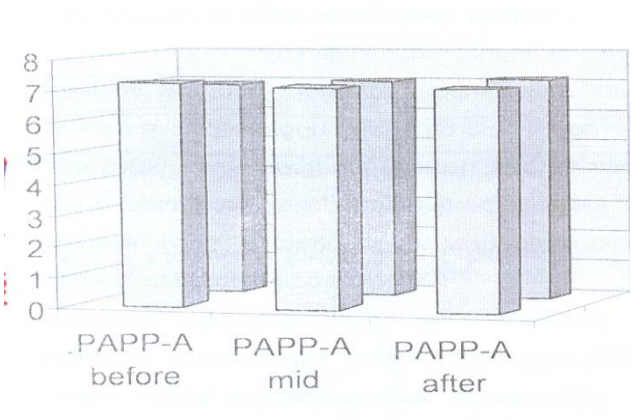

Figure: Comarison of PAPP – A levels of Group 1 & 2
PAPP-A before (ng/ml) = P> 0.05
PAPP-A mid (ng/ml) = P> 0.05
PAPP-A after (ng/ml) = P> 0.05

(ACS). This has lead to the identification of many new markers that can be used in its diagnosis [15].

Pregnancy associated plasma protein –A is a protein associated with plaque activity and morphology in ACS. It appears to be an independent marker of future ischemic events in patients who were troponin negative [12], as well as in patients who are hyperlipidemic but asymptomatic [13]. Smokers are in particular at an increase risk of developing cardiac disease. It has been noted that one out of every five smoking related deaths are caused by cardiovascular disease, especially in people younger then fifty [16]. Cigarette smoke has been found to affect various systems, mainly cardiovascular and immune systems. Cigarette smoke contains a large amount of free radicals which are responsible for initiating lipid peroxidation. These oxidized lipoproteins are responsible for the damage to vascular epithelium [17].

In this study conducted on animals exposed to cigarette smoke we found out that the levels of PAPP-A was not increased. In both the groups the levels remained the same. Side stream smoke the main component of cigarette smoke is associated with increased production of pro inflammatory cytokine production. The exact mechanism of the pathogenesis of vascular disorder in smoking is not known. PAPP-A enzymatically releases IGF-1 from its binding protein IGFBP-4 and makes it available for macrophage activation, chemotaxis, release of proinflammatory cytokines by the cells, and LDL uptake by macrophages [18]. It appears that the endothelial damage caused by smoke is not mediated by IGF-1. This fact is in accordance with the study carried out in smoker pregnant women in whom both beta hCG and PAPP-A levels were found decreased [19]. In another study conducted by shires et al it was noted that PAPP-A levels are decreased in smokers and diabetics [18].

In an histologic study conducted by Bayes –Genis et al [13], specific monoclonal antibodies were used for the detection of PAPP-A, and it was seen that PAPP-A was abundantly expressed in both eroded and ruptured plaques, but was minimally expressed in stable plaques. This means that PAPP-A may be produced by activated cells in unstable plaques and then released into extra cellular matrix [12]. Our study has confirmed to an extent that smoking does not increase the levels of PAPP-A which is proatherosclerotic.

This is in accordance with the studies carried out for the detection of chromosomal anomalies in female smokers, where decrease levels of PAPP-A and beta hCG were found along with increased nuchal translucency in women who smoked 5 or more cigarettes a day compared with non smokers [19]. PAPP-A has recently been documented as a specific IGF-1 dependent protease which cleaves IGFBP-4(insulin like growth factor binding protein) making IGF-1 available at the site. There is now evidence that there may be redundancy in the function of IGFBPs (20). Thus if each of the IGFBPs has a unique function, then they have to be regulated differently in particular pathophysiological situations consistent with their function in specific tissues [21]. Therefore studies are needed to clarify the mechanism for the regulation of PAPP-A due cigarette smoke stimulation.

This study was done to observe the role of this new cardiovascular risk marker in the rats exposed to passive smoke for a duration of 4 weeks, but we could not detect any increase in the levels of PAPP-A.

Future Implications

Future studies are required to be conducted especially in chronic smokers and if possible the study should be human based to further evaluate its role in smokers and to determine the pathological processes which effect the expression of PAPP-A.

CONCLUSION

The levels of PAPP- A are not increased in rats exposed to passive smoke for duration of 4 weeks.

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