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Hemoglobin and C-reactive protein level among smoker and non-smoker individuals of Kathmandu valley, Nepal

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Abstract

Cigarette smoking contribute to the development or progression of numerous chronic and age related disease processes. One major risk factor for morbidity and mortality among smokers is cardiovascular disease and lungs cancer. Hematological abnormalities have been associated with coronary heart disease and other oxidative damage at the tissue level increasing in age is significantly associated with higher hemoglobin concentration and C-reactive protein reactivity. The aim of the study was to find out the level of hemoglobin (Hb) and reactivity of C-reactive protein (CRP) among smoker and non-smoker. A comparative cross sectional community based study was conducted in Kathmandu valley (Swoyambhu, Thankot, Bhaktapur and Balkumari). Other medical records was taken into account and was analyzed. Hemoglobin level of smoker and non-smoker was estimated and it was compared with the qualitative test of CRP. A total of 150 healthy people (both smoker and non-smoker) aged 15-65 years were enrolled in this study for assessment of Hb and CRP. Blood hemoglobin was measured in 150 samples by Cyanmethemoglobin method and reactivity of CRP by latex turbidimetric method. Data were expressed in percentage, mean \pm SD. There was significant difference in mean value of hemoglobin level between smoker and non-smoker with P value 0.005. The difference in the reactivity of serum CRP among smoker and non-smoker was not significant enough with P value >0.05 . The present study showed that the hemoglobin level of smoker group was higher than the non-smoker group as well as CRP showed reactive comparatively more in smoker than the non-smoker.

Keywords: Smoker and non-smoker; Hemoglobin (Hb); C-reactive protein (CRP)

1. Introduction

Smoking is a practice in which substance like tobacco is burned and tasted or inhaled [1, 2]. Other smoking implements include pipes, cigar, bidis, hookahs, vaporizers and bongs. Toxic ingredients in cigarette smoking circulate throughout the body causing damage in several different ways [2]. Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component, including cardiovascular disease and chronic obstructive pulmonary disease [3]. Improvements in assays for protein markers of inflammation have led to many studies on these factors and their roles in disease.

Smoking is known cause of increase in hemoglobin (Hb) concentration. The burning tobacco and paper produce more than four thousand chemical compounds in the form of gases, vapors and particulates like carbon-monoxide, hydrogen cyanide, phenols, ammonia, formaldehyde, benzene, pyrene, nitrosamines, nicotine [2, 4]. Increase in Hb concentration is believed to be mediated by exposure of carbon-monoxide. Carbon-monoxide binds to Hb to form carboxyhemoglobin, an inactive form of hemoglobin having no oxygen carrying capacity resulting in a reduction in ability of Hb to deliver oxygen to the tissue. To compensate the decreased oxygen delivering capacity, smokers maintain a higher hemoglobin level than non-smoker [5].

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Tobacco smoking is the most important risk factor associated with chronic bronchitis and emphysema. Parental smoking to be leading cause of pediatric deaths from low birth weight, short gestation, respiratory distress syndrome and sudden infant death syndrome. Many health problems, hematological and physiological changes are seen in human body due to smoking [6,7]. The links between smoking and increased morbidity and mortality have been long established and current trends indicate that of the one billion smokers worldwide, 500 million will die prematurely from smoking-related diseases [3, 8]. Although the mechanisms linking smoking to these diseases are not well understood, interest in the relationship between inflammatory markers and smoking has been gathering pace in an attempt to provide explanations for smoking-mediated morbidity and mortality [3].

CRP is produced in the liver and its level is measured by testing the blood. CRP is classified as an acute phase reactant, which means that its level will rise in response to inflammation [9]. Smokers have increased number of white blood cells, mainly because of a particular increase in polymorph nuclear neutrophils, which are released from the bone marrow and recruited to inflamed tissue [3,10]. IL- β and IL-6, which are increased in response to lung inflammation and are implicated in the induction of CRP gene expression, may mediate the stimulation of bone marrow cells [3,11]. It is produced principally by hepatocytes, but can also be expressed by adipocytes [12] and cultured coronary artery smooth muscle cells [13], suggesting that localized inflammation can induce CRP expression. Indeed, CRP has been detected by immunofluorescence in atherosclerotic plaques from human coronary arteries [14]. The acute inflammatory response is induced by numerous challenges to the body, including infections and trauma, and leads to gross changes in the levels of CRP and other acute phase proteins [3].

The present study was conducted to compare the effect of cigarette smoking on hemoglobin level and reactivity of CRP among the smoker and non-smoker.

2. Methodology

2.1. Study type

A Comparative cross-sectional community based study was carried out in Kathmandu valley that includes Swoyambhu, Thankot, Balkumari and Bhaktapur. This study was carried out from October to January, 2017 and a random sampling was done on who were smoker and non-smoker. 150 participants were enrolled in the study, whose whole blood (EDTA) sample was taken.

An informed and written consent was taken from all the volunteers. All the information regarding Patient's name, age, address, weight, blood pressure and clinical history were administered by a structured questionnaire.

Blood sample was collected from each participant into EDTA and plain vial. Then the samples were labeled serially according to patient's ID number and the specimen were transported in a Zip lock bag to Hematology laboratory and Biochemistry laboratory of Modern Technical College Sanepa, Lalitpur, Nepal.

People with hemolytic disorder, liver disease, endocrine disorder, under medication for blood disorder and non-residence of Kathmandu valley were excluded from the study.

2.2. Estimation of hemoglobin (By Cyanmethemoglobin method)

When blood was mixed with a solution containing potassium ferricyanide and potassium cyanide, the potassium ferricyanide oxidizes iron to form methemoglobin. The potassium cyanide then combines with methemoglobin to form Cyanmethemoglobin, which is a stable color pigment and the optical density is measured colorimetrically at a wave length of 540 nm.

5 ml of Drabkin's solution in a large size test tube was taken and 20 μ l of well mixed anticoagulated blood was added. It was left for 10 minutes at room temperature for full color development. O.D. was taken at 540 nm against blank (Drabkin's solution) in colorimeter and the value were obtained.

2.3. Estimation of C-reactive protein

CRP Test was based on the **latex agglutination** method introduced by Singer, et. al., in 1957. This is a slide agglutination test for the qualitative and semi-quantitative detection of C - reactive protein (CRP) in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP. When latex particles

coated with human anti-CRP are mixed with a patient's serum containing C – reactive proteins, this results in visible agglutination within 2 minutes.

One drop of serum on the slide using disposable plastic dropper in circled area of the slide, provided in the kit was taken and one drop of CRP latex reagent was added to the above drop and mix with disposable applicator stick. The slide was rocked gently back and forth, observed for agglutination macroscopically after 2 minutes. Agglutination is a positive test result and indicates presence of CRP in the test serum. No agglutination is a negative test result and indicates absence of CRP in the test serum.

2.4. Ethical consideration

Written permission was taken from Institutional Review Board of Modern Technical College after submitting and presenting research proposal. All the information collected remained confidential and would be used for purposes of the study only.

3. Results

This cross-sectional study was done in different places of Kathmandu valley. It was conducted on total of 150 healthy study participants out of which 90 were male and 60 were female. Table 1: shows the socio-demographic distribution of total participants according to gender and reactivity of CRP with respect to smoker and non-smoker. Out of 150 participants, 90 were male and 60 were female participants. Among 90 male, 81 were smoker and 9 were non-smoker where in total of 60 female, 16 were smoker and 44 were non-smoker. Moreover, in total of 150 participants reactive CRP was seen in 30 and rest 120 was non-reactive of CRP. Similarly, among reactive CRP 19 were from smoker group (18 male and 1 female) and 11 were from non-smoker group (1 male and 10 female). On the other hand, among 120 CRP non-reactive participants 78 were from smoker group (63 male and 15 female) and 42 were from non-smoker group (8 male and 34 female).

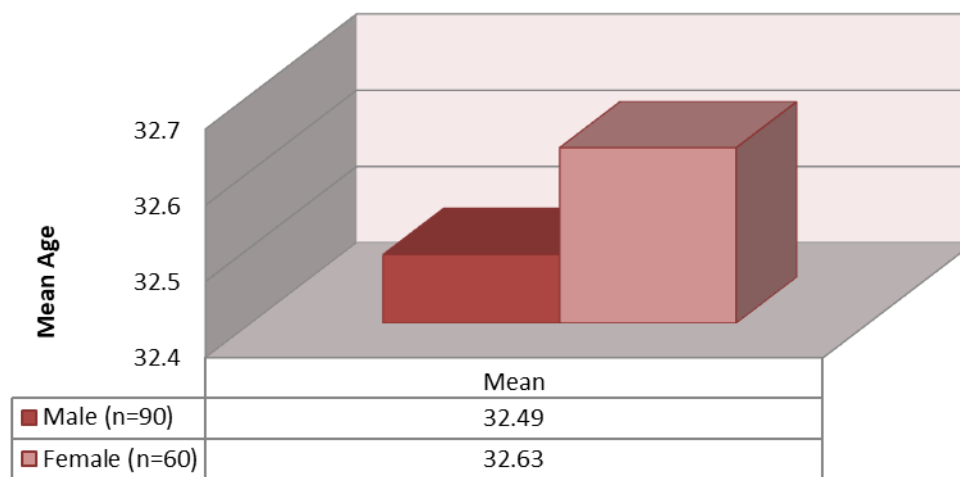
Table 1 Socio-demographic distribution of participants

Characteristic	Smoker (n=97)		Non-smoker (n=53)	
Gender				
Male (n=90)	81		9	
Female (n=60)	16		44	
C-Reactive Protein	(n=19)		(n=11)	
Reactive (n=30)	Male	Female	Male	Female
	18	1	1	10
Non-Reactive (n=120)	(n=78)		(n=42)	
	63	15	8	34

The study population includes people from age group 15 to age group 65. Mean age and standard deviation of male population was (32.49±14.126) and of female was (32.63± 10.583) shown in table 3 below. Among these, total smoker participants were 97 and non-smokers were 53. The mean hemoglobin levels of male and female participant were (13.80±1.585) and (13.02±1.611) respectively. The difference in the mean value of hemoglobin between smokers and non-smokers was prominently significant with p value 0.005 (Table 3). Moreover, all the smoker participants were taken as case population whereas non-smokers were taken as control population.

Table 2 Distribution of participants according to Gender

Sex	Mean	Std. Deviation
Male (n=90)	32.49	14.13
Female (n=60)	32.63	10.58

**Figure 1** Bar diagram comparing gender**Table 3** Descriptive distribution for smoker and non-smoker V/s Hemoglobin

	Mean \pm S.E	Standard Deviation	Sig. (2-tailed)
Smoker (n=97)	13.8 \pm 0.161	1.58	0.005
Non-smoker (n=53)	13.02 \pm 0.221	1.61	

Independent Sample T-test

P value <0.05 is considered to be significant

In the present study, the serum CRP status among the total participants 150 were 30 CRP reactive and 120 CRP non-reactive. 19 (19.6%) of the case within total case (97) were CRP reactive and 78 (80.4%) were CRP non-reactive whereas 11 (20.8%) of the control were found to be CRP reactive within total control (53) along with 42 (79.2%) being CRP non-reactive. The difference in the result of case and control were insignificant with p value > 0.05.

Table 4 Cross tabulation of CRP V/s smoker and non-smoker

Result of CRP	Number of Smoker (n=97)	Number of non-smoker (n=53)	p-value
Reactive CRP	19 (19.6%)	11 (20.8%)	1
Non-reactive CRP	78 (80.4%)	42 (79.2%)	

Chi square Test

P value >0.05 is considered to be insignificant

Smoking population were further divided into different sub groups 1-5, 6-10, 11-20 and 21-50 according to the total number of cigarette sticks consumption per day (n=150). Maximum number of the smoker were found to be in the sub group 11-20 (32) and minimum were in 21-50 sub group (8). Moreover, 31 of the smokers came under 1-5 sub group

and the remaining smokers were in 6-10 (26). On the other hand, the non-smoker participants were grouped as '0' for the diagrammatic representation.

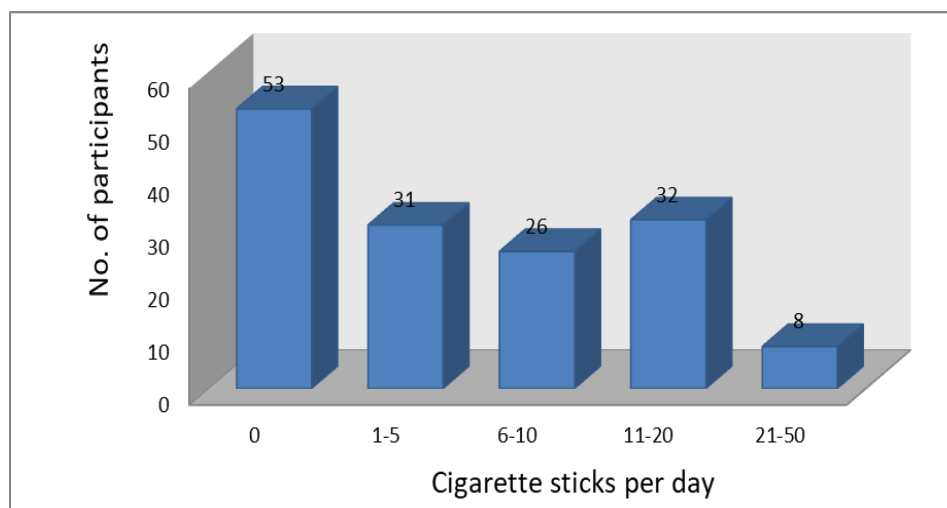


Figure 2 Distribution of population according to cigarette sticks per day

4. Discussion

Consumption of the tobacco through smoking has been correlated to cause morphological and biochemical problems in individuals [15]. In this study, hematological parameter (hemoglobin) and biochemical indicator (CRP) had been used for cross-sectional analysis between smokers and non-smokers among local population of different places of Kathmandu Valley. The experimental results showed significant differences in hemoglobin level between case and control. The mean hemoglobin level of case was significantly high whereas it was low in the control group when compared with each other.

We observed that hemoglobin values were significantly different among smokers than those of non-smokers ($p=0.005$). It is reported that high level of hemoglobin is associated with blood viscosity and clotting in smokers. High level of hemoglobin is termed as polycythemia and very high hemoglobin mass slow blood velocity and increase the risk of intravascular clotting, coronary vascular resistance, decrease coronary blood flow, and predisposition to thrombosis [15, 16]. It has been established that fibrinogen level are higher in smoker than in non-smoker and it has been estimated that the increasing risk of cardiac disease in smoker may be associated with high fibrinogen levels through arterial wall infiltration and effects on blood viscosity, platelet aggregation and fibrin formation [15,16,17]

The mechanism by which polycythemia causes thrombosis is still under investigation but smoking cigarettes create unique condition of combined polycythemia to chronic hypoxia, leading to elevated red cells production due to an elevated carboxyhemoglobin level with concomitant plasma volume reduction. In cigarette smoking, carbon monoxide (CO) is produced by incomplete combustion of carbon containing material. CO has a very high affinity for hemoglobin relative to that for oxygen (approximately 200 folds). Thus CO displaces oxygen from hemoglobin in red cells to produce carboxyhemoglobin (COHb) which reduce the release of oxygen to tissue [15].

We didn't find any significant difference in CRP status between the result of reactivity of CRP test in smoker and non-smoker. Despite the strong evidence on epidemiology that links cigarette smoking with the cardiovascular disease, cancer and chronic obstructive pulmonary disease (COPD), the exact mechanisms by which the smoking causes disease and the components of smoke responsible remain poorly understood.

There is substantial documentation that cigarette smoking can result in both morphological and biochemical disturbances to the endothelium both in vivo and in cell system. Nicotine and carbon monoxide are lesser in hampering than the whole smoke inhaled [18]. But the free radical components of the cigarette sticks are shown to cause more damage to the model system. The injury to the endothelial is taken as the vital component for commencing events on the pathogenesis of atherosclerosis [19] and it is sensible to come up with the assumption that cigarette smoking may employ effects on the cardio vascular system of the body. Smoking also contributes to the development of the secondary

polycythemia and in turn, increases the red cell mass which might be a contributing factor to secondary iron overload disease promoting oxidative stress of the hepatocytes [20].

Numerous studies have been done on the effects of smoking over liver with C-reactive protein as the base of their study taking it as marker for the liver inflammation. Several studies have shown that the CRP happens to be positive among the smoker male and female compared with the non-smokers. Another study found that mean CRP levels were significantly lower in never-smokers than in current smokers. Our study has shown that there is no significant difference in the CRP status between smokers and non-smokers but shown relatively positive in females with compared to the males being non-smokers. Recent study takes in account whether women were taking hormones orally. There is a relation established in the correlation between smoking status of female and increase in CRP concentrations. CRP status was indeed observed to be positive suggesting that such treatments of oral hormones could be masking changes in CRP in female population.

5. Conclusion

On the verge of the study relating smoking with different hematological parameters and smoking related disorders, there have been new reports that demonstrate that cigarette smoking is associated with blood vessel damage and CRP as the marker for the inflammation. Our findings clearly showed that continuous cigarette smoking has severe adverse effect on hemoglobin and CRP. In our experimental result, hemoglobin was significantly elevated in smokers than in non-smokers. Along with this, we also came to find that reactivity indication of CRP status was not significantly different among the smokers and non-smokers. We recommend for further study over this topic including more precise and sensitive technologies in a larger group of people for better results.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declares no conflict of interest.

Statement of ethical approval

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Statement of informed consent

An informed and written consent was taken from all the volunteers. All the information regarding Patient's name, age, address, weight, blood pressure and clinical history were administered by a structured questionnaire.

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