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Leukocyte Telomere length Shortening Among MI patients is Associated with Smoking

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ABSTRACT
Telomeres area heterochromatic region that caps chromosomes ends and have been reported as a marker of age-related disease such as Coronary Heart Disease (CHD). Cigarette smoke contains compounds like nicotine, formaldehyde & acrolein. It also contains some free radicals and oxidants that can cause oxidative damage, a mechanism that causes telomere shortening. Most of the studies ,reporting the effect of smoking on leukocyte telomere length (LTL)in CHD patient specifically Myocardial Infarction (MI),was conducted on white Europeans. No such study has been conducted on the Pakistani population to elucidate the relationship between smoking and telomere length among MI patients. We hypothesized that patients suffering from MI, who use to smoke more than 10 cigarettes per day, may have shorter telomere lengths than non-smoker patients. Epidemiological data and Blood samples were collected from 250 patients suffering from MI. DNA was isolated from peripheral leukocytes and telomere length was measured. Our data showed that the telomere length was significantly reduced among smokers than non-smokers MI patients. This association of cigarette smoking with telomere shortening was consistent among all age groups. This data suggests that smoking might be playing a role in telomere shortening among MI patients. .
Keywords: MI, CHD, LTL, smokers, non-smokers

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INTRODUCTION

Coronary Heart Disease (CHD), the main cause of death worldwide, is caused by formation of plaque in coronary vessels resulting in Fatigue, shortness of breath,angina and Myocardial infarction (MI) [1].A major increase in the deaths is expected because of CHD in South East Asia. Factors like smoking, physical inactivity & unhealthy diet are known to cause CHD but they don't explain the overall burden of CHD in a given population. [1]. The prevalence of MI has decreased because of advance treatment and management of CHD but still, the rate is increasing even in developed countries like Europe and South Asia. Around 15.9 million people worldwide, have suffered from myocardial infarctions in 2015 [2].Telomeres are repetitive nucleotide sequences(TTAGGG) that caps the ends of chromosomes and forms a protective loop by folding back on themselves thus stabilizing them by preventing abnormal recombination and degradation of DNA strands [3]. Telomere gets shorter with every cell cycle by 30 to 200 nucleotides. Telomerase is an enzyme that has a major role in telomere length alteration and causes many diseases as well as aging. Telomerase enzyme is also crucial in DNA repair, apoptosis in mitochondria, regulating gene expression, organization of chromatin and cell growth [4].For these activities, only TERT (telomerase reverse transcriptase) is required instead of TERC (telomerase RNA component) and is thus free of typical telomerase activity[4]. Association of shorter telomeres with CHD could be a result of such activity of telomerase. When telomere length shortens to a critical length, they are not able to serve their protective role and consequently, cell cycle arrest (senescence) or apoptosis is initiated. As increased oxidative stress and cellular senescence are both key indicators of aging, it has been suggested recently that a shortened average telomere length could serve as a biomarker for aging, and age-related diseases [5].

Cardiovascular disorders, most notably atherosclerosis, are found to be closely associated with telomere shortening[6].Telomere shortening is also caused by adopting an unhealthy lifestyle. Degenerative disease, aging, and stress are possible regulators of Telomere Length. Cigarette smoke is one of the factors associated with shorter telomere length in peripheral blood leukocytes. Cigarette smoke consists of many chemicals that are carcinogenic including free radicals, reactive oxygen species (ROS) and gaseous free radical species. These free radicals and oxidants stimulate lipid peroxidation of cellular membranes and promote atherosclerosis. Thus Smoking causes increased levels of oxidative stress and inflammation, both of which are implicated in telomere attrition [7].

The previous studies on the association between leukocyte telomere length(LTL) and CHD have yielded inconsistent results with the majority of studies being conducted with Europeans. No such study has been conducted in Pakistan to evaluate the relationship between LTL, smoking and myocardial infarction (MI). In this study we hypothesized that patients (suffering from MI) who smoke cigarettes may have shorter leukocyte telomere length as compare to patients who were non-smokers, assuming that smoking causes attrition in telomere length.

MATERIAL AND METHODS

Blood samples of 250 MI patients with ages ranging from 30 to 75 were collected from local hospitals of Rawalpindi and Islamabad. All blood samples were collected along with written consent from the patients. The study was approved by the ethics committee of the Biosciences department Comsats University Islamabad Pakistan and respective hospitals. All the research work was conducted in Biosciences Department of Comsats Islamabad. Patients were distributed into 3 groups depending on their age as, Group I (30-45), Group II 45-60and Group III 60-75. A confidential questionnaire form was also filled by each patient that has their bio-data as well as epidemiological factors. Genomic DNA was extracted from blood samples according to the manufacturer's protocol(GF-1 Blood DNA Extraction Kit GF-BD-100 Vivantis USA). Telomere length was measured by real-time PCR (Step 1, Applied Biosystems, USA) by the method described by Cawthon [8] with slight modifications. β-globin was used as single-copy gene or reference gene. For the measurement of telomere length, 35 ng of genomic DNA isolated from the blood samples of MI patients was taken. The reaction for each sample was performed in triplicates. The primer sequence for telomere length and β-globin is described in Table 1. For telomere, the initial denaturation was performed at 95^{o}C for 10 min, followed by 25 cycles of 95^{o}C for 15 sec and 54^{o}C for 2 min. The data acquisition was done at 54^{o}C. Telomere length relative to single copy gene is represented by T/S ratio[9]and was also relative to the average telomere length. In T/S ratio, T represents telomere and S represents single-copy gene. For single-copy gene (β-globin), the concentration of genomic DNA in each sample remained the same. The initial denaturation was done at 95^{o}C for 10 min, followed by 30 cycles of 95^{o}C for 15 sec and 58^{o}C for 1 min with data acquisition. Telomere length was measured in patients suffering from MI of all three age groups. Then based on questionnaire forms we separated MI patients as smokers and non-smokers to analyze the relationship between smoking and telomere length in MI patients and to see if smoking affects telomere length or not.

Results and Discussion:

We collected blood samples from 250 patients suffering from MI. Confidential questionnaire were also filled by the patients and blood samples were taken with the patients consent. Among 250 patients, 65% of patients were smokers and use to smoke on a daily basis as shown in figure 1. These patients use to smoke more than 10 cigarettes per day. Hypertension was present in 89.6% of patients in our study. However, no significant association was found between hypertension and CHD in our study. Overall 59% of patients were suffering from diabetes mellitus. The proportion of risk factors for CHD in the study population is shown in Table 1. Patients were divided into three groups according to their age, group I consist of patients of 30-45 years of age, group II consists of patients of age ranging from 46-60 years and group III consisted of patients of 61-75 years of age(figure 2).Patients were then divided into smokers and nonsmokers based on their epidemiological data. The percentages of smokers and non-smokers in different age groups of patients can be seen in figure 2.

Leukocyte Telomere length was calculated by t/s ratio (fig 3). The data suggests that telomere length shortened among smokers when compared to non-smokers among all age groups. Our observation that leukocyte telomere length declined with increasing age is consistent with previous reports [10]. However, the extent of telomere shortening may vary considerably among individuals within age groups, suggesting that environmental and lifestyle factors could play critical roles in the rate of telomere attrition. Another factor for telomere shortening can be oxidative damage that is caused by the free radicals generated by cigarette smoke (Thorne et al., 2009). LTL of smokers in all three age groups was significantly shorter than nonsmokers of all three age groups (Figure 3).As telomere length shortening is associated with the

aging process, shorter LTL has been associated with CHD.Our study has shown the same trend i.e with increasing age LTL is decreasing among both smokers and non-smokers (figure 3). Numerous studies have shown an association between shorter telomeres in peripheral blood leukocytes and/or buccal cells and risk of bladder, head and neck, lung, and renal cell cancers [11]

There have been several studies conducted on the effect of smoking on LTL but they have reported inconsistent findings. Some of the reports have shown that LTL attrition is faster in smokers [12], whereas in other studies, have shown no effect of smoking on LTL [13]. In one study they have reported faster LTL shortening in nonsmokers [14]. Among the Pakistani cohort, LTL shortens with increasing age in both smokers and non-smokers. Smokers have shorter LTL as compare to non-smokers in all age groups (figure 3).

Table 1. Description of risk factors for CHD among Pakistani population.

Risk Factors	**Frequency**	**Percent**	**P value**
Diabetes mellitus			0.97
Yes	149	59.3%	
No	102	40.6%	
Hypertension			0.39
Yes	224	89.6%	
No	27	10.3%	
Smoking status			0.01*
Yes	163	64.9%	
No	88	35%	
Regular exercise			0.54
Yes	41	16.3%	
No	210	83.6%	
Asthma			0.15
Yes	124	49.4%	
No	127	50.5%	
Anxiety			0.76
Yes	154	61.3%	
No	37	38.6%	

Table 2. Primer sequences of telomere length and β-globin

Primers	**Sequences**
Telomere length	Forward 5'- GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGG-'3 Reverse 5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCT-'3
β-globin	Forward 5'- GCTTCTGACACAACTGTGTTCACTAGC-'3 Reverse 5'- CACCAACTTCATCCACGTTCACC-'3

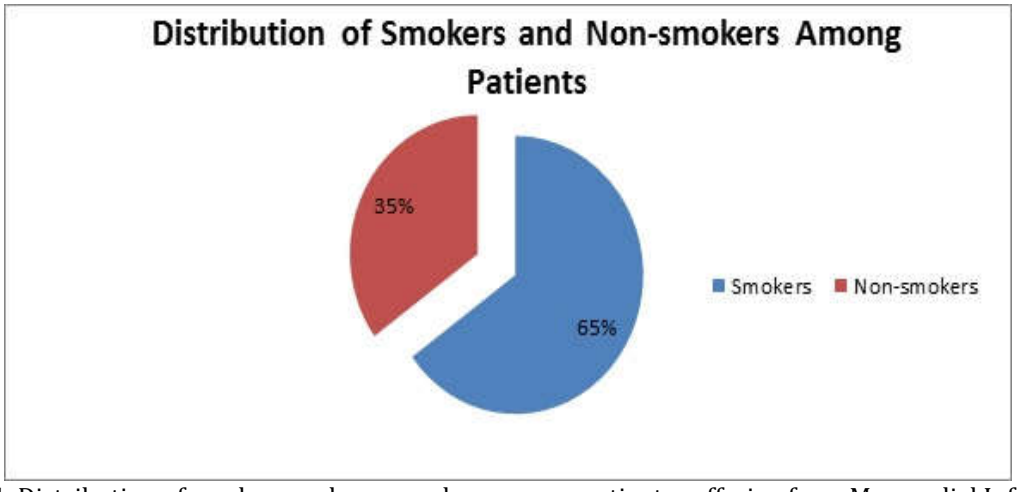

Figure 1. Distribution of smokers and non-smokers among patients suffering from Myocardial Infarction. 65% patients were regular smokers who use to smoke more than 10 cigarettes per day and 35% of the patients were non-smokers.

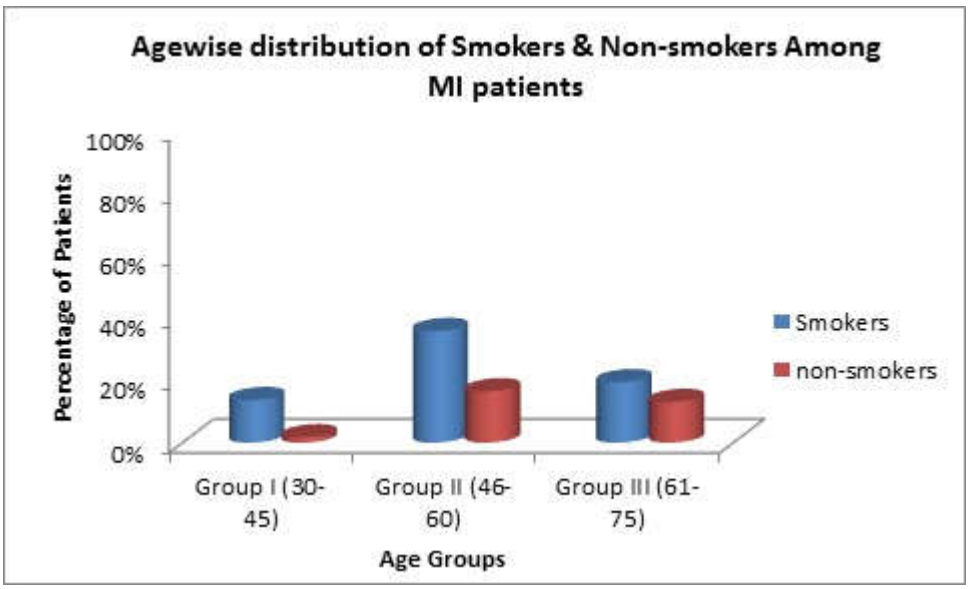

Figure 2. Distribution of smokers and non-smokers among the patients suffering from Myocardial Infarction. Group I consist of patients of 30-45 years of age, group II consists of patients of age ranging from 46-60 years and group III consisted of patients of 61-75 years of age. A high percentage of patients are smokers who smoke more than 10 cigarettes per day.

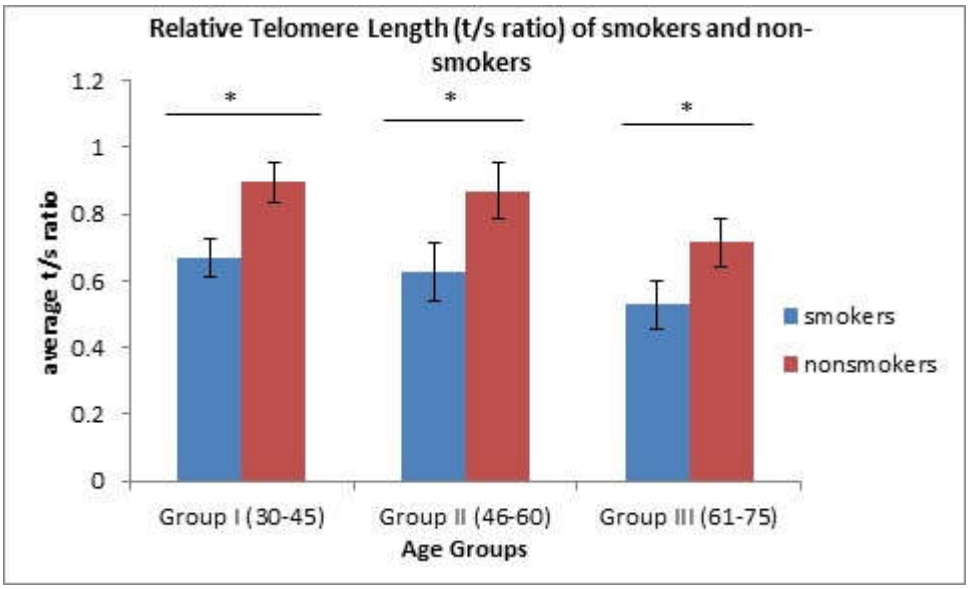

Figure 3. Graph, showing the telomere length normalized with β-globin in groups based on age , and smoking habits. Group I (30-45 years), Group II (46-60 years) and Group III (61-75 years). Error bars show standard error of mean (* $p \leq 0.05$).

CONCLUSION AND RECOMMENDATION

In the Pakistani cohort, Leukocyte Telomere Length (LTL) was associated with smoking, in Myocardial Infarction patients. Smoking is a lifestyle factor that might influence telomere dynamics and cause shortening of leukocyte telomere length. As there have been many contradictory reports on these findings, Longitudinal studies that will measure telomere length on various times points and evaluate the effect of these lifestyle exposures directly on telomere shortening would further elucidate the mechanism . Effective strategies could be adopted for quitting smoking so that the risk of Myocardial Infarction is minimized.

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DECLARATION

The authors declare that they have no conflict of interest

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