SUST
Journal of Natural and Medical Sciences

Journal homepage: http://journals.sustech.edu/

Assessmentof High Sensitive C - reactive protein (hsCRP) and its Association with Cardiovascular Disease among Cigarette Smoking Subjects

Tarig Ahmed Hassan Karar [1], Moawia Ali YousifAbd Allah,[*2], Elyasa Mustafa Elfaki [2]

1. .King Saud bin Abdulaziz University for Health Sciences, College of AppliedMedical Sciences
2. Sudan University of Science and Technology, College of Medical Laboratory Science
Corrsponding Author:
Article history: Recieved: December 2014
Accepted: February 2015

ABSTRACT

Background: Smoking is considered a risk factor for some cardiac diseases and therefore this study aims to assess the serum levels of highly-sensitive C-reactive protein as a marker of cardiovascular inflammatory disease among smokers.
Materials and methods: This cross-sectional study was conducted in Khartoum, Sudan during the period from March to July 2013. Sixty (60) adult-smokers were selected as part of a test group from Khartoum and forty (40) non-smoker adults as control group to assess the serum levels of highly-sensitive C-reactive protein, total cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol. Age and sex of test group was matched with control group. Colorimetric methods were used with commercial kits from Biosystems Company for measuring lipid profile and the hsCRP levels were measured by using fluorescence immune assay technology (sandwich immune detection method). (SPSS) computer software was used for data analysis.Results**:**Results showed a significant increase in the means of serum levels of highly-sensitive C-reactive protein (p=0.000), total cholesterol (p=0.000), and LDLc (p=0.000) of smokers when compared with the control group. Results of this study observed significant decrease in the mean of serum levels of HDLc (p=0.000) of smoker when compared with the control groupand a significantdifference in the mean of serum levels of triglycerides (p=1.371) of smokers when compared with the control group.Also the results of the current study showed significant positive correlation between serum levels of total cholesterol, LDLc with hsCRP (r=0.304, P=0.018) and (r=434, P=0.001) respectively. Furthermore, there is asignificant correlation between serum levels of HDLc with hsCRP (r=0.023, P=0.863).Furthermore the results showed significant positive correlation between serum levels of hsCRP, with duration of smoking(r=0.81, P=0.000) and no significant correlation between serum levels of HDLc with duration of smoking (r=0.109, P=0.407).Conclusion:Study concludes that smoking increase the serum levels of hsCRP, total cholesterol, and LDLc, and decrease the serum levels of HDLc and also the levels of hsCRP, total cholesterol, LDLc affected by duration of smoking. So these parameters can be used as predictors for cardiovascular disease among smokers.

المستخلص

خلفية: يعتبرالتدخينعامل خطرلبعضأمراض القلب، لذلك تهدفالدراسة الحاليةإلىتقييم مستوياتالمصل لبروتين سي التفاعلي عالي الحساسيةكمؤشرلامراض القلب والأوعية الدمويةالالتهابية بينالمدخنين.**المواد والطرق:**اجريت هذه الدراسة المقطعيه في الفتره مابين مارس حتي يوليو2013في60 مدخن بالغبولاية الخرطوم و40 من الأصحاء البالغينتم إختيارهم كمجموعة ضابطة لتحديد وتقييم مستويات مصل بروتين سي التفاعلي عالي الحساسية ، الكولستيرول الكلي، الدهون الثلاثية، كولستيرول البروتين الدهنى عالي الكثافةوكولستيرول البروتين الدهنى منخفض الكثافة. تمت مطابقة الجنس والعمر بين فئتى الدراسة. أستخدمت طرق قياس الضوء بأطقم تجارية من شركة Biosystem التجارية وظائف الدهون واستخدمت الطريقة المناعية المشعة لقياس بروتين سي التفاعلي عالي الحساسية. كما تم استخدام برنامج (SPSS) لتحليل النتائج.
النتائج: اشارت نتائج هذه الدراسة الي زيادة ذات دلالة إحصائية في متوسطات مستويات مصلبروتين سي التفاعلي عالي الحساسية(ب=0.000)، الكولستيرول الكلي (ب=0.000)، وكولستيرول البروتين الدهنى منخفض الكثافة (ب=0.000) للمدخنين لدى مقارنته مع العينة الضابطة (1.15mg/l ± 1.50)مقابل (0.45±0.22mg/l)و (169±24.1mg/dl) مقابل (127±9.9mg/dl)) و (88.0± 18.8 mg/dl) مقابل(37.4±9.7 mg/dl) على التوالى. أشارت النتائج لنقصان زى دلالة إحصائية في متوسطات مستويات مصلكولستيرول البروتين الدهني عالي الكثافة (ب=0.000) للمدخنين لدى مقارنته بالعينة الضابطة (59.7 ± 12.7mg/dl) مقابل (69.1±8.6mg/dl) و فرق غير زى دلالة إحصائية في متوسط مستويمصل الدهون الثلاثية (p=1.371) عند المدخنين لدى مقارنتة بالعينة الضابطة (109±26.2) مقابل (102±19.9). إشارة نتائج هذه الدراسة الى وجود علاقة إرتباط ايجابي ذي دلالة إحصائية بين مستويات مصل الكولستيرول الكلي وكولستيرول البروتين الدهنى منخفض الكثافة مع مستويمصلبروتين سي التفاعلي عالي الحساسية (r=0.304, P=0.018) و (r=434, P=0.001) على التوالى، بالإضافة لعدم وجود إرتباط ذي دلالة إحصائية بين مستويات مصل كولستيرول البروتين الدهني عالي الكثافة مع بروتين سي التفاعلي عالي الحساسية.(r=0.023, P=0.863).أيضا اشارة نتائج هذه الدراسة الى وجود علاقة إرتباط ايجابي ذي دلالة إحصائية بين مستويات مصل بروتين سي التفاعلي عالي الحساسية، الكولستيرول الكلي وكولستيرول البروتين الدهنى منخفض الكثافةمع طول مدة التدخين ((r=0.81, P=0.000)،(r=0.386, P=0.002) و (r=0.427, P=0.001) على التوالى بالإضافة لعدم وجود إرتباط ذي دلالة إحصائية بين مستويات مصل كولستيرول البروتين الدهني عالي الكثافة مع طول مدة التدخين (r=0.109, P=0.407).**الخلاصة:**خلصت الدراسة الى ان التدخين يزيد مستويات مصل بروتين سي التفاعلي عالي الحساسية ، الكولستيرول الكلي و كولستيرول البروتين الدهنى منخفض الكثافةبينما يقلل مستوى مصل كولستيرول البروتين الدهنى عالى الكثافةولايؤثر على مستوى الدهون الثلاثية. بإلاضافة لذلك ان مستويات مصل بروتين سي التفاعلي عالي الحساسية ، الكولستيرول الكلي و كولستيرول البروتين الدهنى منخفض الكثافة تزيد مع طول مدة التدخين.وهذه المعلماتيمكن ان تستخدم كمؤشرات للتنبؤ بامراض القلب عند المدخنين.

KEY WORDS:Cigarette smoking, lipid profile, cholesterol, HDL-c, LDL-c, triglycerides.

INTRODUCTION

Smoking, a global escalating public health problem, is estimated to kill 6 million people, and causes hundreds of billions of dollars of economic damage worldwide each year. Smoking-related diseases cause more deaths each year than all deaths from human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides, and murders combined [(1)].

Cigarette smoking is a complex aerosol that consists of thousands of chemical compounds. Some of the smokers have been identified as carcinogens by International Agency for Research on Cancer. Cigarette smoking is now acknowledged to be one of the leading causes of preventable morbidity and mortality and is one of the largest single preventable causes of ill health particularly associated with coronary artery diseases. Over 30% of the population attributes risk for myocardial infarction is directly attributable to smoking [(2, 3)].

Cigarette smoking is an important and independent risk factor of atherosclerosis, coronary artery disease and peripheral vascular disorders.The mechanism by which smoking increases the cardiovascular diseases are unclear. Recently it has been suggested that smoking adversely affects the concentration of plasma lipids and lipoprotein levels[(4)].

Cigarette smoking was responsible for a large proportion of the increase in cancer mortality in the second half of the 20th Century, a trend with important social consequences, including the widespread misperception that the U.S. is being consumed by a "cancer epidemic" caused by environmental pollution and industrial chemicals. In fact, the "epidemic" consisted almost exclusively of one disease, lung cancer, and was due to one lifestyle factor, cigarette smoking. A retrospective analysis of mortality statistics revealed that, if lung cancer is excluded, the mortality rate from all other forms of cancer combined has declined continuously since 1950[(5)].

Smoking is a risk factor for other malignancies, including cancers of the oral cavity and pharynx, larynx, esophagus, stomach, bladder, kidney, pancreas, uterine cervix and leukemia[(6)].

Highly-sensitive C - reactive protein is considered as a marker of systemic inflammation and this is also assessed for the primary stratification of the general population for the risk of CVD [(7)].Traditionally, dyslipidemia is considered to be one of the most important risk factors for the development of atherogenesis and to assess the cardiovascular risk. Increased LDL cholesterol levels and decreased HDL cholesterol levels are indicative of an atherogenic lipid pattern[(8)].

MATERIALS and METHODS

Across-sectional study conducted during the period March to July 2013 to determine and to evaluate the serum levels of highly-sensitive C-reactive protein, total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein. Sixty smokers were selected as a test group in Khartoum, Sudan. The test group was compared with a control group which included forty non-smoker volunteers.

Detailed history including duration of smoking period, number and type of cigarettes per day, past history of smoking, history and socio-economic status of both the groups were taken and other demographic data questionnaire were used.

All participants were told about the study aims and benefits during their interview and all of them agreed to participate.All samples were taken from the participants after their agreement in

filling the questionnaire.Health educa-education was also provided to all participants. A written consent was obtained from each participates in this study.

Inclusion criteria: Adult Sudanese smokers.

Exclusion criteria: Child smoker, patient with type I and type II diabetes mellitus, hypertension, hyperlipidemia, and those with any inflammatory disorders were excluded from this study.

Sample collection, separation and preservation:

All participants were informed of the aims of study. With all aseptic precautions 5 ml of venous blood had been collected from each smoker and controls at fasting state using a disposable sterile plastic syringe and voided into container without anti-coagulant. Immediately after clot formation, serum was obtained from blood cells after centrifugation for 10 minutes at 5000 r.p.m (round per minute) at room temperature. The serum was collected and kept at -20◦c in different vials till used for analysis.

Analytical procedure:

Serum levels of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein estimated by colorimetric technique while high sensitive C-reactive protein was measured by using fluorescence immunoassay technology.

Estimation of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein were performed by using enzymatic methods, while highly-sensitive C-reactive protein was measured by usingsandwich immuno-detection method. [(9)]

Statistical analysis:

All values were expressed as mean ± SD, t. test was used for comparison of groups andPearson Correlation to measure the strength of a linear association between variables. Statistical analysis was done using SPSS 11.5. P value less than ≤0.05was considered significant and ≤0.001 was taken as highly significant, where the value r = 1 means a perfect positive correlation and the value r = -1 means a perfect negative correlation

Quality control:

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

RESULTS

Table (1) Comparison of means of the serum levels high sensitive C reactive protein, total cholesterol, LDLc, HDLcof thesmokers and non-smokers group

Variables	Test group	Control group	P .value
hsCRP(mg/L)	1.50 ± 1.15	0.45 ± 0.22	0.000**
Cholesterol(mg/dl)	169± 24.1	127 ± 9.9	0.000**
LDLc(mg/dl)	88.01 ± 18.8	37.45 ± 9.7	0.000**
HDLc (mg/dl)	59.71± 12.7	69.1± 8.6	0.000**

- The table shows the mean ± Std. deviation in brackets and probability (P).
- Independent t- test was used for comparison.
- *p<0.05 (Significant); ** p<0.001 (Highly significant)

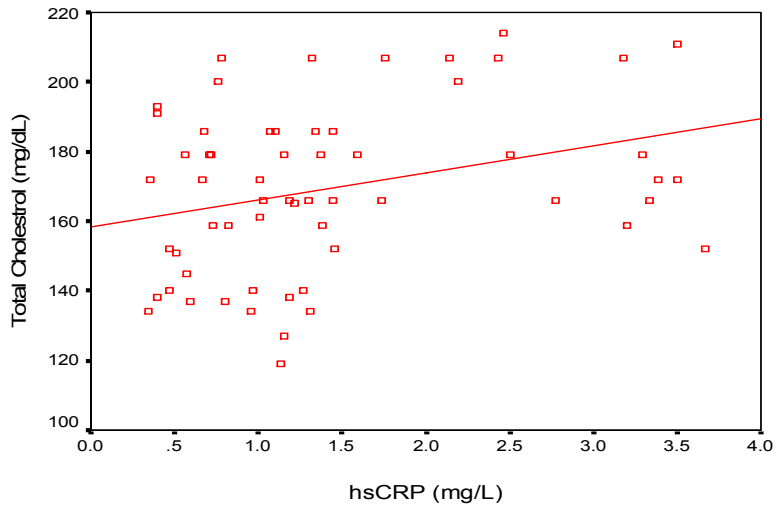

Figure (1): The relationship between the serum levels of high sensitive C. reactive protein and the serum levels of total cholesterol among test group (r=0.304, P=0.018)

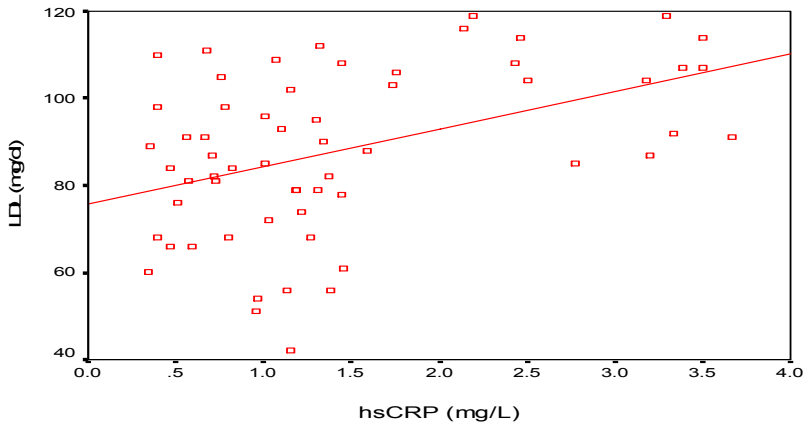

Figure (2): The relationship between the serum levels of high sensitive C. reactive protein and the serum levels of low density lipoprotein cholesterol among test group (r=0.434, P=0.001)

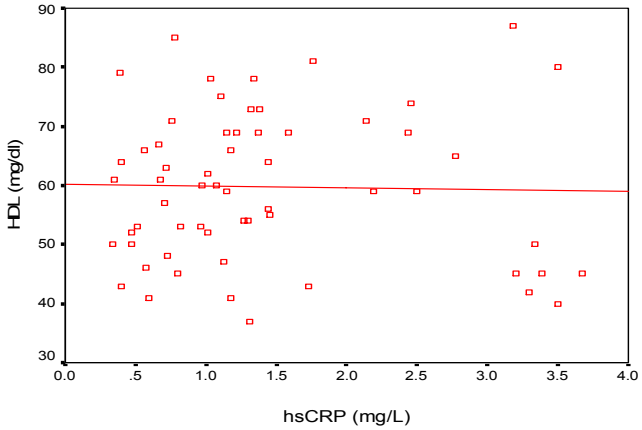

Figure (3): The relationship between the serum levels of high sensitive C. reactive protein and the serum levels of high density lipoprotein cholesterol among test group (r= -0.023, P=0.863)

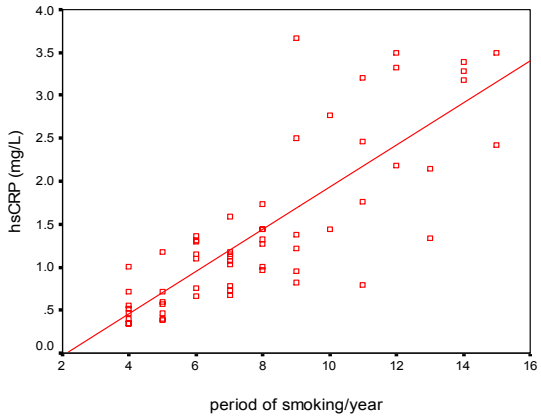

Figure (4): The relationship between the period of smoking and the serumlevels of high sensitive C. reactive proteinamong test group (r=0.810, P=0.000)

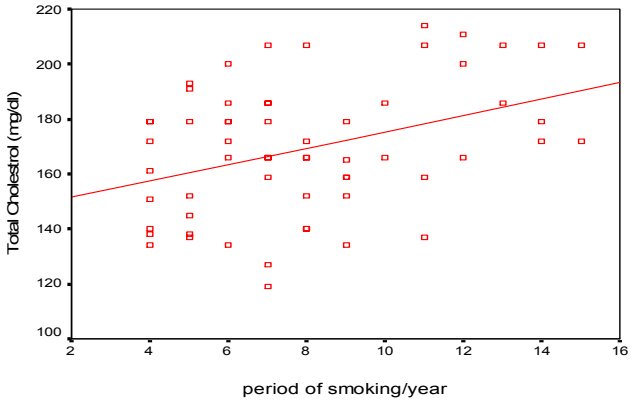

Figure (5): The relationship between the period of smoking and the serumlevels of total cholesterolamong test group (r=0.427, P=0.001)

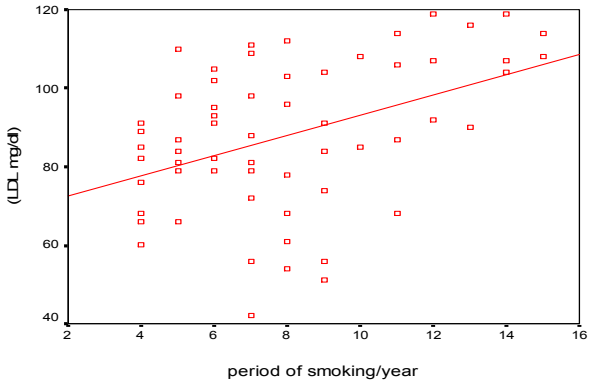

Figure (6): The relationship between the period of smoking and the serum levels of low density lipoprotein cholesterol among test group (r=0.427, P=0.001)

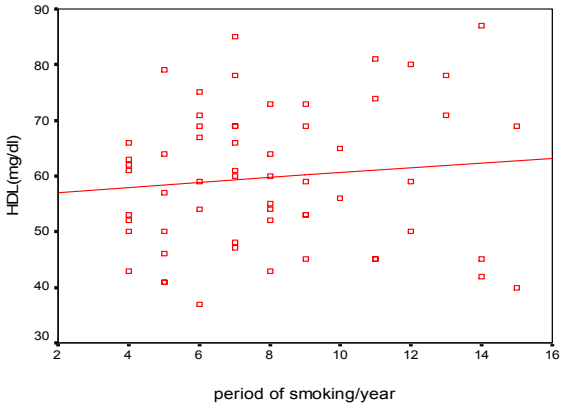

Figure (7): The relationship between the period of smoking and the serum levels of high density lipoprotein cholesterol among test group (r=0.109, P=0.407)

DISCUSSION

Previous study reported that chronic inflammation plays a pivotal role in the development of atherosclerosis. Traditional risk factors are thought to induce inflammatory reaction and to cause the development of atherosclerosis. Cigarette smoking is thought to be one of the major factors responsible for promotion and progression of atherosclerosis, although the mechanisms underlying the pathophysiology of atherogenesis have not been elucidated. Thus, several studies have focused on the association between smoking and inflammatory response [10-14].

Hs-CRP is considered as a marker of systemic inflammation and this is also assessed for the primary stratification of the general population for the risk of CVD [7]. Subsequently, observed elevated levels of hsCRP in smoker individuals, might be assumed as a potential risk factor for heart diseases.

Accordingly the results of present study showed significant increase in hs-CRP of smoker group in comparison with non-smoker group with (*p*-value 0.000). It has long been accepted that cigarette smoking is a classical and major risk factor in the development of cardiovascular disease (CVD) and atherosclerosis [15]. More recently, it has been recognized that CVD contains a component of inflammation and has even been referred to as an inflammatory disease [16]. Based on our study, results indicate that hs-CRP could be a useful predictor marker for cardiovascular disease among cigarette smokers.

The findings of independent t-test showed that mean total cholesterol level is significantly increased in smokers than non-smokers with (P-value 0.000). Furthermore, the test showed that levels of HDL and LDL cholesterol were significantly lower and higher, respectively, in smokers than in non-smokers with (P-value 0.000) for both. The likely justification for our results is that the adverse effect of smoking on cholesterol metabolism which consequently contributes to increased CVD risk seen among smokers[17].

Also our study showed there was insignificant increase in triglyceride level in smokers when compared with non-smokers with (P-value 0.371). This increase might be due to some sort of insulin resistance among smokers which subsequently leads to increased triglyceride [18].

Previous studies indicate that cigarette smoking is a major risk factor for the development of CVD (19) which is usually characterized by dyslipidemia (20), and hs-CRP is considered as a marker for assessing the risk of CVD (7). These findings justified our results which revealed a positive correlation

between cholesterol and LDLc with hs-CRP among smoker with (p-value 0.018, r=0.304), p-value 0.001, r=0.434) respectively, and negative correlation between hs-CRP and HDLc with (p-value 0.863, r= -0.023).

Results of present study demonstrated that duration of smoking is positively correlated with hs-CRP, total cholesterol and LDLc with (p-value 0.000, r=0.810), (p-value 0.001, r=0.427) (p-value 0.001, r=0.427) respectively. Our results were confirmed by previous studies which find that smoking also makes blood vessels and blood cells sticky, allowing cholesterol and other dangerous fatty material to build up inside them. This is called atherosclerosis (21), and consequently leads to increased cholesterol, LDLc and hs-CRP with duration of smoking.

CONCLUSION

This study concludes that hs-CRP is higher in smokers and since hs-CRP is a marker for atherosclerosis it could therefore be a valuable predictor marker for cardiovascular diseases among smokers. Furthermore, long-term smoking leads to increased serum levels of hsCRP, total cholesterol, and LDLc. Therefore long duration of smoking leads to accelerate the complication of smoking.

REFERENCES

1.Parchwani D.N, Upadhyah A.A, Chandan D.H (2013), Effect of Active Smoking on Glucose Tolerance and Lipid Profile, *International Journal of Medical Science and Public Health*, 2 (1) 20-25.

2. Liu J, Liang Q, Pineda K.F, Kah M.R, Rimmer L, Roething H, Mendes P, Sarker M(2011), relation between biomarkers of cigarette smoke exposure and biomarkers of inflammation, oxidative stress, and platelets activation in adult cigarette smokers, *aacr journals*, 20(8) 1760-1770.

3. Yusuf S, Hawaken S, Ounpu U, Danas T, Avezum A, Lanas F, Queen M, Budaj A, Pais P, Varigos J, Lisheng L(2004) effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries. *The Lancet*; 364: 937-952.

4. Devaranavadgi B.B, Aski B.S, Kashinath R.T, Hundekari I.A (2012), Effect of cigarette smoking on blood lipids, *Global Journal of Medical Research*, 12, (6):74-79.

5. Rodu B, Cole P(2001), the fifty year decline of cancer in America, *Journal of Clinical Oncology*, 19:239-241.

6. Rodu B, Godshall W (2006),Tobacco harm reduction: an alternative cessation strategy for inveterate smokers, *Harm Reduction Journal*, 3, (37):1-23

7. Jialal I. Devaraj S (2001), Inflammation and atherosclerosis, *American Journal ofClinical Pathology*, 116, (1): 108-115

8. Nanri A, Moore MA, Kono S (2007) impact of c-reactive protein on disease risk and its relation to dietary factors, *Asian Pacific Journal of Cancer prevention* (8): 167-177.

9.Koening W, Sund M (1999), C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle- aged men. *Circulation* 99; 237-242.

10. R. Ross (1999), Atherosclerosis: An inflammatory disease *N. Engl. J. Med*., 340, pp. 115–126

11. J. Yudkin, M. Kumari, S. Humphries, V. Mohamed Ali (2000): Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis,*148 (2), pp. 209–214

12. W. Kannel(1978), Hypertension, blood lipids, and cigarette smoking as co-risk factors for coronary heart disease Ann. N. Y. Acad. Sci., 304, pp. 128–139

13. The Pooling Project Research Group (1978), Relationship of blood pressure,

serum cholesterol, smoking habit, rela-relative weight and ECG abnormalities to incidence of major coronary events: final report of the pooling project *J. Chron. Dis*:pp. 201–306

14. W. Koenig, M. Sund, M. Frolich, H. Fischer, H. Lowel, A .Doring, W. Hutchinson, M. Pepys(1999): C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged man:*Circulation*, 99, pp. 237–242

15. Smith SC Jr, Milani RV, Arnett DK, Course JR, McDermott MM, Ridker PM, Rosenson RS, Taubert KA, Wilson PW (2004). Atherosclerotic Vascular Disease Conference: Writing Group II: risk factors. *Circulation*; 109: 2613–6.

16. Libby P, Ridker PM, Maseri A(2002). Inflammation and atherosclerosis. *Circulation*; 105: 1135–43.

17. Gossett LK, Johnson HM, Piper ME,(2009) et al. Smoking Intensity and Lipoprotein Abnormalities in Active Smokers. J Clin Lipidol, 3:372–78.

18. Rashidi H, Salesi M, Fatahi F (2010),Effects of Cigarette Smoking on Postprandial Triglyceride in Healthy Smokers*Iranian Journal of Diabetes and Lipid Disorders*, 9: 1-4

19. Akbari MZ, Bhatti MS, ShakoorM (2000), lipid profile in smoking. *JAMC*, 12, 19-21

20. Ahmed SM, Clasen ME, Donnelly JF (1998).Management of Dyslipidemia in Adults. *AmFam Physician*, 57, 2192-2204

21. Pittilo R M (2000). Cigarette smoking, endothelial injury and cardiovascular disease. *International Journal Experimental Pathol*ogy, 81, 219–230.