ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.9, No.04 pp 501-508, 2016

Leukocyte Telomere Length in Heavy Tobacco and Marijuana Egyptian Smokers

Mohamed Abdelmordy Mohamed[1], Khadiga salah Ibrahim[2], Heba Mahdy-Abdallah[2]*, Hanan Abdelgawad Mohamed[2]

[1]Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
[2]Environmental &Occupational Medicine Department, Environmental Research Division, National Research Centre, Egypt.

Abstract : Lifestyle factors such as smoking, obesity, physical inactivity and drug addiction could be related to short telomere length**.** Telomere shortening reflects the balance between cytotoxic stressors and antioxidant defense mechanisms. This study aimed to determine leukocyte telomere length and oxidative stress in Egyptian Tobacco and Marijuana Smokers. The study was carried out on 90 male persons divided into three groups. Group I was composed of 30 non smokers, group II comprised 30 tobacco smokers and group III included 30 tobacco and marijuana smokers. For all participants, Leukocyte Telomere Length (LTL) malondialdehyde (MDA), total antioxidant capacity (TAC), urinary 8-hydroxydeoxyguanosine (8-OHdG), Cotinine and Delta-9-tetrahydrocannabinol (THC) were estimated. There was a highly statistically significant difference between the three groups regarding the LTL. MDA and urinary 8HdG levels were significantly increased in groups II and III compared to the control group On the other hand leukocyte telomere length and the level of total antioxidant capacity were significantly decreased in the two groups as compared to the control one. There were significantly negative correlations between the LTL and levels of MDA and urinary 8HdG in group II and III as compared control group. In conclusion telomere length erosion was greatly accelerated by tobacco smoking and marijuana addiction. Oxidative stress due to heavy smoking of either tobacco or marijuana plays a key role in DNA damage and shortening of telomere length.
Keywords: leukocyte telomere length, cotinine, Delta-9-tetrahydrocannabinol, oxidative stress.

Introduction

Telomeres are regions of tandem arrays of noncoding 5'-TTAGGG-3' repeats. They exist together with their associated proteins, collectively termed the shelterin complex [1,2]. Telomeres has several functions including capping the end of the chromosomes, protecting them from end-to-end fusion and ensuring genome stability [3]. Telomere length is maintained by telomerase, a ribonucleoprotein reverse transcriptase which slows but not entirely prevents telomere erosion, and this is due to end-replication problem [4]**.** Lifestyle factors such as smoking, obesity, physical inactivity and drug addiction could be related to short telomere length [5]**.** Telomere shortening is not only related to the basic biology of aging as a trigger of cellular senescence but also reflects the imbalance between cytotoxic stressors and antioxidant defense mechanisms [6]**.**

Tobacco smoking is one of the great preventable causes of death worldwide, killing approximately five million people who use it each year, it is expected to increase to more than eight million per year by 2030 [7].

Tobacco smoking in Egypt is prevalent with 19 billion cigarettes smoked annually, making it the largest market in the Arab world. Tobacco smoke contain approximately 5,000 chemicals [8], among them are reactive oxygen species and free radicals that are present in the gas phase. In the tar phase radicals are stable and predominantly organic, such as semiquinone which can react to produce superoxide anion. Short lived radicals in the gas phase of cigarette smoke may be quenched immediately in the epithelial lining fluid; however, redox reactions in cigarette smoke condensate may produce reactive oxygen intermediates for a considerable time [9].

Marijuana is the most widely used illegal drug worldwide and is considered the second most commonly smoked substance after tobacco. Marijuana is extracted from the dried flower tops and leaves of the *Cannabis sativa* plant [10, 11]. In Egypt, cannabis plant has been cultivated for almost a thousand years but it has been utilized for hashish production for at least the last eight or nine centuries [12]**.** Marijuana is abused for its mood altering properties and is consumed by different routes, of which, the most common route is smoking. Marijuana is usually smoked in hand-rolled cigarettes called joints or in blunts, which are leaf wrap of a hollowed-out cigars filled with a mixture of tobacco and marijuana [13]**.** In marijuana, more than 500 constituents have been identified; the major psychoactive constituent in it is 1-δelta-9-tetra hydrocannabinol (THC). Marijuana's strength as mind-altering drug is correlated to THC amount it has [10,14]. Other compounds in marijuana include cannabinoids that are chemically related to THC. Some of these cannabinoids have been shown to possess important pharmacological effects [15, 16]**.**

There is a strong link between consumption of cannabis and tobacco smoking as the majority of cannabis users also smoke tobacco [17]**.** It is believed that cannabis use has been found to promote the transition to more profound tobacco smoking [18] and to make it more difficult to quit tobacco smoking [19, 20]**.**

Human beings are continuously exposed to environmental free radicals, including ROS, in the form of radiation, UV lights, smoking, some pesticides as well as certain medications used for cancer treatment**.** ROS may induce damage of DNA, proteins, lipids and carbohydrates ending in change in the structure and functions of the organism as a result of oxidative stress [21, 22]**.** The aim of the present study is to determine the impact of tobacco and marijuana smoking on leukocyte telomere length and oxidant/antioxidant status.

Subjects and Methods

The present study was conducted on 90 male Egyptian persons. They were categorized into three groups matched for age and socioeconomic status. Group I included 30 non smokers as a control group, group II comprised 30 tobacco smokers and group III contained 30 tobacco and marijuana smokers.

All of the participants were free of serious illness, including infectious disease, cardiovascular diseases, mental disorders, and cancer, at the time of participation. Most of the participants were recruited from one of the largest hospitals for psychiatry and addiction in Giza, Egypt.

The present study was approved by the local ethical committee of National Research Centre, Egypt. Informed Consent was taken from each participant in the study and all the included subject were interviewed to fill a questionnaire, including personal, medical and smoking histories.

Samples collection

Peripheral whole blood samples were obtained from each individual into two vacationer tubes; the first tube contained ethylenediaminetetra acetic acid (EDTA) for the determination of leukocyte telomere length (LTL) and the second tube was a plain tube (without anticoagulant) to separate the serum for the determination of Malondialdehyde (MDA) as a marker of oxidative stress and Total Antioxidant Capacity (TAC).

Urine sample was collected from each individual in sterile cups and directed for the determination of levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), as a biomarker of oxidative DNA damage and cotinine as a biomarker of tobacco smoking.

Biochemical and molecular analysis

I -Molecular analysis

Estimation of Leukocyte Telomere Length (LTL):

Telomere length was measured by established quantitative PCR-based technique [23] (using applied biosystems 7500 thermal cycler **(Applied Biosystems, USA).**

DNA extraction:

Leukocyte DNA was extracted using Blood DNA commercial extraction kit **(Vivantis, Malaysia).** Genomic DNA extracted from 200µl whole blood using a specially treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. High-purity DNA is then eluted in water or low salt buffers.

Telomere Length Measurement

The telomere assay contained SYBR green 1x master mix, 300 nmol Telomere F-primers, 300 nmol Telomere R- primers, and 1 ng of template DNA in duplicate10 ml reaction within the same plate.

Telomere primers:

F: 59CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-39

R: 59GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-39.

The single copy assay included estimation of the level of RNase P gene expression as an internal standard using 1x the primers and the TaqMan probe reagent, 1x TaqMan universal master mix, and 3 ng of template DNA in duplicate10 ml reaction within the same plate.

The cycling conditions for the telomere and RNase P assays were as follows: 95°C incubation for 10 min, followed by either 50 cycles of 95°C for 15 sec and 65°C for 1 min.

Each run of the samples also contained a calibrator genomic DNA is used with each run of the samples. Leukocyte telomere length was calculated as the T/S (telomere/single copy) ratio using RNase P gene as a reference for each sample.

II-Biochemical analysis

Estimation of Malondialdehyde (MDA):

MDA was estimated by its thiobarbituric acid reactivity using spectrophotometric method [24] using commercially available kit (Biodiagnostic Giza, Egypt). Briefly, the assay measures the reaction of MDA with thiobarbituric acid (chromogenic substance) in acidic media at temperature 95°C for 30 min to form thiobarbituric acid reactive product, the absorbance of the resultant pink product was measured at 534 nm.

Estimation of Total antioxidant capacity (TAC):

TAC in serum was estimated by using spectrophotometric method [25]. TAC was assayed in serum samples using commercially available kit (Biodiagnostic Giza, Egypt). Briefly, the assay measures the reaction of TAC in the sample with a defined amount of exogenously provided hydrogen peroxide (H2O2). The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5dichloro-2-hydroxy benzene sulphonate to a colored product which can be measured at 505 nm.

Estimation of urinary 8-hydroxydeoxyguanosine (8-OHdG):

8-OHdG was determined using high performance liquid chromatography with electrochemical detection (HPLC-EC) according to [26]. 8-OHdG was assayed in urine sample using commercially available kit (Sigma Chemicals St. Louis, MO, USA).

Extraction:

8-OHdG was extracted from 1ml of each urine sample. The eluents were dried under ultrapure nitrogen stream and were reconstituted in 5 ml deionized water. 20 µl from each sample and also from the different concentrations of the standard were injected in HPLC, and the concentration of urinary 8-OHdG was obtained from the standard curve.

HPLC condition:

HPLC column C18 (260 × 4.6, particle size 5 µl) using mobile phase acetonitrile/methanol/phosphate buffer (25/10/965) v/v. The separation was performed at ambient temperature at a flow rate of 1 ml/min and using electrochemical detector with cell potential 600 mv.

Determination of Cotinine:

Cotinine was estimated by Enzyme-Linked Immunosorbent Assay (ELISA) method [27]**.** Cotinine was assayed in urine samples using commercially available kit (GenWay Biotech, San Diego). Briefly, cotinine in the samples competes with a Cotinine enzyme conjugate for binding sites. Unbound Cotinine and Cotinine enzyme conjugate is washed off by washing step. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Cotinine in the samples. A standard curve is prepared relating color intensity to the concentration of the Cotinine.

Measurement of Delta-9-tetrahydrocannabinol (THC):

THC was estimated by enzyme immunoassay method [28]. THC was assayed in urine samples using commercially available kit (Microgenics Corporation, USA). Briefly, the assay uses specific monoclonal antibody, the specific antibody binds the drug labeled with G6PDH and the enzyme activity is inhibited which can detect the major metabolite of Δ9-THC in urine. This phenomenon creates a direct relationship between the drug concentration in urine and the enzyme activity. The G6PDH activity was determined spectrophotometrically at 340 nm.

Statistical analysis

Statistical analysis of the obtained data was performed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). A One-way analysis of variance test (ANOVA) was used for quantitative comparison between more than two means of the interest, Post Hoc test was used for multiple comparisons between different variables of the interest, Pearson's correlation coefficient (r) test was used for correlating data.

Results

LTL was measured in the three groups of the present study and it was calculated as the T/S ratio. The average leukocyte telomere length was 0.84±0.03 T/S ratio with range 0.8-0.9 T/S ratio in control group, 0.69±0.11 T/S ratio with range 0.47-0.87 T/S ratio in tobacco smokers group and 0.61±0.09 T/S ratio with range 0.46-0.79 T/S ratio in marijuana smokers group and it was observed that there was highly statistically significant difference between the three groups as regards leukocyte telomere length.

Table 1: comparison between the three groups regarding age, cotinine, THC, 8HdG, total antioxidant capacity, malondialdehyde and leukocyte telomere length.

		Groups			LSD		
		Group I	**Group II**	**Group III**	**I vs. II**	**I vs. III**	**II vs. III**
Age (years)	Mean ± SD	40.53±7.52	41.07±6.98	41.83±6.94	0.773	0.483	0.679
	Range	25-53	25-55	27-55			
Cotinine (ug/g creat)	Mean ± SD	37.25±15.21	602.60±138.47	739.82±170.59	**<0.001****	**<0.001****	**<0.001****
	Range	14.2-75.2	356.6-903.9	383.6-1053			
THC (ng/ml)	Mean ±SD	3.23±1.76	3.83±2.94	98.47±23.52	0.866	**<0.001****	**<0.001****
	Range	1-8	1-14	64-155			
8-OHdG (umol/molcreat)	Mean ±SD	0.09±0.03	1.24±0.21	1.48±0.25	**<0.001****	**<0.001****	**<0.001****
	Range	0.02-0.16	0.67-1.65	1.05-2.12			
Total antioxidant capacity (mM/L)	Mean ±SD	1.61±0.26	0.64±0.26	0.58±0.26	**<0.001****	**<0.001****	0.375
	Range	1.01-1.98	0.19-1.21	0.12-0.99			
Malondialdehyde (nmol/ml)	Mean ±SD	2.46±0.78	9.78±3.34	10.60±1.26	**<0.001****	**<0.001****	0.136
	Range	1.1-3.7	5.2-18.6	8.1-12.9			
Leucocyte telomere length (T/S ratio)	Mean ±SD	0.84±0.03	0.69±0.11	0.61±0.09	**<0.001****	**<0.001****	**<0.001****
	Range	0.8-0.9	0.47-0.87	0.46-0.79			

** high significance

According to the statistical data of table1, MDA and urinary 8HdG levels were significantly increased in group II and III compared to the control group. On the other hand leukocyte telomere length and the level of total antioxidant capacity were significantly decreased in the two groups compared to the control one.

There was a significant negative correlation between cotinine level and LTL. Also cotinine level correlated negatively with TAC in groups II and III. Meanwhile, there was a significant positive correlation between cotinine and MDA and 8-OhdG in the smoker groups. In group III, THC level correlated negatively with LTL and TAC level but there was a significant positive correlation between it and MDA and 8-OhdG as showed in table 2.

Table 2: Correlation between THC and cotinine and the other parameters in the three groups.

	Group I				**Group II**				**Group III**			
	THC (ng/ml)		**Cotinine** (ug/g creat)		**THC** (ng/ml)		**Cotinine** (ug/g creat)		**THC** (ng/ml)		**Cotinine** (ug/g creat)	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Age (years)	0.103	0.590	0.066	0.730	-0.297	0.111	0.239	0.204	-0.070	0.714	-0.428	0.078
8-OHdG (umol/mol creat)	-0.117	0.539	0.320	0.059	0.101	0.596	0.344	**0.025***	0.326	**0.029***	0.493	**0.006***
Total antioxidant capacity (mM/L)	-0.162	0.394	0.138	0.468	-0.014	0.942	-0.299	**0.043***	-0.300	**0.031***	-0.257	**0.046***
Malondialdehyde (nmol/ml)	-0.177	0.350	0.009	0.962	-0.015	0.937	0.275	**0.050***	0.368	**0.017***	0.381	**0.016***
Leukocyte telomere length (T/S ratio)	0.178	0.346	-0.167	0.376	-0.031	0.873	-0.343	**0.024***	-0.294	**0.041***	-0.295	**0.040***

*p<0.05 = Significant

Discussion

In the present study the levels of MDA were significantly increased in both Group II (tobacco smokers) and Group III (P-value <0.001) as compared to the control group. The obtained results correlate with those published by [29] who reported that MDA levels were significantly higher in smokers than in non–smokers. Elevated plasma levels of MDA indicate increase in the level of production of oxygen free radicals. Therefore, quitting smoking represents an irreplaceable preventive strategy against tobacco– induced oxidative stress. In the same concern, [30] reported an increase in the level of MDA in marijuana smokers. Marijuana cigarettes stimulated the formation of reactive oxygen species (ROS) by 80% over control levels and lowered intracellular glutathione levels by 81%. Also, activation of endothelial cells, neutrophils, and hepatocytes, results in amplified reactive oxygen and nitrogen species.

In the current study the levels of 8-OHdG were significantly increased in groups II and III (P-value <0.001) as compared with the control group. The urinary biomarker, 8-OHdG is generated by the interaction of hydroxyl radical with guanine. It acts as a biomarker to detect systemic oxidative DNA damage associated with oxidative stress [31]**.** Previous studies have reported that the increase in the concentration of 8-OHdG in the urine of smokers, indicates DNA damage and the genotoxic action of cigarette smoke [32].

A comparison of TAC level among the three groups of the present study demonstrated that there was significantly decrease in TAC level in both tobacco smokers and tobacco and marijuana smokers. Those findings are in accordance with a study by [33] who reported that smokers had lower levels of total antioxidant capacity. This could be due to the effects of cigarette smoking on antioxidant defense system, also the progress of oxidative stress with cigarette smoking. Also [30] reported that reduction of the TAC confirm the involvement of hepatic damage as a result of marijuana smoking. TAC was decreased indicating the presence of a state of oxidative stress. The reduction in the antioxidants mechanisms in the blood of marijuana smokers may favor the reaction of NO with the excessive free radicals; including superoxide anion forming peroxynitrite which results in decreased NO bioavailability.

In the present study, the comparison between the three groups as regards leukocyte telomere length (T/S ratio) showed highly statistically significant difference between them. It reflected significant decrease in leukocyte telomere length (T/S ratio) in smokers of both groups compared to the control group. This could be probably due to disturbances which occur during cell division, occurrence of oxidative stress, impairment of antioxidant function, and or interference with telomerase activity [34]**.**

Our results agreed with those obtained by [35] who studied the effects of cigarette smoking and nicotine metabolite ratio on LTL [36] also reported that heroin; amphetamine, cocaine, and marijuana addiction significantly increased the levels of oxidants and decreased the levels of antioxidants. When oxidant/antioxidant balance is affected, oxidative damage to telomeric DNA occurs.

The rate of telomeric erosion has been reported to be associated with oxidative stress. The latter provides a potential mechanism by which it could interfere with replication of telomeric DNA and accelerating its erosion [37]**.**

The present results showed a positive significant correlation between LTL and THC and cotinine in smokers confirming the relation between telomere shortening and smoking habit of tobacco and marijuana. MDA levels in tobacco smokers showed significant negative correlation with cotinine in group Π and with both cotinine and THC in group III. Moreover, biomarkers of tobacco and marijuana correlated negatively with TAC. Those findings confirm results of previous studies [34, 35, 36, 38].

Conclusion

Telomere length erosion was greatly accelerated by smoking habit and addiction. The present study indicates that imbalance of oxidant/antioxidant status (oxidative stress) due to heavy tobacco smoking and marijuana addiction plays a key role in DNA damage and shortening of telomere length. Since the telomeric damage is hard to repair, the process of cell senescence and early aging will occur. Those findings may help in explanation of many diseases and cancers which are associated with shortening of leukocyte telomere length resulting from smoking and addiction.

References

1. Houben, J.M., Moonen, H.J., vanSchooten, F.J., and Hageman, G. J.. Telomere length assessment: biomarker of chronic oxidative stress? Free Radic. Biol. Med., 2008, 44:235–246.
2. Nasir NFM, Kannan TP, Sulaiman SA, Shamsuddin S, Ahmad A and Stangaciu S. Telomeres and Oxidative Stress. A Review. Brit. J. Med. Med. Res., 2014, 4 (1): 57-67.
3. Derradji, H., Bekaert, S., Van oostveldt, P. and Baatout, S. Comparison of Different Protocols for Telomere Length Estimation by Combination of Quantitative Fluorescence *In Situ* Hybridization (Q-FISH) and Flow Cytometry in Human Cancer Cell Lines. Anticancer Res., 2005, 25: 1039-1050.
4. Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM and Dimmeler S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. Circ Res., 2004, 94(6):768-75.
5. Chen LS, Bloom AJ, Baker TB, Smith SS, Piper ME, Martinez M. et al. Pharmacotherapy effects on smoking cessation vary with nicotine metabolism gene (CYP2A6). Addiction., 2014,109: 128–137.
6. Aviv A, Susser E . Leukocyte telomere length and the father's age enigma: implications for population health and for life course. Int. J. Epidemiol., 2013, 42(2):457-62.
7. Mathers CD and Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med., 2006, 3:e442.
8. Borgerding, M. and Klus, H. (). Analysis of complex mixtures—cigarette smoke. Exp. Toxicol. Pathol., 2005,57: 43-73.
9. Valavanidis A, Vlachogianni T and Fiotakis C. 8-Hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J. Environ. Sci. Health C Environ. Carcinog. Ectoxicol Rev., 2009, 27 (2): 120–139.
10. Tashkin DP . Effects of marijuana smoking on the lung. Ann. Am. Thorac. Soc., 2013, 10(3):239–247.
11. Owen KP, Sutter ME and Albertson TE . Marijuana: Respiratory tract effects. Clin. Rev. Allergy Immunol., 2014, 46(1):65–81.
12. Clarke, R. and Merlin, M. Cannabis: Evolution and Ethnobotany. EBL ebooks online. University of California Press.2013, p. 234
13. Baggio S, Deline S, Studer J, Mohler-Kuo, M., Daeppen, J.B. and Gmel, G. Routes of administration of cannabis used for nonmedical purposes and associations with patterns of drug use. J. Adolescent Health., 2014,54: 235-240.
14. Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, Ross SA, Khan IA and ElSohly MA . Potency trends of Δ9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J. Forensic Sci., 2010, 55(5):1209-1217.
15. Ashton CH . Pharmacology and effects of cannabis: A brief review. Brit. J. Psychiat., 2001, 178:101–106.
16. Pertwee, R.G. Pharmacological actions of cannabinoids. Handb. Exp. Pharmacol., 2005, 168:1–51.
17. Leatherdale ST, Hammond DG, Kaiserman M and Ahmed R. Marijuana and tobacco use among young adults in Canada: are they smoking what we think they are smoking? Cancer Causes & Control., 2007, 18 (4): 391–397.
18. Agrawal KH . Catch them young. Natl. J. Community Med. 2011,2: 498–499.
19. Ford D E, Vu H T and Anthony JC. Marijuana use and cessation of tobacco smoking in adults from a community sample. Drug Alc. Depend., 2002, 67: 243–248.
20. Amos A, Wiltshire S, Bostock, Y, Haw S and McNeill A. 'You can't go without a fag...you need it for your hash'—a qualitative exploration of smoking, cannabis and young people. Addiction., 2004, 99: 77–81.
21. Prakash R, Singapalli T and Gokulnath . Review of oxidative stress in relevance to uremia. Clin. Queries Nephrol., 2012,1 (3):215–221.
22. Small DM, Coombes JS, BennettN, Johnson DW and Gobe GC . Oxidative stress, antioxidant therapies and chronic kidney disease. Nephrology., 2012,17 (4):311–321.
23. Gil ME and Coetzer TL. Real-time quantitative PCR of telomere length. Mol. Biotechol., 2004, 27: 169–172.
24. Satoh K . Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica. Chimica. Acta., 1978,90: 37-43.
25. Koracevic D, Koracevic G, Djordjevic V, AndrejevicS and Cosic V. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 2001, 54(5):356-361.

26. Kim JY, Mukherjee S, Ngo L. and Christiani DC. Urinary 8-hydroxy-2-deoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to fine particulates. Environ. Health Persp., 2004,112 (6): 666-671.
27. Wielkoszynski T, Tyrpien K and Szumska M. The enzyme-linked immunosorbent assay (ELISA) method for nicotine metabolites determination in biological fluids. J. Pharm. Biomed. Anal.,2009, 49 (5):1256–1260.
28. Rubenstein KE, Schneider RS, and Ullman EF . Homogeneous Enzyme Immunoassay: A New Immunochemical Technique. Biochem. Biophys. Res. Commun., 1972, 47 (4): 846-851.
29. Shankar H, Kalaiselvi K . Study of plasma levels of Malondialdehyde as an oxidative stress marker" so it comes as marker in smokers & non-smokers. Paripex-Ind. J. Res., 2016, 5(1): 69-70.
30. Toson EA . Impact of marijuana smoking on liver and sex hormones: Correlation with oxidative stress. Nature and Science., 2011,9(12):76-87.
31. Valavanidis A, Vlachogianni T and Fiotakis K . Tobacco Smoke: Involvement of Reactive Oxygen Species and Stable Free Radicals in Mechanisms of Oxidative Damage, Carcinogenesis and Synergistic Effects with Other Respirable Particles. Int. J. Environ. Res. Public Health., 2009,6: 445-462.
32. Lu C, Ma Y, Chen P, Wu C and Chen Y. Oxidative Stress of Office Workers Relevant to Tobacco Smoking and Inner Air Quality. Int. J. Environ. Res. Public Health., 2014, 11 (6): 5586-5597.
33. Mojtaba, E.; Hussein, D. M.; Ghodratollah, B. Association of C-reactive protein and total antioxidant capacity in smoker. Int. J. Biosci., 2014,5(12) 357-363.
34. Mather KA, JormA F, Parslow R A and Christensen H. Is telomere length a biomarker of aging? A review. J. gerontol. A. Biol. Sci. Med. Sci., 2011, 66 (2): 202–213.
35. Verde Z, Reinoso-Barbero L, Chicharro L, Garatachea N, Resano P, Sánchez-Hernández I, Rodríguez González-Moro JM, Bandrés F, Santiago C, Gómez-Gallego F. Effects of cigarette smoking and nicotine metabolite ratio on leukocyte telomere length. Environ Res., 2015,140: 488-494.
36. Yang Z, Ye J, Li C, Zhou D, Shen Q, Wu J, Cao L, Wang T, Cui D, He S, Qi G, He L, Liu Y. Drug addiction is associated with leukocyte telomere length. Sci. Rep., 2013, 3:1542.
37. Salpea K, Talmud PJ, Cooper JA, Maubaret CG, Stephens JW, Abelak K and Humphries SE. Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. Atherosclerosis., 2010, 209:42–50.
38. Fotiadou P, Henegariu O and Sweasy JB. DNA polymerase β interacts with trf2 and induces telomere dysfunction in a murine mammary cell line. Cancer Res., 2004, 64(11):3830-3837.

*****