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MEASUREMENT OF INFLAMMATION AND OXIDATIVE STRESS BIOMARKERS INDUCED BY CIGARETTE SMOKE AMONG IRAQI SMOKERS

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ABSTRACT : To determine the association between cigarette smoking and oxidative stress, a study was conducted in the period from January 2020 to April 2021, at College of Medicine, Al-Nahrain University, Baghdad, Iraq. The Enzyme-linked immunosorbent assay (ELISA) technique was utilized for measurement the antioxidant enzymes including: Glutathione superoxide (GPX) and catalase (CAT) and the biomarker of lipid peroxidation Malondialdehyde (MDA). Also, the gene expression of Nrf2 and HO-1were determined by using RT-PCR technique. The results indicate lower level of both GPX and CAT ($p \leq 0.001$) in smokers compared with non-smokers. While the result of MDA indicate higher level in smokers ($p \leq 0.001$) compared with non-smokers. The Nrf2 and HO-1 gene expression was lower in smokers ($p \leq 0.001$) compared with non-smokers. Thus, we may conclude that smokers are exposed to more oxidative stress compared to the non-smokers group led to alterations in gene expression, enzymatic activities and MDA level.

Key words : Smokers, free radical production, Lipid peroxidation, oxidative damage, gene expression.

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INTRODUCTION

Cigarette smoke contains many oxidants and prooxidants compounds, which are lead increase in the production of free radicals and increase the oxidative stress (Arinola *et al*, 2011). The puff of cigarette smoke (CS) contains numerous Reactive oxygen species (ROS) like peroxyl (ROO•) radicals, hydroxyl (OH•), hydrogen peroxide (H_2O_2) and superoxide (O_2•) (Nagamma *et al*, 2011). These free radicals are super reactive molecules produced normally in cells by biochemical redox reactions leading to the oxidative stress and harm to lipids, DNA and proteins (Taati *et al*, 2020). The body of human has an enzymatic and non- enzymatic inherent defense mechanism, which protect the body against ROS. The enzymatic part, Free radical scavenger enzymes, called Glutathione peroxidase (GPx) and Catalase (CAT). The dangerous effects of these free radicals are controlled by a precise balance between the average of free radicals production and the average of their removal by the action of these defense enzymes. When the exogenous sources causes an addition of free radicals plus the endogenous production, the tissue defense system becomes discompose and lead to tissue oxidative damage (Pasupathi *et al*, 2009).

The normal balance between oxidants and antioxidants is shift by Smoking tobacco, impacting the endogenous system of oxidative stress (Fischer *et al*., 2015). Oxidative stress imbalance and the resulting tissue inflammation are caused disease and billions of deaths per year are due to cigarettes smoking (WHO, 2017).

Lipid peroxidation process is occurring naturally in an organism initiated by reactive oxygen species and generating malondialdehyde (MDA) in small amounts in the body. In this process, the ROS attack the polyunsaturated fatty acids (PUFA) of the plasma membrane changing the plasma membrane fluidity, increasing permeability and losing integrity of membrane, finally lowering the viability of cells. The increased free radicals results excess production of Malondialdehyde MDA. In human, level of MDA is used as an oxidative stress biomarker (Raut *et al*, 2019). Therefore, tobacco smoke thought to cause different chronic pulmonary and cardiovascular diseases (Reejamol *et al*, 2013). Also The

alterations in the activity and expression of NRF2 as a general mechanism in many diseases like autoimmune, pulmonary, digestive, cardiovascular, metabolic and neurodegenerative diseases, and cancer (Cuadrado *et al*, 2018).

The acute exposure of cigarette smoke increased the amount of antioxidant enzymes (Ignatowicz *et al*, 2013), but chronic exposure reduced these amount (Chan *et al*, 2009). Researchers thought that with continuous exposures of cigarette smoke, Nrf2 will be less sensitive to the oxidants of smoke (Chan *et al*, 2004). In contrast, Aldehydes resulted from lipid peroxidation originate protein carbonyl or present in CSE adduct with sulfhydryl groups of Nrf2/Keap1 and resulting modification in the sulfhydryl groups. This might be the reason of Nrf2 failure to localize into the nucleus or reservation of Nrf2 in the cytoplasm (Ciencewicki *et al*, 2008). Other results suggest that Glutathione GSH could be form conjugates with electrophilic materials contained in CS and inhibitory action of CS on Nrf2 or its upstream regulator genes expression by interaction of CSE electrophilic materials with the cysteine group in the active site (Kode *et al*, 2008).

Gene expression of HO-1

HO-1 expression was detected in the blood cells, the result in Table 4 demonstrated decreased in HO expression in smoker compared with smokeless group and the differences is highly significant ($p<0.007$). The mean of smoker was (1.84±0.26), while for non-smoker was (2.51±0.22).

This result agree with previous results of Goven *et al* (2009), who evaluated the effects of cigarette smoke condensate (CS) in the human monocyte/macrophage cell line in vitro. After (6 h) of CS exposure the expression of HO-1 and nuclear Nrf2 increased, but the expression of both HO-1 and Nrf2 decreased after (72h). Also, Dang *et al* (2020) found the exposure of human bronchial epithelial cell lines to 5% CSE suppressed the protein expression of Nrf2 and the downstream HO-1 gene. Other study evaluated the expressions of HO-1 by RT-qPCR on days 2, 4 and 6. The results indicated that the expressions of HO-1 mRNA increased with cell exposed to CSE on days 2 and 4, while on day 6 they decreased. Cigarette smoke decreased the mRNA expression of HO (Dianat *et al*, 2018). But, Pace *et al* (2013) disagree with result in Table 3 and revealed the CSE increased the HO-1 gene expression in bronchial epithelial cells. In this regard, it has been revealed that an aldehyde compound contained in CS called acrolein, induces HO-1 expression in bronchial epithelial cells (Zhang and Forman, 2008).

Many research revealed a decreased expression of HO-1 resulted from modulation in the expression of Nrf2 in cigarette smoker suffering from lung disease like COPD (Goven *et al*, 2009).

The regulation of cigarette smoke extract on the pathway of Nrf2/HO-1 is controversial. Some evidence proved that CSE can initiate the Nrf2 and HO-1 expression (Lee *et al*, 2017). In contrast, it has also been suggested that CSE can suppress the Nrf2 and HO-1 expression (Cui *et al*, 2018).

The high expression of HO-1 is associated to the inactivation of (Keap1) protein, producing in nuclear accumulation of Nrf2. CSE oxidizes Keap1 thiol groups, leading to the separated of Nrf2 from Keap1 and the leave of Nrf2 into the nucleus increasing the HO-1 mRNA in human macrophages, followed by decreased in the expression of HO-1 gene after continuous CS exposure concomitant with a delayed nuclear transport of Bach1 (transcription factor that suppress the transcription of HO-1) and a cytosolic accumulation of Keap1 and Nrf2 (Goven *et al*, 2013).

The pathologic variation of HO-1–related factors were associated with intensity of smoking. Smoking increased the expression of HO-1 and HO-1 was associated with malignant behavior of cancer. The clinical correlations of HO-1 were regulated by a complicated mechanism that depended on intensity of smoking (Miyata *et al*, 2014).

CONCLUSION

In conclusion, the current information revealed that the exposure to cigarette smoke is fit for stimulating free radicals, marked harmful modification in some enzymatic functions including GPX, CAT and the level of MDA which caused deleterious effect on human health. In addition, the continuous exposure of smokers to cigarette smoke, the smokers were subjected to continuous oxidative stress as shown in gene expression alterations of HO-1 and Nrf2 genes. The results illustrated that changes in gene expression may has an important role in susceptibility of smokers to various diseases.

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REFERENCES

Abdulhamid R E, Sharief A H and Omer S A (2015) Association of smoking and IgE levels among smoker women in Khartoum. *Am*

J Res Com. **3**, 48-54.

Abou-Seif M A M (2016) Blood antioxidant status and urine sulfate and thiocyanate levels in smokers. *J Biochem Toxicol*. **11**(3), 133-138.

Agarwal P, Bagewadi A, Keluskar V and Vinuth D P (2019) Superoxide dismutase, glutathione peroxidase and catalase antioxidant enzymes in chronic tobacco smokers and chewers: A case–control study. *Indian J Dent Res*. **30**, 219-225.

Ahmed N J, Husen A Z, Khoshnaw N, Getta H A, Hussein Z S, Yassin A K, Jalal S D, Mohammed R N and Alwan A F (2020) The effects of Smoking on IgE, Oxidative Stress and Haemoglobin Concentration. *Asian Pac. J. Cancer Prevention : APJCP* **21**(4), 1069–1072. https://doi.org/10.31557/APJCP.2020.21.4.1069.

Arbabi-Kalati F, Salimi S, Nabavi S, Rigi S and Miri-Moghaddam M (2017) Effects of Tobacco on Salivary antioxidative and Immunologic Systems. *Asian Pac. J. Cancer Prevention : APJCP* **18**(5), 1215–1218.

Arcavi L and Benowitz N L (2004) Cigarette smoking and infection. Archives of Internal Medicine. *Arch Intern Med*. **164**(20), 2206-2216.

Arinola O G, Akinosun O M and Olaniyi J A (2011) Passive- and active- cigarette smoking: Effects on the levels of antioxidant vitamins, immunoglobulin classes and acute phase reactants. *Afr. J Biotech*. **10**, 6130-6132.

Bello H and Dandare A D (2017) Effects of Cigarette Smoking on Lipid Peroxidation and Serum antioxidant vitamins. *IOSR J. Pharm. Biol. Sci*. **12**, 40-44. 10.9790/3008-1202044044.

Bizo´n A, Milnerowicz H, Kowalska-Piastun K and Milnerowicz-Nabzdyk E (2021) The Impact of Early Pregnancy and Exposure to Tobacco Smoke on Blood Antioxidant Status and Copper, Zinc, Cadmium Concentration—A Pilot study. *Antioxidants* **10**, 493.

Boehm R E, Do Nascimento S N, Cohen C R, Bandiera S, Pulcinelli R R, Balsan A M, Fao N S, Peruzzi C, Garcia S C, Sekine L, Onsten T and Gomez R (2020) Cigarette smoking and antioxidant defences in packed red blood cells prior to storage. *Blood transfusion = Trasfusione del sangue* **18**(1), 40–48.

Buege J and Aust S (1978) Microsomal Lipid Peroxidation. *Methods-Enzymol* **52**, 302- 310 https://www.abcam.com/catalase-activity-assay-kit www.elabscience.com.

Caliri A W, Tommasi S and Besaratinia A (2021) Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutation Research. *Reviews in Mutation Research* **787**.

Chan K H, Ho S P, Yeung S C, So W H, Cho C H and Koo M W (2009) Chinese green tea ameliorates lung injury in cigarette smoke-exposed rats. *Respir Med.* **103**, 1746–1754.

Chan K H, Ho S P, Yeung S C, So W H, Cho C H, Koo M W, Lam W K, Ip M S, Man R Y and Mak J C (2004) Gene expression profiling in respiratory tissues from rats exposed to mainstream cigarette smoke. *Carcinogenesis* **25**, 169–178.

Ciencewicki J, Trivedi S and Kleeberger S R (2008) Oxidants and the pathogenesis of lung diseases. *J Allergy Clin Immunol.* **122**, 456–468.

Cuadrado A, Manda G, Hassan A, José Alcaraz M, Barbas C, Daiber A, Ghezzi P and León R (2018) Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine approach. *Pharmacol Rev*. **70**, 348–383.

Cui W, Zhang Z, Zhang P, Qu J, Zheng C and Mo X (2018) Nrf2 attenuates inflammatory response in COPD/emphysema: crosstalk with Wnt3a/betacatenin and AMPK pathways. *J Cell Mol Med*. **22**, 3514–3525.

Dang X, He B, Ning Q, Liu Y, Guo J, Niu G and Chen M (2020) Alantolactone suppresses inflammation, apoptosis and oxidative stress in cigarette smoke-induced human bronchial epithelial cells through activation of Nrf2/HO-1 and inhibition of the NF-κB pathways. *Resp. Res*. **21**(95), 1-11.

Dianat M, Radan M, Badavi M, Mard S A, Bayati V and Ahmadizadeh M (2018) Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress. *Resp. Res.* **19**(1), 58. https://doi.org/10.1186/s12931-018-0766-3.

Durak I, Yalçin S, Burak Cimen MY, Büyükkoçak S, Kaçmaz M and Oztürk H S (2013) Effects of smoking on plasma and erythrocyte antioxidant defense systems. *J Toxicol Environ Health A* **5**(6), 373-378.

Erguder I B, Ucar A and Ariturk I (2009) The effects of cigarette smoking on serum oxidant status, and cholesterol, homocysteine, folic acid, copper and zinc levels in university students. *Turk J Med Sci*. **39**, 503-513.

Fischer B M, Voynow J A and Ghio A J (2015) COPD: balancing oxidants and antioxidants. *Int J Chron Obstruct Pulmon Dis*. **10**, 261–276.

Garbin U, Fratta Pasini A, Stranieri C, Cominacini M and Pasini A (2009) Cigarette Smoking Blocks the Protective expression of Nrf2/ARE Pathway in Peripheral Mononuclear Cells of Young Heavy Smokers favouring inflammation. *PLoS ONE* **4**(12), e8225.

Garg N, Singh R, Dixit J, Jain Amita and Tewari V (2006) Levels of lipid peroxides and antioxidants in smokers and nonsmokers. *J. Periodontal Res*. **41**, 405-10. 10.1111/j.1600-0765.2006.00889.x.

Goven D, Boutten A, Lecon-Malas V, Boczkowski J and Bonay M (2009) Prolonged cigarette smoke exposure decreases heme oxygenase-1 and alters Nrf2 and Bach1 expression in human macrophages: roles of the MAP kinases ERK1/2 and JNK. *FEBS Lett*. **583**, 3508–3518.

Hemalatha A, Venkatesan A, Bobby Z, Selvaraj N and Sathiyapriya V (2006) Antioxidant response to oxidative stress induced by smoking. *Indian J Physiol Pharmacol*. **50**, 416-420.

Hou S M, Yang K, Nyberg F, Hemminki K, Pershagen G and Lambert B (2013) Hprt mutant frequency and aromatic DNA adduct level in nonsmoking and smoking lung cancer patients and population controls. *Carcinogenesis* **20**, 437-444.

Ignatowicz E, WoŸniak A, Kulza M, Señczuk-Przyby³owska M, Cimino F, Piekoszewski W, Chuchracki M and Florek E (2013) Exposure to alcohol and tobacco smoke causes oxidative stress in rats. *Pharmacol Rep*. **65**, 906–913.

Joshi B, Singh S, Sharma P, Mohapatra T and Kumar P (2020) Effect of Cigarette Smoking on selected Antioxidant Enzymes and Oxidative Stress Biomarkers. *J. Clin. Diag. Res*. **14**(10), 19-23.

Kalaiselvi K (2016) Study of plasma levels of Malondialdehyde as an oxidative stress marker, so it comes as a marker in smokers & non-smokers. *PARIPEX-Ind J Res*. **5**, 69-70.

Kamceva G, Arsova-Sarafinovska Z, Ruskovska T, Zdravkovska M, Kamceva-Panova L and Stikova E (2016) Cigarette Smoking and Oxidative Stress in Patients with Coronary Artery Disease. *Open*

Access Macedonian J. Med. Sci. **4**(4), 636–640.

Kode Aruna, Rajendrasozhan, Saravanan Caito, Samuel Yang, Se-Ran Megson, Ian Rahman and Irfan (2008) Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells: implications in COPD. *Am. J. Physiol. : Lung Cellular and Molecular Physiology* **294**, 478-488.

Kole E, Ozkan S O, Eraldemir C, Akar F Y, Ozbek S K, Kole M C, Kum T and Filiz P C (2020) Effects of melatonin on ovarian reserve in cigarette smoking: an experimental study. *Arch Med Sci*. **6**, 1377- 1386.

Kurku H, Kacmaz M, Kisa U, Dogan O and Caglayan O (2015) Acute and chronic impact of smoking on salivary and serum total antioxidant capacity. *J Pak Med Assoc*. **65**(2), 164-169.

Landis G N and Tower J (2005) Superoxide dismutase evolution and life span regulation. *Mech Ageing Dev*. **126**, 365.

Lee K H, Jeong J, Koo Y J, Jang A H, Lee C H and Yoo C G (2017) Exogenous neutrophil elastase enters bronchial epithelial cells and suppresses cigarette smoke extract–induced heme oxygenase-1 by cleaving sirtuin 1. *J Biol Chem*. **292**, 11970–11979.

Loboda A, Damulewicz M, Pyza E, Jozkowicz A and Dulak J (2016) Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci*. **73**, 3221–3247.

Mahapatra S K, Das S and Dey S K (2008) Smoking induced oxidative stress in serum and neutrophil of the university students. *Al Ameen J Med Sci*. **1**, 20-31.

Metta S, Basalingappa D R, Uppal S N and Mittal G (2015) Erythrocyte Antioxidant Defenses Against Cigarette Smoking in Ischemic Heart Disease. *Clin Diagn Res*. **9**, 8-11.

Miyata Y, Kanda S, Mitsunari K, Asai A and Sakai H (2014) Heme oxygenase-1 expression is associated with tumor aggressiveness and outcomes in patients with bladder cancer: A correlation with smoking intensity. *Transl Res*. **164** (6), 468-476.

Moore T A, Samson K, Ahmad I M, Case A J and Zimmerman M C (2019) Oxidative Stress in Pregnant Women between 12–20 weeks Gestation and Preterm Birth. *Nurs. Res*. **69**, 244–248.

Naga C V and Manohar R M (2013) Study of antioxidant enzymes superoxide dismutase and glutathione peroxidase levels in tobacco chewers and smokers:a pilot study. *J Cancer Res Ther*. **9**, 210–214.

Nagamma T, Anjaneyulu K, Baxi J, Dayaram P and Singh P P (2011) Effects of Cigarette smoking on Lipid Peroxidation and Antioxidant status in Cancer Patients from Western Nepal. *Asian Pasific J Cancer Prev.* **12**, 313-316.

Naresh C K, Rao S M, Shetty P R, Ranganath V, Patil A S and Anu A J (2019) Salivary antioxidant enzymes and lipid peroxidation product malondialdehyde and sialic acid levels among smokers and non-smokers with chronic periodontitis-A clinico-biochemical study. *J. Fam. Med. Primary Care* **8**(9), 2960–2964. https://doi.org/10.4103/jfmpc.jfmpc_438_19.

Nur A, Nusrat J and Rafiquzzaman (2013) Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *SPJ* **21**(2), 143-152.

Pace E, Ferraro M, Vincenzo S D, Cipollina Ch, Gerbino S, Cigna D, Caputo V, Balsamo R, Lanata L and Gjomarkaj M (2013) Comparative cytoprotective effects of carbocysteine and fluticasone propionate in cigarette smoke extract-stimulated bronchial epithelial cells. *Cell Stress Chaperones* **18**(6), 733-743.

Pannuru P, Vaddi D R, Kindinti R R and Varadacharyulu N (2011) Increased erythrocyte antioxidant status protects against smoking induced hemolysis in moderate smokers. *Hum Exp Toxicol.* **30**, 1475-1481.

Pasini A, Ferrari M, Stranieri Ch, Vallerio P, Mozzini Ch, Garbin U, Zambon G and Cominacini L (2016) Nrf2 expression is increased in peripheral blood mononuclear cells derived from mild–moderate ex-smoker COPD patients with persistent oxidative stress. *Int. J. COPD* **11**, 1733-1743.

Pasupathi P, Saravanan G and Farook J (2009) Oxidative stress Bio Markers and antioxidant Status in Cigarette Smokers compared to nonsmokers. *J Pharm.Sci & Res.* **1**, 55-62.

Patel B P, Rawal U M, Rawal R M, Shukla S N and Patel P S (2008) Tobacco, antioxidant enzymes, oxidative stress and genetic susceptibility in oral cancer. *Am J Clin Oncol*. **31**, 454-459.

Raut A M, Andure D V, Padalkar R K, Patil S M and Bhagat S S (2019) Study of interaction of cigarette smoke with thiol (-SH) group of sulfhydryl proteins in smokers. *Int J Clin Biochem Res*. **6**(1), 25-28.

Reejamol M K and Swaminathan M (2013) Estimation of lipid peroxides and antioxidants in smokers and non-smokers with periodontitis. *King Saudi Univ J Dent Sci*. **4**, 53–56.

Russo M, Cocco S and Secondo A (2011) Cigarette smoking condensate causes a decrease of the gene expression of CuZn-SOD, glutathione peroxidase, catalase and free radical induced cell injury in SH-SYSY human neuroblastoma cells. *Neurotoxicity Research* **19**, 49-54.

Saha S, Buttari B, Panieri E, Profumo E and Saso L (2020) An overview of Nrf2 Signaling Pathway and its Role in Inflammation. *Molecules* **25**, 1-31.

Safyudin S and Subandrate S (2016) Smoking tends to decrease glutathione and increase malondialdehyde levels in medical students. *Univ Med*. **35**(2), 89- 95.

Taati B, Arazi H and Suzuki K (2020) Oxidative Stress and Inflammation Induced by Waterpipe Tobacco Smoking despite Possible Protective effects of Exercise Training: A review of the literature. *Antioxidants* **9**, 777.

Vlahos R and Bozinovski S (2013) Glutathione peroxidase-1 as a novel therapeutic target for COPD. *Redox Rep*. **18**, 142–149.

Wei J, Liu C-X, Gong T-T, Wu Q-J and Wu L (2015) Cigarette smoking during pregnancy and preeclampsia risk: A systematic review and meta-analysis of prospective studies. *Oncotarget* **6**, 43667–43678.

WHO (2017) Report on the global tobacco epidemic: monitoring tobacco use and prevention policies. Available at :https://www.who.int/tobacco/global_report/2017/en/

Zhang H and Forman H J (2008) Acrolein induces heme oxygenase-1through PKC-delta and PI3K in human bronchial epithelial cells. *Am J Respir Cell Mol Biol*. **38**, 483–490.