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GSTM1, GSTT1 genotyping and their frequency with selected physiological parameters of smokers

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Abstract

Smoking is considered one of the most dangerous addictions that ingests through respiratory, hematology and digestive systems, due to the substances that tobacco contains, which are harmful to health, the most important component is nicotine. Tobacco smoke has chemical compounds that caused genomic DNA damage. Glutathione-s-transferees enzymes have the ability to metabolize numerous number of chemicals. GSTs of human include *GSTM1*/T1. This enzymes involved in providing a defense mechanism against oxidative stress. Therefore, this project was designed to exam the effect of smoking on hematological, biochemical markers and the frequency of *GSTT1* and *GSTM1* genes in smokers men. Data showed a significant decrease weight, BMI, total protein, albumin, bilirubin, HDL and GSH. However, hematological parameters, blood pressure, lipid profiles, liver enzymes and MAD were significantly increase in cigarette smoking men.

GSTM1 frequency was deleted up to 2.25 time and *GSTT1* absences at 1.3 time in smoking. In addition, both genes were absence at 2.2 in smokers. It was observed that physiological parameters decreased when null genotype off *GSTM1* and *GSTT1* in cigarette smoking.

Key words: *smoking, physiological index, polymorphism, GSTT1, GSTM1*

Introduction

Smoking is one of a common health risk factor and considered as the most factor causing the death that resulted from different non-communicable diseases (Jafari *et al*., 2021). annually,

smoking contributed in killing greater than eight millions of people in the world (Briskin, 2023). In 2018, tobacco use was considered as a major risk factor to cause lung cancer that resulted in greater than (1.5 million) deaths of people while 20% of deaths resulted from smoking (Walser *et al*., 2008). However, deaths risk caused by cancer was increased between (2-10) times in female while was increased between (8-15) times in men compared with control samples (Khuder and Mutgi, 2001). Tobacco smoke has different carcinogenesis components and chemical compounds that caused genomic DNA damage including aromatic amines, phenols, alcohols, esters, carboxylic acids, nitrogen oxides, ketones, N-nitrosamines and nicotine (Khuder and Mutgi, 2001; Rodgman and Perfetti, 2008). Tobacco smoke has a positive correlation with WBC, RBC and platelet number and hematocrit level in smokers (Charles *et al*., 2007; Kung *et al*., 2008). Metabolizing enzymes may have a key role in defense system against diseases, which related tobacco smoke. Phase II (Glutathione-s-transferees) enzymes have the ability to metabolize numerous number of chemicals (Saadat and Saadat, 2010). GSTs of human include three families including mitochondrial, systolic and microsomal. GSTM1 (Mu) and GSTT1 (theta) are members of the cytosolic family. This family of enzymes involved in providing a defense mechanism against oxidative stress products and electrophiles (Almoshabek *et al*., 2016).

A difference in human genomic material sequences among populations groups and individuals (existed in greater than 1% of the group) named as polymorphism that could was induced via spontaneous processes or by external factors such as radiation or viruses (Bruhn *et al*., 1998).

GSTM1 gene implicated in the carcinogens metabolizes detoxification that existed in the atmosphere including tobacco smoke (Zakiullah *et al*., 2020). This gene has 10 exons and located on chromosome1 short arm 13(p13) band (3). The null variant of GSTM1- (homozygous deletion) refers to lose the activity of the enzyme and increased the risk of tumor (Lao *et al.*, 2014).

GSTT1 gene contains six exons and located on chromosome twenty-two, short arm (p11.2). GSTT1 includes most two frequent alleles null allele and wild type allele (Bruhn *et al*., 1998; Singh *et al*., 2022). Chemicals in cigarettes smoke produce some of free radicals that cause oxidative stress. This oxidative cause the weakness to the defense system of host and can lead to lung carcinogenesis. According to previous assays that demonstrated GSTs (M, THETA) modify risk of pulmonary, atherosclerosis and some diseases in those who are smoke. GSTT1 and

GSTM1 have the possibility to reduce the effect of oxidative damage and oxidative stress. These genes protect the cells from the detoxification. current study was conducted to test if there is any modulation in polymorphism of GSTT1 and GSTM1 genes frequency in the smokers compared with non-smokers participants and test if tobacco smoke increased or decreased selected physiological parameters.

Materials and methods

Collection of blood samples (60) blood samples were collected from smokers whose ages ranged between (20-60) years, and (45) other blood samples were collected from non-smokers as a control group, aged between (20-60) years. The information was collected based on a questionnaire that includes many information, age, gender, weight, amount of smoking, duration of smoking. The size of the blood samples taken from each person (2-5) ml. 2 ml of blood was kept in tubes containing the anticoagulant EDTA for the purpose of conducting genotype and physiological tests. The remaining blood was placed in tubes free of blood clotting for the purpose of conducting biochemical tests.

Physiological tests: Blood pressure levels were measured after five minutes of rest using a Mercury sphygmomanometer. Blood profile including red blood cells RBC, leukocyte WBC, PCV, Hb, platelet counts and red blood cell indicators (MCV, MCH, MCHC) were estimated using automatic hematological analyzer (CELL-DYN3700). Biochemical parameters including lipids profiles, protein, bilirubin and liver enzymes (GOT, GPT and ALP) were measured by following the steps of the instructure attached to the test kits (Bio Merieux). Malondialdehyde (MDA) and glutathione (GSH) were estimated according to the test kit prepared for this purpose.

Molecular test: DNA was isolated from blood cells using DNA blood Minikit (Qiagen, USA). Olegonuclutaid sequences and polymorphisms GSTs genes were done by multiples PCR amplification a ccording to the protocol of (Al-Badran, 2003). 2% gaarose gel used to separated PCR product for visualized specific bands.

Statistical results: the analysis data carried out using graph pad presim version 8 program. Chi square, T-test were applied to analyses all the results for statistical significant.

Results

No significant variances were detected in the ages of smokers ($p>0.05$) compared to the ages of non-smokers. Weight and BMI were significantly decreasednmm ($p<0.05$) among smoking men compared to control.. A significant increase ($p<0.05$) in smoker blood parameters RBC, Hb, PCV, WBC and platelets compared to non-smoker. No significant variances ($p>0.05$) in the red blood indicators including MCV, MCH and MCHC for smokers group compare with control, Table (1). A significant increasing ($p<0.05$) was showed in systolic and diastolic pressure for smoker's men compared to non-smokers.

parameters	**Non-Smoking**	**Smoking**
Age (years)	42.16±12.11	41.18±10.21 n
Weight (Kg)	58.69±22.71	54.61±22.28*
BMI kg/m^2	23.11±8.31	22.60±8.93*
Systolic pressure (mmHg)	120.17±2.6	138.20±2.48*
Diastolic pressure (mmHg)	80.00±1.6	96.11±1.3*
Red blood cells (10^{12})	4.68±0.04	4.87±0.48*
Hb (g/dl)	12.4±1.3	13.9±1.2*
PCV (%)	40.8±4.1	43.10±2.6*
WBC (10^9/l)	6.76±2.8	7.80±2.6*
Platelet	249±50.2	268±45.0*
MCV	87.17±5.0	88.50±50.2 n
MCH	29.48±50.2	29.77±50.2 n
MCHC	294±87	297.74±50.2 n

Table 1 Explains some physiological and hematological parameters for smokers and non-smokers (control).

In serum blood of smokers, the statistical analysis presented a significant increase in TC, TG, LDL and VLDL compare with they didn't smoke. While, HDL level was decrease significantly ($p<0.01$), Table (2). Total protein and bilirubin concentration were significantly lowered ($p<0.05$, $p<0.01$) in serum of smoking group in compared with control. However, albumin was

non-significantly decreased (p>0.05) in smokers men compared with they did not smoke, Table (2).

Parameters	Non-Smoking	Smoking
TC (g/dl)	161.40±18.20	272.80±10.70 **
TG (g/dl)	120.81±7.10	139.53±6.81*
HDL-C(g/dl)	42.35±3.60	23.31±3.86**
LDL-C(g/dl)	95.71±4.70	228.60±16.34***
VLDL-C(g/dl)	23.85±2.18	27.60±2.20*
Total proteins (g/dl)	8.12±0.81	6.80±1.13*
Albumin (g/dl)	46.9±3.5	45.20±3.4n
Bilirubin (g/dl)	0.58±0.081	0.28±0.061**

Table 2 Shows some biochemical parameters and lipid levels for smokers and non-smokers (control).

Table (3) presented a significant elevates (p<0.05, (p<0.01) in liver enzymes (ALP, ALT and AST) of smoker men compare with control group. LDH enzyme also increased significantly (p<0.05) in the serum of smoking compared with their concentration in non-smokers, Table (3). GSH level was significantly decreased (p<0.05) in serum of smoking group in compared with control. However, MAD concentration was significantly increased in the serum of these smokers, (p<0.05).

Enzymes	Non-Smoking	Smoking
AST (U/L)	30.60±4.20	44.60±3.87*
ALT (U/L)	29.86±4.83	54.80±12.02**
ALP (U/L)	60.42±6.23	84.10±5.60*
LDH (U/L)	320.18±18.11	419.62±23.41*
MDA (mmol/ml)	5.68±0.48	8.44±0.38*
GSH (mmol/ml)	142.10±26.02	103.11±18.14**

Table 3 shows some liver enzymes and indicators of oxidative stress in smokers and non-

Genotypic study by a multiplex-PCR analyzed simultaneously the absence or presence of GSTs genes. Electrophoresis of PCR product on agarose gel 2% showed *GSTM1* (present) by 215bp and GSTT1 by 480bp, Figure (1). The frequencies null of *GSTM1*- was found 20% in the smokers and 10% in the control, (p-value= 0.031, OR= 2.25, CI= 26.0-28.15). *GSTT1* null genotype showed 53.3% for smoking men, while 45% for non-smoking. The variance was significantly presented at p-value= 0.015, OR=1.3, CI= 35.94-57.93. Null genotype of

combination genes null *GSTM1, GSTT1* were 20% in the smoking and 10% in control (p-value= 0.032, OR=2.2, CI=43.03-51.40).

Genotype	Non-Smoking **n=40**	Smoking **N=60**	OR	p-CI 95%	p-value
GSTM1 (+)	36 (90%)	48 (80%)	1.0		
GSTM1 (-)	4 (10%)	12 (20%)	2.25	26.0-28.15	0.031
GSTT1 (+)	22 (55%)	28 (46.6%)	1.0		
GSTT1 (-)	18(45%)	32 (53.3%)	1.3	35.94-57.93	0.015
GSTM1, GSTT1 (+)	22 (55%)	30 (50%)	1.0		
GSTM1, GSTT1 (-)	4 (10%)	12 (20%)	2.2	43.03-51-40	0.032

Table 4 GSTM1 and GSTT1 gene polymorphisms distribution between smokers and non-smokers (control)

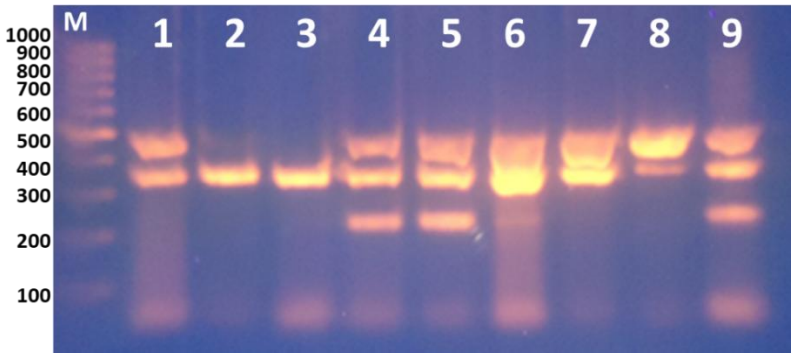

Figure 1: PCR amplification presented on agarose gel 2%. *M: DNA marker. Lines 1,8 null genotype of GSTM1. Lines 6,7 null genotype of GSTT1. Lines 2,3 null genotype both genes. Lines 4,5,9 presences both genes.*

Hemoglobin and packed cell volume values significantly decreased when *GSTM1*- and *GSTT1*- were absence in smokers compared to the presences of both genes. Concentration TC and TG significantly higher in *GSTM1*-, *GSTT1*- null genotype compared with genotype of *GSTM1*+, *GSTT1*+. GSH level were significantly decrease with the absence of *GSTM1. GSTT1* than presence both of genes, Table (5). However, MAD was significantly higher in null genotypes of GSTM1/*T1* compared with the presence genotype of *GSTM1/T1*.

Genotype **parameters**	*GSTM1 (+)*	*GSTM1 (-)*	p-value	*GSTT1 (+)*	*GSTT1 (-)*	p-value
Hb	11.95±1.28	10.29±1.45	0.041	11.65±1.38	10.14±1.54	0.034
PCV	38.12±2.67	37.64±2.61	0.023	37.00±3.39	35.01±3.43	0.046

TC	1.88±0.91	5.46±1.28	0.043	1.98±0.32	5.34±1.33	0.042
TG	63.09±4.48	74.26±5.39	0.031	61.26±5.10	70.12±6.23	0.042
GSH	0.54±0.11	0.34±0.03	0.06	1.12±0.08	0.41±0.06	0.051
MAD	9.43±2.41	80.98±5.58	0.031	8.97±5.65	84.58±8.66	0.012

Table 5 Correlated between physiological parameters and combination of GSTM1, GSTT1 genotype among smokers.

Discussion

Smoking is one of the most dangerous forms of addiction that a person ingests through his respiratory and digestive systems due to the substances that tobacco contains that are harmful to health, the most important of which is nicotine. This substance is considered to have a characteristic superior to cyanide (Omare *et al*., 2021). Nicotine has the effect of reducing the appetite for food. The chemicals attached to cigarette smoke, it causes a direct inverse deterrent to stomach secretions, in addition to the fact that it numbs the sense of taste and taste in the tongue and the mucous tissue in the mouth, and it also numbs the olfactory centers in the nose (Bloom *et al*., 2019). Nicotine effects on the stomach and intestines, it increases the movement of the intestines and colon, causing diarrhea sometimes. It may also cause nausea accompanied by vomiting, which is a common symptom among smokers (Berkowitz *et al*., 2018; Mishra *et al*., 2015). The reason for this is attributed to a decrease in body weight, the decrease in BMI may be due to oxidative stress generated by smoking, which generates free radicals, affecting the metabolism of fats and proteins. Data were consistent with the results of (Chhabra and Chhabra, 2011; Mahapatra *et al*., 2008; Taylor *et al.*, 2019).

Smoking had a negative impact on blood parameters, led to an increase in the RBC, Hb, and PCV since cigarette consists of many gases like carbon monoxide (CO), which accumulates. This gas collects in the body tissues of smokers (Al Salhen, 2014; Malenica *et al*., 2017). Andersson and Møller (2010) indicated that the rate of CO combining with blood Hb is 50% in smokers compared to healthy, carboxyhemoglobinemia component is 1%. People who smoke heavily develop suffocation (hypoxia) (Kafhage *et al*., 2024; Malenica *et al.,* 2017; Umahi-Ottah *et al.*, 2022). The cause of suffocation is a decrease in the effectiveness of RBC in transporting

oxygen O_2 to tissues, which stimulates an increase in the secretion of the hormone erythropoietin, which affects bone marrow cells to stimulate them to produce more of these cells, leads to an elevate in the percentage of hemoglobin in the blood, resulting in its superiority in smokers (Kafhage *et al*., 2024). PCV and erythrocyte were connected to hypoxia brought on by high level carbohydratescon. Data of the current project are agreed with the results of (Lakshmanan and Saravanan, 2014; Malenica *et al*., 2017). Smoking lead to leukocytosis by different factors and several pathways. Increasing WBC could be resulting of nicotine stimulated release of steroid hormones and catecholamine via adrenal gland. Increasing levels of endogenic hormones like epinephrine and cortisol consequence in raise in leukocyte count (Deutsch *et al*., 2007; Kapoor and Jones, 2005). Further, tobacco smoking can be effect on the respiratory system that resulting inflammation activation induces the up regulate of the inflammatory markers in circulation like cytokine that effect on the WBC account (Malenica *et al*., 2017). This result is agreed with the data of (Higuchi *et al*., 2016; Inal *et al*., 2014). Estimating the contents of red blood cells in smokers showed no a significant increase in MCV, MCH and MCHC compared to non-smokers. This data are agreed with Malenica *et al.* (2017), they reported no variation in MCV and MCH smoking. In contradiction to our data Kafhage *et al.* (2024) found significant increase in MCV, MCH and MCHC. Smoking leads to high blood pressure (diastolic and systolic) for smokers. Data of this project are consistent with the finding of (Dong-Qing *et al.*, 2014; Primatesta *et al*., 2001; Vallée, 2023). The reason is that nicotine and some compounds that are included in the composition of tobacco contribute to the increase in LDL and VLDL, which leads to their deposition in the walls of the arteries and increases their thickness, resulting in a narrowing of these arteries, leads to an up-regulate in blood pressure (Klein, 2022).

Proteins and albumin in the serum of smokers were lower compared with control, which due to the fact that cigarette smoke contains carbon monoxide (CO), leads to an imbalance in the metabolism and synthesis of proteins (Roohi and Mehjabeen, 2017). The decrease in the concentration of total proteins and albumin to the dangerous effects of cigarette smoke, as it contains some oxidants that cause oxidative stress to plasma proteins and tissues in humans (Jaafar, 2020; Roohi and Mehjabeen, 2017). Lower protein level could be due to cigarette

smoking have several hepatotoxic substances that impact on liver function via its effect on proteins function through synthesis and metabolism in the liver therefore it could effect on protein (Abdul-Razaq and Ahmed, 2013; Neki, 2002). The decrease in albumin may be due to the fact that it is considered an antioxidant, and when it scavenges free radicals, it may cause a rapid reaction with the –SH group on this albumin, thus oxidizing the albumin. This is clear because of its rapid breakdown in the circulatory system (Hu *et al.*, 1993). Bilirubin levels of smokers showed a significant decrease in smokers men compared to control. It could be due to the decrease in albumin concentration in the blood, as it works to transfer bilirubin to the hepatic splanchnic cells. It may be due to a decrease in the activity of the bilirubin enzyme UGT-glucurony transferase, which binds bilirubin to glucuronic acid in order to transfer bilirubin from the liver to bile, leading to a decrease in bilirubin secretion (Mahmood and Salih, 2018). Data is consistent with other studies (Abdul-Razaq and Ahmed, 2013; Mahmood and Salih, 2018)..

AST, ALT and ALP levels were high in the blood serum of smokers. The reason for this is that chronic smoking leads to a change in the membranes of tissues and organs, which leads to changes in the transport of electrolytes and enzymes (Rukhsana *et al*., 2022). It may be that the metabolic processes in the liver cells are affected when exposed to toxic substances. Increased levier enzymes could due to exposure to smoke release increasing levels of the cellular oxidative radicals (Wannamethee and Shaper, 2010). The reason for the increase in ALP in the blood serum of smokers may be the negative effect of smoking on the tissue and organic like liver, kidneys and bones, which are the main source of this enzyme (Mahmood and Salih, 2018). The results are agreed with other studies (Abdul-Razaq and Ahmed, 2013; Dhahir and Noaman, 2017; Mahmood and Salih, 2018; Rukhsana *et al*., 2022). Serum LDH was higher in cigarette smokers compared to control. This increasing could lead to elevate oxidative stress via nicotine. Our data greed with study of (Rao *et al*., 2017)

The level of lipids increased, while HDL in blood serum decreased of smokers. It could be the smoking changing lipids through several mechanisms, the most important of which is nicotine stimulates the marrow of the adrenaline gland, leads to an increase in lipid levels. Alteration of lipids levels of cigarette smoking probably due to nicotine that activation the release catecholamine resulting in raise level of plasma free fatty acids that additional consequence in

raise the stimulating of free fatty acids and triglycerides additionally to VLDL to the blood stream (Abdul-Razaq and Ahmed, 2013; Pannuru Padmavathi *et al*., 2009). A decrease in the level of estrogen due to smoking leads to a decrease in HDL in the blood (Benowitz, 2009). High insulin in smokers leads to an up-regulate in the level of cholesterol and lipoproteins due to a decrease in the level of the lipoprotein lipase enzyme (Bodaghi *et al.*, 2023). These results agreed with other studies (Abdul-Razaq and Ahmed, 2013; Rashan *et al*., 2016).

An increase in the level of MDA, decrease in GSH in the serum of smokers. The result of this study agreed with other results of (Rabiu *et al*., 2022; Singh *et al.*, 2022). It could be cigarette smoke consists of a several chemical compounds, which are able to pass into cells and interact with biological systems in the human. These compounds work to increase free radicals, especially hydrogen peroxide (Seo *et al*., 2023). Smoker could be probable to have evaluate concentration of lipid peroxidation. In addition, cigarette smoking could lead to increase oxidase stress through activation NADPH oxidase and reducing antioxidant which lead to lipid peroxidation (Broekhuizen *et al*., 2006; Karadag *et al*., 2008). The decrease in GSH is due to it being an antioxidant that plays an essential role in protecting cells detoxification of cigarette smoking, as well it works to scavenge free radicals which generate by smoking (Rabiu *et al*., 2022).

A significant effect of smoking on the *GSTM1* gene, absent by (2.25) times related to the control, while a slight impact of smoking appeared on the *GSTT1* gene, absent by (1.3) times compared to the control. Data of the our work agreed with the project of (Tamer *et al*., 2004). Singh *et al.* (2022) noted 284 cigarette smoking, *GSTM1* was present in 59.9% and absent in 40.1 %, whereas *GSTT1* was present in 51.1% and absent in 48.9% as compared with they didn't smoking. Chiurillo *et al*. (2013) analyzed 120 smokers samples, found *GSTM1* absence of 51% and *GSTT1* null genotype frequency of 11%. The reason may be attributed to the fact that this substance is considered the main substance that works to remove the toxicity of the *GSTM11* gene because it removes the toxicity of large hydrocarbon substances (Noda *et al*., 2004; Sheikhha *et al.*, 2005). The *GSTT1* gene is responsible for removing toxic substances (Monohalomethanes, Epoxy butanes) and some other compounds found in cigarette smoke (Al-Badran, 2003). The reason is that the loss of genes leads, as a result to the loss of enzymes

responsible for removing the toxicity of hydrocarbon compounds found in cigarette smoke, which leads to an increased risk of contracting the disease (AL-Safi and AL-Rikabi, 2013). The loss of the *GSTM1* and *GSTT1* genes indicates that evidence of atherosclerosis and the occurrence of clinical complications among those exposed to cigarette smoke (AL-Safi and AL-Rikabi, 2013; Olshan *et al*., 2003). Smoke excessively have more cases of loss of three genes (*GSTM1, GSTT1, GSTP1*) by four times, which increases the possibility of lung tumor (Masetti *et al*., 2003). Smoking increases the loss of the *GSTM1* and *GSTT1* genes at different rates, which leads to an increased risk of coronary artery disease (Masetti *et al*., 2003). The results of researcher AL-Safi and AL-Rikabi (2013) study showed the effect of smoking the loss of *GSTM1*, GSTT1. Kim *et al*. (2008), they found that the risk of myocardial dysfunction doubled in smokers who lacked the *GSTM1* gene by 2.07 times and lost the *GSTT1* gene by 2 times In humans, the GSTs detoxification family is linked to the removal of toxins resulting from chemical metabolic processes present in cigarette smoke (van der Hel *et al*., 2003). GSTs enzymes alone *GSTM1* and *GSTT1* or both combination null genotype promote the rsik of bladder, gastric, colon and lung cancers (Singh *et al*., 2022). In addation, smoking is a main possibility reason for cardiovascular and atherosclerotis diseases (Gallucci *et al*., 2020). The polymorphism of *GSTT1*, *GSTM1* gene are deletion variations that complete absence of the enzymes or produce a functional enzyme (Singh *et al*., 2022). Studies observed that impact of air pollution exposure lead to several genotype of *GSTT1*, *GSTM1* (Dai *et al.,* 2018). He *et al*. (2004) showed the association cigarette smoking with null genotypes of GSTs have a clear weakening in functions of lung compare with GSTs abundant genotype. Ford *et al*. (2000) indicated that the risk of developing cancer is due to smoking causes loss of the *GSTM1* gene by 2.1 times. Previous studies have also confirmed that smoking is due to the loss of the *GSTM1* gene, which leads to an amplified risk of lung tumor (Losi-Guembarovski *et al*., 2002). GSTs contribute in the preserves of cell integrity, detoxification of exogenous and endogenous compounds, DNA damage and defense against oxidative stress in the cell (Chatterjee and Gupta, 2018). Saadat and Saadat (2010) showed hematological parameters were significantly decreased among null genotypes of *GSTM1* and *GSTT1* in smokers. They reported hematological profile (RBC,PCV, Hb) raised associated with risk heart diseases. It propably be established *GSTM1,GSTT1* null genotype have protective roles for developing cardiovascular diseases. Ho *et*

al. (2021) reported increasing oxidative stress and connected lipid peroxidation indicators in smokers. Deficiency of antioxidant proteins expression in the *GSTM1*null and *GSTT1* null genotypes moderates the antioxdant capacity (Ding *et al*., 2019). Pašalić and Marinković (2017) showed that a significant impact of *GSTT1* polymorphism on levels of lipid indicates such as cholesterol, triglycerids in smoking status. However , Letonja *et al*. (2012) reported not signficant correlated lipids parmaters with *GSTM1* and *GSTT1* polymorphsims. Rabiu *et al*. (2022) reported the levels lipid peroxidation such as MDA significantly increased in case null genotype individuals of both *GSTM1* and *GSTT1*. The reduce activity of the antioxidant enzymes, GSH levels and elevate MDA levels might be additional_reasoned by deletion *GSTM1* and *GSTT1*, leads to reduce GSTs activity and high release reactive oxygen species clearly showed through prolonged smoker's (Rabiu *et al*., 2022). Data agreed with our previous work that found the correlation between polymorphisms of GSTM1, GSTT1 with oxidative stress enzymes include MAD, GST and GSH (AL-Khaledy, 2014; Wawi and Ahmed, 2014).

Conclusion physiological parameters could be changed when null genotypes of GSTM1 and GSTT1 among cigarette smoking.

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