

N.B: My review is within the paper text as following:

In Yellow: text to remove (to change)

In Red: suggested text to replace the removed one

In blue: sentences need to be reformulated

In Green: questions to the authors, comments and suggestions to improve the paper

## COMPARITIVE STUDY OF GLUTATHIONE-S-TRANSFERASE ISOENZYME AND VITAMIN D LEVELS IN SMOKERS AND NON-SMOKERS

### ABSTRACT

#### BACKGROUND:

In developed countries, cigarette smoking is the leading preventable cause of morbidity and mortality [ref]. In the second half of this century, dramatic changes in the prevalence of cigarette smoking in the United States reduced current smoking levels to approximately one quarter of the adult population, reducing gender differences in smoking prevalence and smoking-attributable diseases [ref]. Cigarette smoking is a serious risk factor for lung cancer, which is the leading cause of cancer-related deaths in both men and women in the United States and around the world.

**AIM:** Comparative Study of Glutathione-S-Transferase Isoenzyme mu and Vitamin-D Levels in Smokers and Non-Smokers

(Should give the importance of this work)

#### MATERIAL AND METHODS:

A total of 100 people aged 20 to 55 years old who came to Shalinitai Meghe hospital in Nagpur for a health check-up were chosen for the research. Non-smokers make up the control group, while smokers make up the research group (did those people informed that their data will be used for this publication?). There are 50 patients in each group. ELISA was used to determine vitamin D status. An enzyme-linked immunosorbent assay was used to detect GST- $\mu$  in heparinized whole blood.

#### RESULTS:

GST- $\mu$  was found to be mostly positive in smokers, and it was also found to be raised in heavy smokers over smokers ( $6.39 \pm 3.2$  unit) than light smokers ( $4.56 \pm 0.78$ ). GST- $\mu$  is positive in light smokers. GST- $\mu$  is nearly equal in smokers ( $5.24 \pm 0.95$ ) and heavy smokers relative to others.

#### CONCLUSION:

Quitting give up smoking for a longer period of time was related to higher vitamin D levels than current smoking (start the sentence with the effect). Furthermore, the GST- $\mu$  measure used in our research may be used to show differences in cytogenetic damage between smokers who have a genetically defined detoxification enzyme and those who do not.

**KEYWORDS:** 25-hydroxyvitamin D, GST- $\mu$ , cytogenetic damage, smoker, and GST

## **INTRODUCTION**

In developing countries, cigarette smoking (hereafter referred to as "smoking") is the leading cause of premature death. Smoking is responsible for more than 400 000 deaths each year in the United States, as well as 30% of all cancers. <sup>1</sup> Lung cancer is the leading cause of cancer-related death in the United States, and it is also the leading cause of smoking-related death. <sup>2,3</sup> There are an estimated 1.1 billion smokers in the world, with 900 million men and 200 million women (ref). Men outnumber women 2:1 in developed countries and 7:1 in developing countries. In developed countries, smoking prevalence rates for men and women are 42 percent and 24 percent, respectively, and 48 percent and 7 percent, respectively, in less advanced countries. <sup>4</sup>

After China, India has the world's second-largest tobacco consumer (ref). Tobacco kills more than 7 million people per year, according to the World Health Organization (ref). Direct intake causes over 6 million deaths, while passive smoking causes 890,000 deaths. In the twentieth century, nearly 100 million premature deaths were reported, with the number projected to rise to 1 billion by the twenty-first century (ref). In India, smoking kills over one million people every year and is the fourth leading cause of non-communicable diseases (NCDs) including cancer and heart disease, which account for 53% of all deaths.<sup>5</sup>

Please give references for all those statistics that you provide!!

In cancer epidemiology, biomarkers are increasingly being used to estimate exposure to carcinogens or putative anti-carcinogens, preclinical biological effects, and genetic factors that may affect individual susceptibility. <sup>6</sup> DNA damage markers are of particular interest because DNA damage is a critical step in carcinogenesis. <sup>7</sup> In smokers, who have a documented elevated risk of lung cancer, DNA damage markers such as GST- $\mu$  are reduced or absent. <sup>3,8</sup> As a consequence, it's fair to believe that higher antioxidant levels and improved detoxification would result in less DNA damage in smokers. We demonstrate an association between deficiency in the detoxification enzyme GST- $\mu$  and increased cytogenetic damage in smokers by tobacco smoke. <sup>3,9</sup>

Low 25-hydroxyvitamin D (vitamin D) levels in the blood have been linked to a variety of chronic diseases, including fractures,<sup>10</sup> diabetes <sup>11</sup>, and cardiovascular disease.<sup>12</sup> The majority of recent studies have shown that current smokers have lower serum vitamin D levels than never smokers. <sup>13</sup> (if this was already showed? what is the originality of this work?) Lung destruction is mediated in part by inflammation <sup>14, 15</sup> oxidative stress <sup>16, 17</sup> and increased proteases in smoking-related lung disease. <sup>16, 18</sup> many of these processes are modulated by vitamin D. <sup>19, 20</sup>

The purpose of the study was to compare the serum glutathione s transferase- $\mu$  and vitamin D levels in smokers and non-smokers patients of Nagpur City.

**AIM:** Comparative Study of Glutathione-S- Transferase Isoenzyme mu and Vitamin-D Levels in Smokers and Non-Smokers

The introduction same too long, not well write, it should be re-rewrite!!

The originality and aims of the work should be well underlined!

## **MATERIAL AND METHODS-**

Present community based study UHTC & RHTC (defined in the first use) was carried out in the Biochemistry Department of DMMC&SMHRC Nagpur from August 2020 to February 2021. A total of 100 subjects aged 20 to 55 years old were enrolled in this study. There were 50 smokers in the study group and 50 non-smokers in the control group out of a total of 100 participants. For this study, 100 patients between the ages of 20 to 55, both sexes, who smoked and suffered from lung or respiratory issues and came to Shalinitai Meghe Hospital Nagpur for their regular checkup was chosen.

The ethical issue is if those patients are informed and all agreed about the use of their personal information in this study?

### **Inclusion criteria**

- Age group more than 20 years and less than 55 years
- This study includes the smokers who smoke more than 5 cigarettes in per day

### **Exclusion criteria**

- Less than 20 yrs and more than 55 year age group were excluded
- No underweight participants, pregnant women, individuals with malignancies/infections.
- Candidates not willing to participate.

You should justify this choice of the age range!

Give the total of patients inside of each age range class? It gives an idea about the distribution of patients inside of each the group according to the age?

### **Blood sample collection and processing**

Non fasting blood sample was obtained in a vacutainer containing gel clot activator (give the technical details or the commercial reference) from the median cubital vein with tourniquet attached to the limb and fingers squeezed. Blood was centrifuged for 10 min at 10,000 rpm to settle all the formed elements and separate serum. Samples were analyzed in the clinical chemistry laboratory of Shalinitai Meghe Hospital Nagpur. Glutathione-s-transferase- $\mu$  and vitamin D were measured within 24 h from samples obtained from the patients. Aliquots of the samples were frozen at  $-80^{\circ}\text{C}$  for subsequent assessment of glutathione isoenzyme- $\mu$ , and vitamin D.

### **Biochemical analysis**

Vitamin D status was assessed by ELISA. GST- $\mu$  was established in heparinized whole blood using an Enzyme-Linked Immunosorbent Assay.

Give the method used for the analysis of the glutathione isoenzyme- $\mu$ , and vitamin D.

This part should be more developed?

### **Statistical Analysis**

Smoking and nonsmoking groups, as well as GST- $\mu$ -deficient and non-deficient groups were compared using the Student t test and the  $\chi^2$  test. Data for all parameters was evaluated for means and standard deviation. Data processing was carried out by Microsoft Excel and the social sciences statistical kit (SPSS version 22).

## RESULT

**Table 1 Smokers and non-smokers' characteristics**

Sr.No	Characteristics	Smokers (n= 50)	Non-smokers (n=50)
1	Age	35.5±6.7	34.2± 7.9
2	Body mass index	22.5±6.3	22.21±2.1
3	Cigarette per day	13.8±7.3	0
4	Duration of smoking (years)	10.5±5.0	0

It will be better to present those data in chart

The data for the smoking and nonsmoking groups are shown in Table 1. In that table, there is no distinction between smokers and nonsmokers in terms of age group or (Body mass index) BMI. (it should be proved statistically) Some smokers smoke more than 5 cigarettes per day and have been smoking for more than 3 years (whay 3 ?), so we labeled them as heavy smokers, while those who smoke 5 or less cigarettes per day and have been smoking for less than 3 years are labeled as light smokers.

**Table 2: GST-μ status in heavy and light smokers.**

Please give the unite

Sr.No	Smoking status	GST-μ Positive ?	GST-μ Negative ?	P- Value
1	Smokers (n=50)	5.24±0.95 (unit)	2.94±0.35	P < 0.0001
2	Light smokers (n=25)	4.56±0.78	3.85±1.3	P = 0.0234
3	Heavy smokers (n=25)	6.39±3.2	1.25±0.27	P < 0.0001
4	Non-smokers (n=50)	2.11±1.13	2.01±1.07	P = 0.6506

Defined the difference between positive and negative?

The table is not clear if the smokers (Smokers (n=50)) group is divided to 2 and 3 whay you present them in same time?? The total then according to table will be 150 ?

Table 2 reveals that smokers have two separate smoking statuses. GST-μ was found to be mostly positive in smokers, and it was also found to be more significant in heavy smokers than light smokers. GST-μ is positive in light smokers, but not significantly so when compared to smokers and heavy smokers. GST-μ is nearly equal in smokers and heavy smokers relative to others.

**Table 3: Vitamin D levels in smokers and non-smokers.**

Sr.No	Biochemical Parameter	Smokers (n=50)	Non-smokers (n=50)	P-Value
1	Vitamin D(25(OH)D) (ng/ml)	20.5±5.2	28.40±10.5	P < 0.0001

Table 3 indicates that non-smokers have substantially higher vitamin D levels than smokers. We find vitamin D levels in all smokers, both light and heavy where are the result? Since smokers have very low vitamin D levels give the optimum value? they must take a vitamin D supplement to meet their needs.

It will be better to present those data in chart, and not in table

## DISCUSSION

Should explain what is the link between the oxidative stress enzyme like GST and smoking (mechanism of action...), same for vit D?

Since the GST- $\mu$  isozyme is inherited in an autosomal dominant manner, the findings for the GST- $\mu$  phenotype imply that a portion of the variance in glutathione S transferase isoenzymes in smokers is genetically determined.<sup>21</sup> Glutathione-S-transferase detoxifies reactive electrophiles, especially epoxides, and GST- $\mu$  deficiency can indicate a reduced detoxification capacity and increased carcinogen-mediated DNA damage.<sup>3,22</sup> Our findings back up this theory, suggesting that increased DNA damage in GST- $\mu$  deficient heavy smokers can play a role in the case-control studies that show a correlation between GST and lung cancer. Should be careful with such constation?

As per the Table 2 demonstrate that the two different smoking status in smokers. Here showed that GST- $\mu$  mostly positive in smokers in that also it is more significant in heavy smokers ( $P < 0.0001$ ) as compare to light smokers ( $P = 0.0234$ ). In light smokers GST- $\mu$  is positive but it is not significant as compared to smokers and heavy smokers. Smokers and heavy smokers GST- $\mu$  is almost similar as compared to others ( $P < 0.0001$ ).

One study found a strong inverse association between GST deficiency and lung cancer in heavy smokers but not in light smokers,<sup>23</sup> which appears to fit our findings. Another research<sup>24</sup>, which was more ambiguous, found an inverse association (though not statistically significant) only in heavy smokers. Squamous carcinoma had an inverse association with adeno-carcinoma of the lung, but not with adeno-carcinoma of the lung, according to **Zhong et al., 1991**<sup>25</sup>. The micronuclei counts findings do not support the theory, as micronuclei counts were also lower in GST-deficient subjects.

Smoking has an adverse effect on the synthesis of steroid hormones, including vitamin D, according to **Soldin and colleagues (2011)**<sup>26</sup>. The exact mechanisms by which smoking has an effect on vitamin D metabolism are unknown.

One explanation is that current smokers had a lower vitamin D dietary pattern than never smokers, which may explain the negative relationship between smoking and vitamin D in our study in part, if not entirely. The chemicals in tobacco smoke can have a direct impact on vitamin D metabolism and function, according to **Brot C, 1999**<sup>27</sup>. According to **O'Shaughnessy PJ, 2011**<sup>28</sup>, there is evidence that smoking changes the expression of certain genes involved in the vitamin D metabolic pathway. Due to residual confounding, it was difficult to decide if smoking decreases vitamin D levels or whether there is a correlation between smoking and vitamin D levels.

This discussion should be reworked and more developed?

## Conclusion:

In summary, current smokers had lower vitamin D serum level than non-smokers, and the associations showed that smoking more cigarettes a day, for longer periods of time, and for more pack-years was correlated with lower vitamin D. Quitting smoking for a longer period of time was related to higher vitamin D levels than current smoking.

Those are the results not a conclusion? Furthermore, the GST- $\mu$  measure used in our research may be used to show differences in cytogenetic damage between smokers who have a genetically defined detoxification enzyme and those who do not.

Conclusion should be reworked?

## References: the majority of those reference are old try to use the new references?

- 1) Cigarette brand use among adult smokers—United States, 1986. MMWR Morb Mortal Wkly Rep 1990; 39:665, 671-3.
- 2) Travis WD, Travis LB, Devesa SS. Lung cancer [published erratum appears in Cancer 1995;15;75:2979]. Cancer 1995;75(1 Suppl):191-202.
- 3) Ranjit S. Ambad, Priya Koundal, Akansha Singh, Roshan Kumar Jha. Association between Glutathione-S-Transferase and Gastric Carcinoma: A Case Control Study. Evolution Med. Dent. Sci. Vol. 9 / Issue 39 / Sept. 28, 2020, 2783-2786.
- 4) The Health Consequences of Smoking: A Report of the Surgeon General. <<http://www.surgeongeneral.gov/library/smokingconsequences/>> (Version current at August 25, 2008).
- 5) World Health Organization Tobacco Fact Sheet. Available from: <http://www.who.int/mediacentre/factsheets/fs339/en/>.
- 6) Hulka, B. S., Wilcosky, T. C., and Griffith, J. D. Biological Markers in Epidemiology New York: Oxford University Press, 1990.
- 7) Weinstein, I. B. The origins of human cancer: molecular mechanisms and their implications for cancer prevention and treatment. Cancer Res. 1988; 48: 4135-4143.
- 8) Wilcosky, T. C., and Rynard, S. M. Sister chromatid et.al. In: B. S. Hulka, . C. Wilcosky, and J. D. Griffith, Biological Markers in Epidemiology. New York: Oxford University Press, 1990.
- 9) Vine, M. F. Micronuclei. In: B. S. Hulka, T. C. Wilcosky, and J. D. Griffith, Biological Markers in Epidemiology. New York: Oxford University Press, 1990.
- 10) S. IARC Monograph on the evaluation of the carcinogenic risk of chemicals to humans, Tobacco Smoking. WHO/International Agency for Research on Cancer, Lyon, 1986;38.
- 11) Bischoff-Ferrari HA, Willett WC, Wong JB, et al. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. JAMA 2005; 293:2257-64.
- 12) Song Y, Wang L, Pittas AG, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2013; 36:1422-8.
- 13) Elamin MB, Abu Elnour NO, Elamin KB, et al. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011; 96:1931-42.

- 14) Shinkov A, Borissova AM, Dakovska L, et al. Winter 25-hydroxyvitamin D levels in young urban adults are affected by smoking, body mass index and educational level. *Eur J Clin Nutr* 2015;69:355–60.
- 15) Xu L, Jiang CQ, Schooling CM, et al. Prediction of 4-year incident diabetes in older Chinese: recalibration of the Framingham diabetes score on Guangzhou Biobank Cohort Study. *Prev Med* 2014; 69:63–8.
- 16) Xu L, Jiang CQ, Lam TH, et al. Sleep duration and memory in the elderly Chinese: longitudinal analysis of the Guangzhou Biobank Cohort Study. *Sleep* 2014; 37:1737–44.
- 17) Jiang CQ, Lao XQ, Yin P, et al. Smoking, smoking cessation and aortic arch calcification in older Chinese: the Guangzhou Biobank Cohort Study. *Atherosclerosis* 2009; 202:529–34.
- 18) Jiang CQ, Xu L, Lam TH, et al. Smoking cessation and carotid atherosclerosis: the Guangzhou Biobank Cohort Study—CVD. *J Epidemiol Community Health* 2010; 64:1004–9.
- 19) Lam KB, Jiang CQ, Jordan RE, et al. Prior TB, smoking, and airflow obstruction: a cross-sectional analysis of the Guangzhou Biobank Cohort Study. *Chest* 2010; 137:593–600.
- 20) Shi L, Nechuta S, Gao YT, et al. Correlates of 25-Hydroxyvitamin D among Chinese breast cancer patients. *PLoS ONE* 2014; 9:864–67.
- 21) Seidegrd, J., and Pero, P. W. The hereditary transmission of high glutathione transferase activity towards trans-stilbene oxide in human mononuclear leukocytes. *Hum. Genet.*, 1985;69: 66–68.
- 22) Mannervik, B., and Danielson, U. H. Glutathione-S-transferase: structure and catalytic activity. *CRC Crit. Rev. Biochem.* 1988; 23: 283–337.
- 23) Perry, P. E., and Thomson, E. J. The methodology of sister chromatid exchanges. In: B. J. Kilbey, M. Legator, W. Nichols, and C. Ramel, *Handbook of Muta-genicity Test Procedures*, Amsterdam: Elsevier's Sciences Publishers, 1984.
- 24) Heckbert, S. R., Weiss, N. S., Hornung, S. K., Eaton, D. L., and Motulsky, A. C. Glutathione S-transferase and epoxide hydrolase activity in human leukocytes in relation to risk of lung cancer and other smoking-related cancers. *J. Natl. Cancer Inst.* 1992; 84:414–422.
- 25) Zhong, S., Howie, A. F., Ketterer, B., Taylor, J., Hayes, I. D., Beckett, C. J., Wathen, C. C., Wolf, C. R., and Spurr, N. K. Glutathione S-transferase  $\mu$  locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. *Carcinogens* 1991; 12: 1 533–1 537.
- 26) Soldin OP, Makambi KH, Soldin SJ, et al. Steroid hormone levels associated with passive and active smoking. *Steroids* 2011;76:653–9.
- 27) Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. *Eur J Clin Nutr* 1999;53:920–6.
- 28) O'Shaughnessy PJ, Monteiro A, Bhattacharya S, et al. Maternal smoking and fetal sex significantly affect metabolic enzyme expression in the human fetal liver. *J Clin Endocrinol Metab* 2011;96:2851–60.