Comparative Study of Glutathione-S-Transferase Isoenzyme and Vitamin D Levels in Smokers and Non-smokers

Ranjit S. Ambad a#, Suryakant Nagtilak b*, Dattu Hawale c¤ and Ashish Anjankar c#

a Department of Biochemistry, Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur (Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha), India.
b Department of Biochemistry, NAMO Medical Education and Research Institute Silvassa DNH, India.
c Department of Biochemistry, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha, India.

Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: In developed countries, cigarette smoking is the leading preventable cause of morbidity and mortality. 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. 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.

Materials 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. 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-µ in heparinized whole blood.

**Results:** GST-µ was found to be mostly positive in smokers, and it was also found to be raised in heavy smokers (6.39±3.2) than light smokers (4.56±0.78). GST-µ is positive in light smokers. GST-µ is nearly equal in smokers (5.24±0.95) and heavy smokers relative to others.

**Conclusion:** Quitting smoking for a longer period of time was related to higher vitamin D levels than current smoking. Furthermore, the GST-µ 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-µ; cytogenetic damage; smoker; and GST.

1. 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 [1]. 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 [2,3]. There are an estimated 1.1 billion smokers in the world, with 900 million men and 200 million women. 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 [4].

After China, India has the world's second-largest tobacco consumer. Tobacco kills more than 7 million people per year, according to the World Health Organization. Direct intake causes over 6 million deaths, while passive smoking causes 880,000 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. 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 55% of all deaths [5].

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 [6]. DNA damage markers are of particular interest because DNA damage is a critical step in carcinogenesis [7]. In smokers, who have a documented elevated risk of lung cancer, DNA damage markers such as GST-µ are reduced or absent [3, 8]. 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-µ and increased cytogenetic damage in smokers by tobacco smoke [3, 9].

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

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

2. MATERIALS AND METHODS

Present community based study UHTC & RHTC 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.

2.1 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

2.2 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.

2.3 Blood Sample Collection and Processing

Non fasting blood sample was obtained in a vacutainer containing gel clot activator 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 Shaliniti Meghe Hospital Nagpur. Glutathione-s-transferase-μ and vitamin D were measured within 24 h from samples obtained from the patients. Aliquots of the samples were frozen at -80°C for subsequent assessment of glutathione isoenzyme-μ, and vitamin D.

2.4 Biochemical Analysis

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

2.5 Statistical Analysis

Smoking and nonsmoking groups, as well as GST-μ-deficient and non-deficient groups were compared using the Student t test and the χ² 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).

3. RESULTS

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 BMI. Some smokers smoke more than 5 cigarettes per day and have been smoking for more than 3 years, 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 reveals that smokers have two separate smoking status. 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>
<thead>
<tr>
<th>Sr. No</th>
<th>Characteristics</th>
<th>Smokers (n= 50)</th>
<th>Non-smokers (n=50)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>35.5±6.7</td>
<td>34.2± 7.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Body mass index</td>
<td>22.5±6.3</td>
<td>22.21±2.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cigarette per day</td>
<td>13.8±7.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Duration of smoking (years)</td>
<td>10.5±5.0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Smoking status</th>
<th>GST-μ Positive</th>
<th>GST-μ Negative</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Smokers (n=50)</td>
<td>5.24±0.95</td>
<td>2.94±0.35</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>2</td>
<td>Light smokers (n=25)</td>
<td>4.56±0.78</td>
<td>3.85±1.3</td>
<td>P = 0.0234</td>
</tr>
<tr>
<td>3</td>
<td>Heavy smokers (n=25)</td>
<td>6.39±3.2</td>
<td>1.25±0.27</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>4</td>
<td>Non-smokers (n=50)</td>
<td>2.11±1.13</td>
<td>2.01±1.07</td>
<td>P = 0.6506</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Biochemical Parameter</th>
<th>Smokers (n=50)</th>
<th>Non-smokers (n=50)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitamin D(25(OH)D) (ng/ml)</td>
<td>20.5±5.2</td>
<td>28.40±10.5</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 3 indicates that non-smokers have significantly higher vitamin D levels than smokers (P <0.001). We find vitamin D levels in all smokers, both light and heavy. Since smokers have very low vitamin D levels, they must take a vitamin D supplement to meet their needs.

4. DISCUSSION

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

As per the Table 2 demonstrate that the two different smoking status in smokers. Here showed that GST-μ 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-μ is positive but it is not significant as compared to smokers and heavy smokers. Smokers and heavy smokers GST-μ 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, [23] which appears to fit our findings. Another research [24], 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 [25]. 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) [26]. 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 [27]. According to O'Shaughnessy PJ, 2011 [28], 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.

5. 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. Furthermore, the GST-μ 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.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Institutional ethic committee approval was taken.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


