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Original article

Exposure to Volatile organic compounds (VOCs) on Oxidative stress among domestic painters – A Salivary biomarker study.

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ABSTRACT

Occupational exposure to volatile organic compounds (VOCs) and its health implications has emerged as a significant concern in the past few decades. VOCs include benzene, toluene, xylene, styrene, ethyl benzene, polycyclic aromatic hydrocarbons, chlorinated solvents and aromatic azo dyes and are present in paints, varnishes, paint pigments and adhesives. Domestic painters are exposed to diverse types of paints that contain varied levels of VOCs which is believed to induce DNA damage brought on by oxidative stress.

Studies report saliva to be a better analytical tool in the evaluation of oxidative stress related to occupational exposure of VOCs. This study may further appreciate VOC exposure that cause oxidative stress which can be determined by the level of the Salivary biomarker – 8hydroxy deoxyguanosine (8-OHdG). Domestic painters between the age group 18 -70 were involved in the study. With the help of a questionnaire, the personal details, occupational history, educational status, working exposure and medical history were collected. Saliva of 52 domestic painters and 50 age matched controls with the informed consent signed have been collected by passive drool method. The levels of salivary 8OHdG were analysed with the Elisa kit. Mann-Whitney test showed significant relationship between VOC exposure and oxidative stress damage among the domestic painters who had work experience for more than 10 years when compared to the non-exposed group. The present study suggests that chronic exposure to VOCs may contribute to the oxidative DNA deterioration among domestic painters which might be a signal for various pathological conditions.

Keywords: *Salivary 8-hydroxy deoxyguanosine, Oxidative stress, domestic painters*

INTRODUCTION

Painting profession is an integral part of construction and maintenance industry. Domestic painters play a significant role in this sector. Exposure to organic substances that are volatile and its effect on human population is of health concern in the recent decades. They are exposed to a range of harmful chemical substances which can be collectively called ad Volatile Organic Compounds (VOCs). Volatile organic compounds are the chemicals that rapidly evaporate at ambient temperature.[1] These compounds are widely used in paints, paint pigments, adhesives, and cleaning products. The VOCs present in paints are benzene, toluene, styrene, ethylbenzene, polycyclic aromatic hydrocarbons and aromatic azodyes.[2] Maintenance of older homes includes scraping off old paint, repairing cracks and holes or addressing issues like peeling that require more solvents than working with new homes or buildings. Water-based paints have been developed as an alternative in response to environmental and health concern. Regardless of the emergence of the alternatives, solvent-based paints are preferred due to their adhesion, duration and resistance to molds for older homes than for new buildings. There are studies existing on automobile painters, paint industry workers, carpenters, histopathology technicians, personal exposure to individual VOCs in India. There is a notable dearth in understanding the health effects of VOC exposure among domestic painters in India. The present study might help us to understand better if long-term or short-term exposure that can lead to oxidative damage among domestic painters of Coimbatore.

The disparity between the production of oxygen reactive species (free radicals) and the ability of the body to detoxify these reactive products is called oxidative stress.[3] This imbalance can contribute to various

pathological conditions that affects cellular function and exert a broader impact on overall health.[4,5] VOC exposure is a significant occupational hazard and can lead to both genotoxicity as well as oxidative stress, which can cause damage to cellular components like DNA and lead to adverse health effects.[6] Saliva is found to be an emerging and a promising analytical tool because of the non-invasive, less harmful procedure for detection of oxidative DNA damage.[7,8,9] Various studies have reported on different salivary biomarkers to be useful in diagnosing systemic diseases like Cardiovascular disorders, diabetes and cancer.[10] Few studies mention that water-based paints are safer than solvent based traditional paints with regards to health while few others of opinion that even low VOCs as a long-term exposure is a threat to human population especially to painters.[11] In the current research we aimed to investigate the association of VOCs with oxidative damage by measuring Salivary 8 – Hydroxydeoxyguanosine (8-OHdG) levels among domestic painters who are exposed to different kinds of paints with varied level of VOCs. The findings of the study may contribute to the potential use of salivary biomarkers in monitoring occupationally induced VOC exposure and the resulting health hazards.

MATERIALS AND METHODS:

Study Population:

Domestic painters and control population of 102 between the age group of 18-70 were involved in this study. With the help of a generalized structured questionnaire the personal data that included age, occupation, nature of painting, number of years of painting, education and social background were collected. Also, the past and present history of illness and if there is any medication for the same were all recorded. Possible confounding factors like smoking and alcohol was also made clear from the questionnaire. All the above-mentioned details were collected for the control population except for the painting exposure details.

Study design:

This is a cross-sectional comparative study conducted among the domestic painters and controls in the city of Coimbatore, Tamilnadu between December 2022 till August 2023.The study the participants were evaluated for the study and met the inclusion criteria. Saliva is collected by passive drool method into a sterile disposable collection tube between 6.30-8.30 in the morning and the participants were instructed to avoid eating, drinking, or smoking for a minimum of 30 minutes before the sample collection.[12]

Principle – This assay adopts the technique of competitive inhibition enzyme immunoassay method. The microtiter in this test kit has been pre-coated with 8-Hydroxyguanosine (8OHdG) protein. Standards or samples were added to the appropriate microtiter plate wells the with a biotin-conjugated antibody specific to 8OHdG.

Saliva samples were centrifuged at 3000 rpm for 10 minutes to remove any debris or impurities. The supernatant was then collected and stored at -80°C until analysis. 50µl of sample along with 50 µl Biotinylated -conjugate (1X) is added to each well and incubated for 1hr at 37ºC. Each well is sucked up and washed thrice. 100 µl of Streptavidin- HRP (1X) is added to each well and incubated for an hour at 37ºC. Each well was aspirated and washed for 5 times and complete removal of liquid was done at each step for a good performance. 90 µl of substrate solution was added to each well and incubated for 20 minutes at 37ºC. Then 50 µl of stop solution was added to each well. The wells containing highest concentration of standard develops blue colour. The absorbance of each well was measured using a microplate reader at a wavelength of 450 nm.

Ethics:

According to Indian Council of Medical Research rules (Ref No. 61/MMCH&RI/2021), the protocol was authorized by the Institution Committee of Ethics in Human Research, which is part of Chettinad Academy of Research and Education (CARE) with the reference number - IHEC-II/0160/22. After being informed, every patient signed a written informed consent form for willingness to take part in the research.

Sample size:

The sample size for this study was determined based on the available literature on oxidative stress among workers exposed to organic solvents. Using the standard formula for comparing two means, the required sample size was calculated considering a confidence level of 95%. The expected mean difference and standard

deviation were derived from previous studies investigating cognitive function impairments among occupationally exposed populations. Based on these parameters, the estimated sample size per group was 100 with 50.

AGE	RESULT (pg/ml)			Chi-Square	p-value
	N	Mean±SD	Median		
<30	15	532.67±205.6	784	10.90	0.00*
31-40	21	366.05±149.98	1130		
>40	14	417.86±220.911	1178		

STATISTICAL ANALYSIS

The values were observed and analysed using IBM Corp. Released 2013. IBM SPSS Statistics for windows, version 22.0. Armonk, NY: IBM Corp.

Salivary 8-OHdG exposed group and not exposed group comparison: Table 1

Group	RESULT (pg/ml)			Mann Whitney U value	p-value
	N	Mean±SD	Median		
Painters	**52**	**1898.67±1791.05**	**1006**	**214.5**	**<0.001**
Controls	**50**	**430.54±198.07**	**456**		

*Note: **.P <0.01*

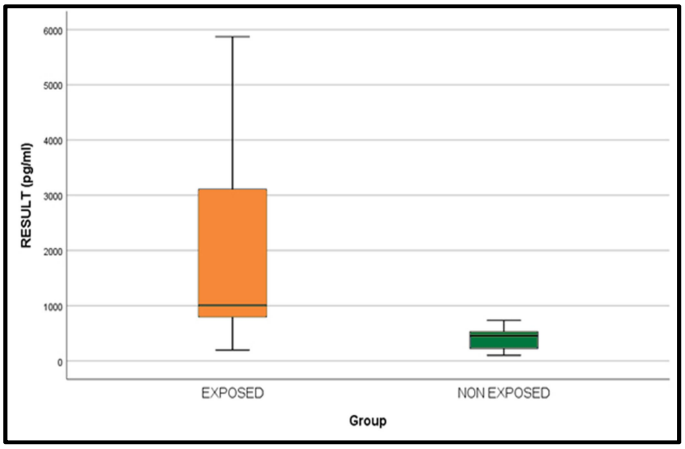

Fig. 1: Salivary 8-OHdG painters Vs control

The value of salivary 8-OHdG was higher among the exposed group when compared with the non-exposed and the Mann-Whitney test showed significance with the P value <0.01 as shown in Table 1& Fig. 1.

Table 2: Salivary 8OHdG among painters compared for age groups

*Note: **.P <0.01*

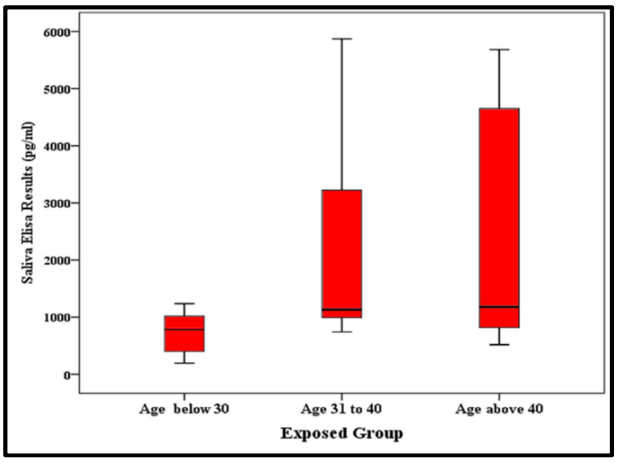

Fig.2: Age group comparison among exposed

Table 2 & Fig. 2 shows the values among domestic painters with different age groups and the P value is significant.

Table 3: Salivary 8OHdG among controls

AGE	RESULT (pg/ml)			Chi-square	p-value
	N	Mean±SD	Median		
<30	15	749.87±331.04	525	5.87	0.053
31-40	21	2221.48±1873.88	456		
>40	16	2552±2049.7	412		

Table 3 & Fig. 3 shows no significance among the non-exposed group with the value of P<0.13 when compared to the exposed population. Post hoc analysis was done using Tukey HSD to understand high significance. Significant differences were observed in the age group above 40 when compared to the other age groups like below 30 and between 31-40 among domestic painters. There was no significant difference among controls of any age group.

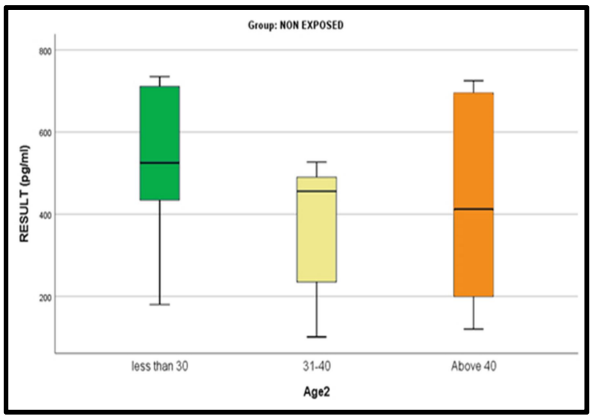

Fig.3: Age group comparison among control population

Table 4 : Salivary 8OHdG among exposed compared for working experience

Experience	N	Mean ± SD	Median	Mean Rank	Chi-Square	p value
Below 5	16	791.25 ± 359.32	798.00	17.16	19.22	.00**
5 to 10	17	1099.35 ± 756.78	978.00	22.03		
Above 10	19	3546.42 ± 1978.10	4205.00	38.37		

*Note: ** P <0.01*

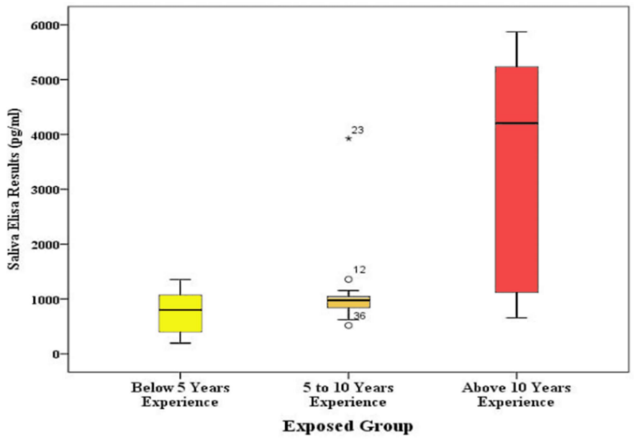

Fig.4 Saliva 8-OHdG Painter Vs Years of Experience

Table 4 & Fig. 4.shows the comparison among painters with regards to their work experience which intern indicates the years of exposure. The workers who have greater work experience might have chronic exposure to the chemicals and the comparison shows significant P value.

DISCUSSION

The present study was to understand the relationship between oxidative stress and exposure to volatile organic compounds among domestic painters. Domestic painters of 52 and control population of 50 participated in the study (n=100). All the study participants were informed regarding the study in detail and possible health implications. Oxidative stress and presence of free radicals are acknowledged as significant threat to human health. When VOCs are inhaled or ingested, they can enter the blood stream and cause various health effects, including neurological deficit, respiratory problems, cancer and DNA damage.[13] The detrimental impact of reactive oxygen species (ROS) extends to the impairment of lipids, nucleic acids and proteins leading to alterations in their functions. The consequential imbalance between production of ROS and the body's ability to fight against, gives rise to oxidative stress.[14]

In this study salivary 8-OHdG was analysed for 52 painters and 36 age matched controls (n=88) to understand the status of oxidative stress among the domestic painters exposed to VOCs in paints. When compared with the control population, domestic painters exhibited increased 8-OHdG values and was statistically significant *(P <0.01).* The rise in salivary 8-OHdG coincides with findings from several studies indicating oxidative stress among solvent exposed. [15, 16]

Oxidative damage caused by individual solvents of paints like benzene, toluene, ethylbenzene and xylene were reported in various studies. In a study among spray painters, urinary methyl hippuric acid and mandelic acid were assessed as metabolites of xylene and ethylbenzene respectively along with urinary 8-OHdG. The urinary 8OHdG level was positively correlated to ethylbenzene indicating oxidative damage

similar to the current study.[17] Parallel to the present study on increased salivary biomarker among painters, another study confirmed higher levels of toluene exposure in painting, printing and automotive industries. This was evidenced by the presence of biomarkers like metabolites of toluene in urine (methyl hippuric acid) and the parent compound (toluene) in blood which contributed to oxidative damage.[18]

The study participants were categorised into different age groups like below 30 years, 31-40 years and above 40 years. Painters above 40 years. Increased levels of salivary 8-OHdG were observed among the painters above 40 years compared to controls, highlighting the impact of exposure to organic solvents in causing DNA damage. A study among painters of Egypt over the age of 50 showed significant increase in Malondialdehyde (MDA), a biological indicator of oxidative stress which is harmonious with the present study.[19] Similarly, exposure to polyaromatic hydrocarbons (PAHs) in paints showed increased 8OHdG in urine in a study which correlated with urinary PAH level.[20] Few other studies that reassured occupational exposure to PAHs for prolonged period expresses increase in 8OHdG levels in urine suggesting strong association with oxidative damage to DNA along with lipids and proteins.[21]

With regard to the work experience that can give the years of exposure among painters, they were categorised into three groups of below 5 years, 5-10 and above 10 years. Painters over 10 years of experience exhibited greater level of 8OHdG when compared with the other two groups. This reaffirms that extended period of exposure induce more pronounced oxidative distress which is similar to previous studies that reported exposure over 20 years and increased DNA damage.[22] There are studies that have documented low level exposure to the solvents like toluene, xylene and ethylbenzene which revealed increased oxidative damage.[23] The above studies are incoherent with the current study which observed significant values of 8OHdG among younger painters and had lesser years of exposure (less than 5 years). The possible confounding factors were smoking and alcohol in the present study. Benzene is one of the important sources of smoking apart from a component of paint leading to oxidative stress. Certain studies discovered increased levels of 8OHdG in the working environment among smokers when compared to non-smokers.[24] In order to avoid confounders, in this study the controls were also having similar habits of smoking and alcohol intoxication but to a lesser magnitude.

CONCLUSION:

The findings conclude that both low level exposure and long-term exposure were significant among domestic painters. Occupational exposure in the field of painting were categorised as a Group I carcinogen according to the IARC monograph volume 47 (IARC 1989) which was established due to an elevated risk of lung cancer and reaffirmed in Monograph Volume 98 (IARC,2010, a). Persistent daily exposure to VOCs among domestic painters in their job in an unprotected and uncontrolled atmosphere may end up adverse health effects. Larger number of studies have acknowledged interaction with mixed organic solvents or individual solvent exposure to cause increased oxidative DNA damage that can lead to cancer. The effect of exposure may be extended even after they retire and may affect the quality of their retired life. Domestic painters are often unaware of the harmful effects of VOCs and they underscore the need for safety measures, regular health assessment that could help them identify early signs of health issues. Occupational safety protocols should be strengthened to address the specific risks faced in this profession.

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