RESEARCH ARTICLE

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Genetic Polymorphism in Xenobiotic Metabolising Genes and Increased Oxidative Stress among Pesticides Exposed Agricultural Workers Diagnosed with Cancers

Arun Pandiyan[1], Summaiya Lari[1,2], Janardhan Vanka[1], Balakrishnan Senthil Kumar[1], Sudip Ghosh[1], Babban Jee[3], Padmaja R Jonnalagadda[1]*

Abstract

Background: Oxidative stress combined with nullity of xenobiotic metabolizing *GSTT1/GSTM1/CYP2E1* genes may increase the susceptibility of agricultural workers to adverse health effects including cancer. The present study was conducted to determine; the prevalence of polymorphisms in *GSTM1*, *GSTT1* and *CYP2E1* genes, serum 8-hydroxy-2'-deoxygunosine levels, and the role of these markers in risk of cancer among agricultural workers occupationally exposed to pesticides. **Methods:** A total of 360 participants, of which 180 belonging to farming group diagnosed with leukemia (n=60), lymphoma (n=60) and breast cancers (n=60), 90 in non-farming group diagnosed with similar cancers and the other 90 as healthy controls with neither history of occupational exposure nor diagnosed with any type of cancers were recruited. Following the questionnaire survey, serum 8-OHdG and genetic polymorphisms in the three genes were determined using ELISA and PCR methods respectively. **Results:** The results of the study revealed that farm workers carrying GSTT1 null genotype had increased risk for lymphoma (OR = 5.34; 95% CI = 1.80-15.82) and breast cancer (OR=4.04; 95% CI = 1.24-13.07). For farm workers carrying *GSTM1* null genotype, the risk was six-fold for breast cancer (OR = 6.88; 95% CI =1.88-25.99). Further, there found a significant difference between 8-OHdG and nullity of *CYP2E1* among the farm workers diagnosed with leukemia. **Conclusion:** The findings of the present study suggest that the polymorphisms in detoxifying genes among farm workers occupationally exposed to pesticides and the oxidative stress may likely be responsible for triggering the mechanism of malignancy.

Keywords: Occupational exposure- pesticides- oxidative stress- genetic polymorphism- cancer- agricultural worker

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Introduction

Genes that encode xenobiotic metabolizing enzymes (XMEs) are being reviewed continuously as they play a key role in the detoxification of various chemicals including pesticides. Among them, the cytochrome P450 (CYP) and the glutathione S-transferase μ1 (GSTM1), glutathione S-transferase θ1 (GSTT1) in the phase I and phase II respectively are the most widely reviewed genes of the detoxifying enzymes that are implicated in the conjugation of numerous carcinogenic compounds into excretable hydrophilic compounds (Sharma et al., 2012). Further, null alleles of these genes corresponding to the deletion of whole protein-coding region have been identified to exist and are common across major human populations. However, those who completely lack GSTM1 and GSTT1 enzyme activity due to the inherited homozygous loss of these genes are at a higher risk for cancer (Agúndez and Ladero, 2008; Li et al., 2019). These enzymes also play a major role in mechanism related to reduction of cancer risk by a process of deactivation of the mutagenic compounds and they also prevent the formation of DNA adducts (Wang et al., 2010). Similarly, due to its ability to metabolize bioactivate compounds which are potentially carcinogenic, polymorphism in CYP2E1 is also thought to be associated with the development of certain chemically mediated cancers (Trafalis et al., 2010).

Several studies confirmed that pesticide exposure could induce oxidative damage by producing reactive oxygen species (ROS) that can damage the DNA to produce nucleoside modifications (Koureas et al., 2014: Lushchak et al., 2018). The most commonly found and measured DNA lesions in human biological samples is 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a vital biomarker for assessing the levels of oxidative stress and mechanism of carcinogenesis (Valavanidis

*[1]ICMR-National Institute of Nutrition, Tarnaka, Osmania University, PO, Hyderabad, Telangana 500007, India. [2]Department of Biochemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh 522510, India. [3]Department of Health Research, Ministry of Health and Family Welfare, Government of India, Red Cross Road, New Delhi, 110001, India. *For Correspondence: drpadmajaj@gmail.com*

et al., 2009). Further, individuals identified with these genetic polymorphisms were also found to be at higher risk to pesticide induced oxidative stress (Costa et al., 2019). Therefore, the combination of exposure and polymorphisms in xenobiotic metabolizing genes might influence individual's susceptibility to cancer (Figure 1) (Teodoro et al., 2019). Farm workers while engaged in farming activities are not only exposed to pesticides but also to heat, ultraviolet radiation, biologically active dusts, fuel engine exhaust and other viral and bacterial exposures via farm animals which may have impact on their health (Ramirez et al., 2005; Tual et al., 2016).

The U.S. National Cancer Institute (NCI) and the European Food Safety Authority (EFSA) had published reviews of the cancer burden with respect to pesticide exposure and found limitations in drawing firm conclusions (Burns and Juberg, 2021). In India, higher incidence of cancer among agricultural workers was observed in some pockets of the country where many banned and restricted pesticides are still being used (Mittal et al., 2014). However, the occupation associated risk factor for cancer among farm workers is sparsely explored in Indian context. Very little information is available on the association between pesticides induced oxidative stress and polymorphism in key genes of detoxification in relation to the development of malignancies among farm workers. Considering the need of the time as well as the increasing global prevalence of cancer among agricultural workers, the present study was conducted among farm workers/non-farm workers visiting a tertiary cancer care hospital in Hyderabad, Telangana State, India. Following a questionnaire-based survey, the levels of oxidative stress (8-OHdG) and genetic polymorphisms in three key genes of detoxification viz., GSTM1, GSTT1 and CYP2E1 were determined among both the groups diagnosed with leukaemia, lymphoma and breast cancers and to compare with healthy controls who were the family members of farmers with no history of exposure to pesticides and not diagnosed with any types of cancers.

Materials and Methods

Study design and ethical clearance

This hospital-based cross-sectional study was approved by the Institutional and Hospital Ethics Committees. The data were collected from the farm workers/non-farm workers visiting a regional tertiary cancer care hospital in Hyderabad, Telangana State, India by administering the pre-tested questionnaire through an interview at the hospital premises during their periodical visits. The potential participants were also explained the study objectives and written informed consent was also obtained from them before the collection of samples.

Sampling

Prior to the collection of data, a preliminary survey was conducted to assess the frequency and the types of different cancers reported in the regional tertiary cancer care hospital in Hyderabad, India. The findings from the preliminary survey revealed that the patients belonging to the farming community were most commonly diagnosed with either leukaemia, lymphoma and breast cancers while the other types of cancers were negligible. Based on the prevalence (81%), using the formula $n = (z)^2 p (1 – p) / d^2$, the estimated sample size calculated was 90 with a 5% level of significance at 10% precision. Stratified random sampling was used to assign the participants to a 2:1:1 ratio and this allocation supported the distinction between agricultural workers and other participants. Hence, the total number of participants was 360 i.e., 180 farm workers, 90 non-farm workers and 90 healthy controls were set as target for the present investigation (Figure 2).

Inclusion and exclusion criteria

Farm workers between the age group of 18 to 60 years with a previous history of involvement in farming activities and diagnosed with leukaemia (n=60), lymphoma (n=60), and breast cancers (n=60) were included as cases, while non-farm workers (n=90) diagnosed with similar types of cancers were included as controls. The healthy controls (n=90) were those with neither history of exposure to pesticides nor diagnosed with any types of cancers but belonging to the members of the farmer's family. Subjects diagnosed with any other cancers and involved in other occupations were not considered for the study.

Questionnaire data

A questionnaire was prepared based on the previously published literature supported by the validated questionnaires used for our earlier studies and was pre-tested among 30 subjects before its administration (Medithi et al., 2017; Lari et al., 2021). It sought to obtain information on different variables such as demographic particulars; socio-economic status; occupational exposure to pesticides; knowledge, attitude, and practices related to the use of pesticides. The questions employed were both open-ended type and 'yes' or no' kind and the information were collected through face-to-face interviews by trained investigators in the local regional language of the participants or the language of their comfort.

The questionnaire was divided into five sections with a total of fifty-seven questions. The first section included questions on socio-demographic particulars such as sex, age and the extent of land holding by the farm workers, their principal occupation and their educational status. The second section constituted questions on the particulars of pesticide exposure such as their participation in spraying activity followed by the third section containing a set of questions on their involvement in different farming activities namely watering, sowing, cutting, thrashing, weeding followed by harvesting and the types and brand names of pesticides used by them in different forms for the cultivation of major crops such as wheat, paddy, cotton, and vegetables. The fourth section dealt with personal histories such as dietary habits, consumption of alcohol and smoking and the respective quantities consumed per day. Further, the fifth section contained questions related to the participant's attitude, awareness and practice, knowledge of the routes of pesticides exposure, frequency of spraying and protective measures adopted such as PPE, if any, while handling pesticides, reading of the precautions on the label, and storage/sanitary practices

adopted such as washing hands and clothes immediately after handling the pesticides or reuse the same without washing.

Sample collection and processing

A whole blood sample (5 mL) was collected from all the subjects in EDTA vacutainers and was subjected to centrifugation (Multifuge, Heraeus, Thermo Scientific, Germany) at 3,000 rpm for five minutes to obtain the serum and stored at – 20°C for further analyses. Prior to centrifugation, the DNA from all the blood samples were isolated as per the protocol using NucleoSpin® Blood Kit (Machery-Nagel, Germany). The quality of DNA and its concentrations were evaluated using a NanoDrop spectrophotometer and were stored at – 20°C till further use.

Determination of genetic polymorphisms in GSTT1, GSTM1 and CYP2E1 genes

The primer sequences used for the detection of *GSTM1, GSTT1* and *CYP2E1* genotypes were obtained from previous literature (Kalakas et al., 2019). The *GSTT1* and *GSTM1* genes were determined simultaneously in a single assay, using a multiplex PCR method with Albumin as an internal control. The detection of *GSTM1* and *GSTT1* genotypes were carried out using the following primers:

Gene	Primer sequence	Length (bp)
GSTT1	5'-GAACTCCCTGAAAAGCTAAAGC-3' (forward)	459
	5'-GTTGGGCTCAAATATACGGTGG-3' (reverse)	
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC-3' (forward)	219
	5'-GTTGGGCTCAAATATACGGTGG-3' (reverse)	
Albumin	5'-GCCCTCTGCTAACAAGTCCTAC-3' (forward)	350
	5'-GCCCTAAAAAGAAAATCGCCAATC-3' (reverse)	

Briefly, the PCR was carried out using 100 ng of template DNA, dNTP (Sigma-Aldrich, USA), and 5 µL of each forward and reverse primer of GSTT1 and GSTM1 (Bioartis Life Sciences, India) and Taq DNA polymerase (ABM, Canada) in a 20 µL reaction volume. PCR cycle conditions were set with an initial melting step at 95°C for 10 min, followed by an annealing temperature of 56°C for 30 s, thus allowing 35 cycles of amplification and to the final stage where the temperature was maintained at 4°C. The amplified PCR product bands were recognized on 2% agarose gel (Thermo Fischer Scientific, USA). The absence of either one of the bands indicated the genetic polymorphism in the respective genes. The CYP2E1 genotyping was carried out separately in which the isolated DNA was genotyped by using PCR-RFLP. The Rsa1 restriction enzyme specificity (GT/AC) and cut position were obtained from NEB cutter V 2.0. Essentially, the primers were designed in such a way that in the mutant alleles, a Rsa1 restriction site will be inactivated and hence, cannot be cleaved, while, the wild-type alleles were digested by Rsa1 restriction enzyme (New England Biolabs, USA). The primer sequences for CYP2E1 were as follows:

Gene	Primer sequence	Length (bp)
CYP2E1	5'- CCAGTCGAGTCTACATTGTCA -3' (forward)	413
	5'-TTCATTCTGTCTTCTAACTGG-3' (reverse)	

The PCR for CYP2E1 was carried out in a 20 µL volume reaction mixture containing the buffer, 100 ng of genomic DNA, dNTP, 5 µL of each forward and reverse primer and Taq DNA polymerase. The conditions were as follows: initial denaturation at 95°C for 4 min followed by annealing at 46°C for 30 s and extension at 54°C for 30 s. Subsequently, the allelic variant of the polymorphic site was studied using 1 U Rsa1 enzyme digestion of the PCR products for 24 hours in a water bath at 37°C followed by resolution on 1.5% agarose gel with Ethidium Bromide (EtBr) (Invitrogen, USA).

Determination of serum 8-hydroxy-2'-deoxygunosine (8-OHdG):

The serum levels of 8-OHdG were analyzed using commercially available ELISA kits (Elabscience, USA) and the method was followed as per the manufacturer's instructions provided in the leaflet. Briefly, 50 µL each of standard and serum samples were added in respective wells of a 96-well plate. Subsequently, biotinylated detection Ab (1:100 dilutions) working solution was added to the wells, followed by incubation for 45 min at 37°C. After incubation, the plates were washed and aspirated thrice using a wash buffer followed by the addition of 100 µL of HRP-streptavidin conjugate solution and were incubated again for 30 min at 37°C. Subsequently, the plates were aspirated and washed again using wash buffer followed by the addition of 90 µL substrate reagent and incubated at 37°C for 15 min. The incubated wells were added with 50µL of stop solution and the absorbance was read at 450 nm using a microplate reader (BioTek Instruments). The samples were estimated in duplicates as per the standard protocol.

Statistical analysis

Data were verified for completeness and consistency and analyzed using SPSS v28.0 (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.). Descriptive variables were represented as mean, standard deviation, frequency and percentages. As part of our exploratory analysis, we assessed group-wise differences in mean levels of 8-OHdG with the prevalence of polymorphisms in three genes using Paired t-test. Multivariable analysis was performed to find out the significant association, if any, between serum 8-OHdG and other confounding variables such as age, gender, duration of pesticide exposure, and personal habits. The violin plot was presented to depict the mean levels of serum 8-OHdG. The data were considered significant when $p < 0.05$ at 95% CI.

Results

Socio-demographic features and characteristics of the study population

The demographic particulars of the farm workers who

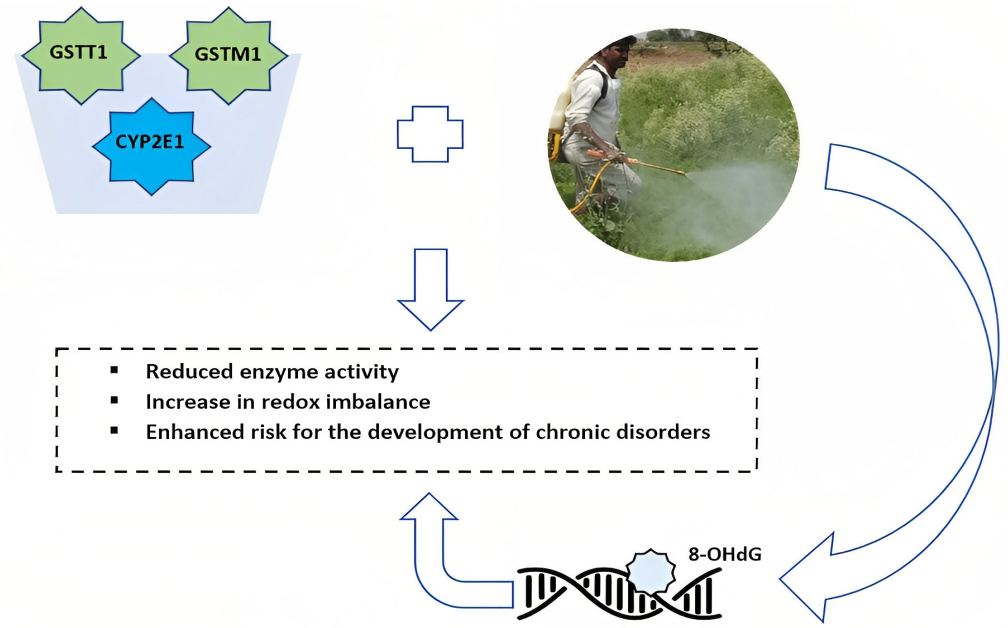

Figure 1. The Combinational Effect of Genetic Polymorphisms and Pesticide-Induced Oxidative Stress

participated in the study are presented (Table 1). The average duration of participation in pesticide spraying by farm workers diagnosed with leukemia, lymphoma and breast cancers was 1.2, 0.9 and 1.6 months respectively in the past one year prior to their diagnosis with an average duration of exposure of 3 hours in a day. The mean quantity of pesticides used per acre was found to be 36 mL. Majority of the farm workers (n=76) were involved in wheat cultivation, followed by paddy (n=50). The remaining farm workers were engaged in the cultivation of cotton (n=35), vegetables (n=11) and fruits (n=8) (Table 2). The farm workers reported to have frequently used monocrotophos, dichlorovos, chlorpyrifos and acephate. In addition, quinalphos was also predominantly used for crops such as cotton and wheat respectively while lambda-cyhalothrin was used on tomato and eggplant. However, for paddy, the most commonly used pesticide was found to be chlorpyrifos. All the pesticides used were found to belong to the insecticides class. None of the farm workers reported to have used fungicides or herbicides.

None of the farm women diagnosed with breast cancer (n=60) reported to have had the habit of smoking cigarettes, cigar or beedi and consumption of alcohol. Among the farm workers diagnosed with lymphoma, 15% (n=9) of them reported to have consumed alcohol every day, while 28% (n=17) of the farm women diagnosed

Table 1. Demographic Particulars of the Participants Belonging to farm workers Group (n=180)

Variable	Leukaemia (n=60)	Lymphoma (n=60)	Breast Cancer (n=60)
Mean age (years)	38.92	44.07	45.45
Sex	Male (n=38)	Male (n=32)	Male (n=0)
	Female (n=22)	Female (n=28)	Female (n=60)
Type of house	Pucca (n=20)	Pucca (n=10)	Pucca (n=19)
	Semi pucca (n=34)	Semi pucca (n=43)	Semi pucca (n=30)
	Kutcha (n=6)	Kutcha (n=7)	Kutcha (n=11)
Location of the house	In the farm (n=6)	In the farm (n=8)	In the farm (n=14)
	Away from the farm (n=54)	Away from the farm (n=52)	Away from the farm (n=46)
Educational status	Illiterate (n=18)	Illiterate (n=19)	Illiterate (n=26)
	Read & Write (n=41)	Read & Write (n=31)	Read & Write (n=34)
	Primary (n=1)	Primary (n=10)	Primary (n=0)
Major occupation	Own cultivation (n=29)	Own cultivation (n=12)	Own cultivation (n=4)
	Tenant cultivation (n=8)	Tenant cultivation (n=31)	Tenant cultivation (n=14)
	Agricultural labour (n=20)	Agricultural labour (n=17)	Agricultural labour (n=42)
	Other labour (n=3)		
Family engaged in agricultural activities	Yes (n=19)	Yes (n=36)	Yes (n=31)
	No (n=41)	No (n=24)	No (n=29)
Land holding in acre (mean)	0.958	0.533	0.117

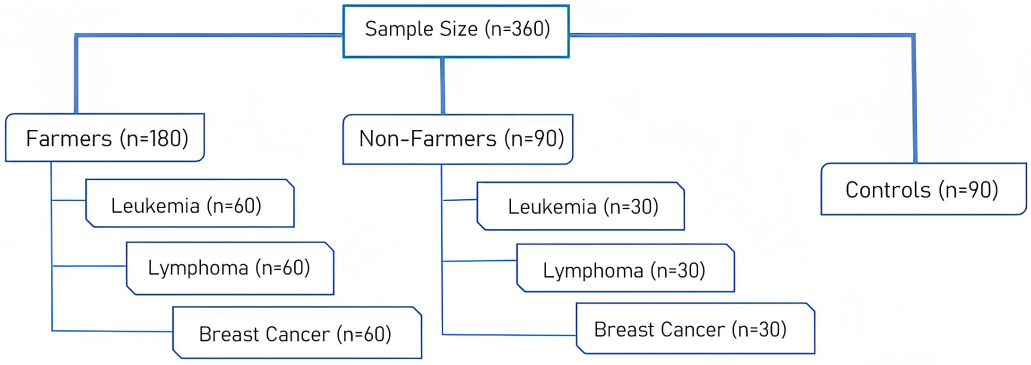

Figure 2. Study Design & Sample Size

Table 2. Particulars Pesticide Usage and Crops Cultivated

Farmers diagnosed with	Particulars of pesticide exposure	Mean±SD	Crops cultivated (n)	Quantity of pesticide used mL/acre (mean)
Leukemia (n=60)	No. of months in last one year	1.2±1.08	Rice (22), Wheat (23), Cotton (8), Vegetables (5) & Fruits (2)	36.41
	Duration of spraying (in hours/day)	3.7±1.04		
	No. of days of spraying in a month	3.5±0.78		
	No. of months in a year	3.8±0.84		
Lymphoma (n=60)	No. of months in last one year	0.9±1.00	Rice (14), Wheat (25), Cotton (16), Vegetables (2) & Fruits (3)	47.29
	Duration of spraying (in hours/day)	3.2±0.09		
	No. of days of spraying in a month	3.8±0.76		
	No. of months in a year	4.1±0.96		
Breast Cancer (n=60)	No. of months in last one year	1.6±1.17	Rice (14), Wheat (28), Cotton (11), Vegetables (4) & Fruits (3)	25.75
	Duration of spraying (in hours/day)	2.5±1.12		
	No. of days of spraying in a month	3.5±0.83		
	No. of months in a year	4.2±1.37		

with breast cancer reported to have consumed tobacco every day. In the present study, none of the farm workers were able to recall their past history of morbidity due to exposure to any specific pesticide and hence, no information on the morbidity symptoms experienced, duration of the symptoms, and the system of medication underwent and place of treatment to alleviate symptoms were gathered.

Genetic polymorphisms in GSTT1, GSTM1 and CYP2E1

The presence of a 219 bp band of GSTM1 or 459 bp band of GSTT1 in the amplified PCR product,

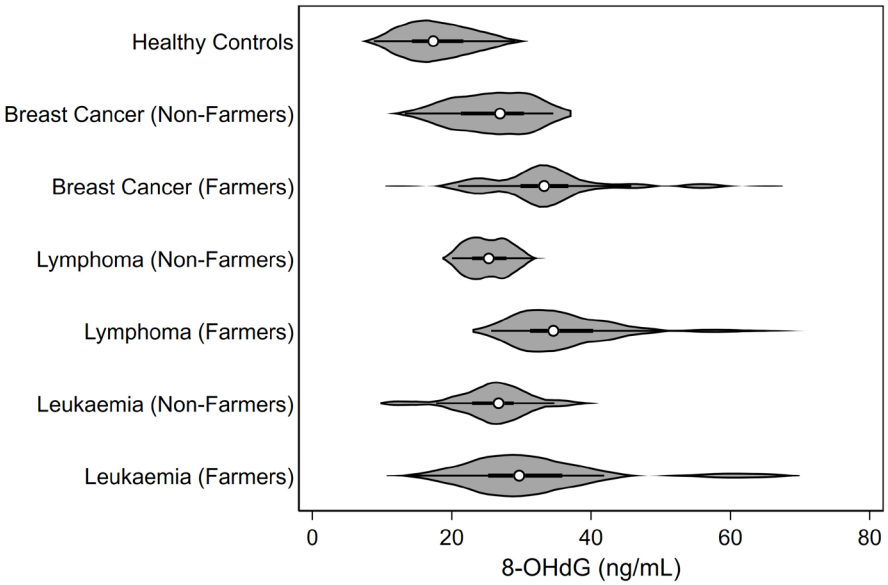

Figure 3. Levels of Serum 8-OHdG in (1) farm workers; (2) non-farm workers and (3) control subjects

Table 4. Comparison between the Genetic Polymorphisms in Three Genes and Serum 8-OHdG

	Leukaemia (n=90)				Lymphoma (n=90)				Breast Cancer (n=90)				Healthy Controls (n=90)	
	farm workers (n=60)		Non-ffarm workers (n=30)		farm workers (n=60)		Non-farm workers (n=30)		farm workers (n=60)		Non-farm workers (n=30)			
	Null	Wild	Null	Wild	Null	Wild	Null	Wild	Null	Wild	Null	Wild	Null	Wild
GSTT1	15	45	3	27	31	29	5	25	23	37	4	26	4	86
8-OHdG (ng/mL) (mean±SD)	34.34±13.0	31.79±10.74	21.92±8.40	26.15±6.63	36.24±8.47	38.71±10.02	26.15±2.73	25.09±3.11	33.23 ±5.21	34.74±11.71	29.16±7.65	25.11±5.30	19.76±2.78	17.96±5.04
Sig.	p>0.05		p>0.05		p>0.05		p>0.05		p>0.05		p>0.05		p>0.05	
							p<0.001							
GSTM1	22	38	6	24	25	35	8	22	26	34	3	27	11	79
8-OHdG (ng/mL) (mean±SD)	35.94±15.35	30.40±7.63	21.22±7.03	26.86±6.63	36.96±6.12	37.78±11.04	25.05±2.51	25.34±3.25	36.13±10.91	32.67±8.54	29.77±3.58	25.19±5.72	16.25±3.53	18.29±5.103
Sig.	p>0.05		p>0.05		p>0.05		p>0.05		p>0.05		p>0.05		p>0.05	
							p<0.001							
CYP2E1	12	48	3	27	12	48	6	24	11	49	5	25	4	86
8-OHdG (ng/mL) (mean±SD)	30.13±4.62	37.82±9.44	32.97±5.39	24.92±6.50	35.90±8.65	37.82±9.44	26.63±2.74	24.98±3.09	35.18±8.91	33.95±9.95	23.48±5.70	26.08±5.69	19.76±2.78	17.89±5.00
Sig.	p<0.05		p<0.05		p>0.05		p>0.05		p>0.05		p>0.05		p>0.05	
							p<0.015							

*p<0.01 denotes significance at 95% CI

Table 3. Comparison of Odds Ratio (OR)

		GSTT1				*GSTM1*				*CYP2E1*			
Cancer	Group	Null	Wild	OR	p-value	Null	Wild	OR	p-value	Null	Wild	OR	p-value
Leukaemia (n=90)	farm workers (n=60)	15	45	3 (0.795-11.323)	0.09	22	38	2.31 (0.821-6.534)	0.1	12	48	2.25 (0.583-8.681)	0.23
	Non-farm workers (n=30)	3	27			6	24			3	27		
Lymphoma (n=90)	farm workers (n=60)	31	29	5.34 (1.805-15.826)	0.001*	25	35	1.96 (0.753-5.121)	0.16	12	48	1 (0.334-2.991)	1
	Non-farm workers (n=30)	5	25			8	22			6	24		
Breast Cancer (n=90)	farm workers (n=60)	23	37	4.04 (1.249-13.07)	0.015*	26	34	6.88	0.01*	11	49	1.12 (0.351-3.587)	0.84
	Non-farm workers (n=30)	4	26			3	27	(1.880 -25.190)		5	25		

*odds ratio (OR) at 95% CI, two-sided p-value <0.01

with reference to 350 bp Albumin (as positive internal control) indicated a positive genotype, while their absence indicated a null genotype. The allelic variants of *CYP2E1* gene Rsa1 polymorphism were determined according to the patterns created following 24-hour enzymatic digestion of amplified fragments and resulting genotypic distributions in cases and controls. Since conventional genotyping was used in the present study, distinguishing the GSTT1 and GSTM1 positive genotypes as heterozygous or homozygous genotypes were not done. Further, the Hardy–Weinberg equilibrium could not be tested because of the inability of the present PCR protocol to separate heterozygous carriers of the null polymorphisms. The study found a significant difference ($p<0.05$) in the frequency of GSTT1 and GSTM1 between the farm workers and non-farm workers diagnosed breast cancer. There also found a significant difference ($p<0.01$) in the frequency of GSTT1 null genotype between the farm workers and non-farm workers diagnosed with lymphoma. It was observed that farm workers carrying GSTT1 null genotype had an increased risk for lymphoma (OR = 5.34; 95% CI = 1.80-15.82) and breast cancers (OR=4.04; 95% CI =1.24-13.07). Similarly, there found a six-fold increased risk for breast cancer among farm workers carrying GSTM1 null genotype (OR = 6.88; 95% CI =1.88-25.99) (Table 3).

Serum 8-OHdG among farm workers, non-farm workers and controls

The farm workers (n=180) showed significantly ($p<0.05$) higher levels of serum 8-OHdG when compared to the non-farm workers (n=90) and healthy controls (Figure 3). With respect to the relationship between other confounding variables such as age, sex, smoking, alcohol consumption and duration of spraying of pesticides and serum 8-OHdG levels, the regression analysis performed showed no significant associations. Higher levels of 8-OHdG were observed among the farm workers diagnosed with leukaemia having null polymorphism in the GSTT1 gene. Further, the farm workers diagnosed with leukaemia and breast cancers showed higher levels of 8-OHdG with null polymorphism in the *GSTM1* gene. There found a significant difference ($p<0.05$) between the levels of serum 8-OHdG and the prevalence of genetic polymorphism in CYP2E1 among the farm workers and non-farm workers diagnosed with leukaemia. It was observed that the farm women diagnosed with breast cancer showed higher mean levels of 8-OHdG with null polymorphism in the *CYP2E1* gene. Moreover, there also found a significant difference between the subjects diagnosed with cancers and the healthy controls with regard to the prevalence of polymorphism in all the three genes and serum 8-OHdG levels (Table 4).

Discussion

Occupational exposure to pesticides is common in developing countries because farm workers are often under-trained and illiterate. The improper use of pesticides followed by operational procedures, their frequent application above the recommended dose, and the amount of time spent in applying the pesticides leave plausible amount of their residues not only on crops but also in the environment, leading to adverse effects on the environment and human health (Ali et al., 2017). There is currently a knowledge gap in the field of occupational health and safety among farm workers, as researchers tend to lack vital information on the collective factors affecting the individual's susceptibility to the risk and the underlying mechanism behind malignancy.

The nullity of GSTT1/GSTM1 may increase the susceptibility of agricultural workers to adverse health effects including cancer due to lack of efficient detoxification mechanism of pesticides. Further, oxidative stress induced due to exposure and GSTT1/GSTM1 null polymorphism may in turn lead to mRNA dysregulation resulting in the development of cancer (Usman et al., 2018). In the present study, it was found that the farm workers showed 40% and 38% null genotype in GSTT1 and GSTM1 genes respectively. The distribution of genotypes for GSTT1 and GSTM1 genes in the present study was in line with the findings reported earlier among agricultural workers occupationally exposed to pesticides (Singh et al., 2011; Ahluwalia and Kaur, 2018). Earlier studies have also shown similar significant difference in the prevalence of CYP2E1 polymorphism between farm workers cultivating soybean and the non-farmers (Godoy et al., 2019).

A systematic review reported that the exposure to pesticides at household level showed significant association between *GSTM1* polymorphisms and childhood leukaemia (Brisson et al., 2015). However, in the present study, no such association was observed among farm workers diagnosed with leukaemia. Further, in the present study, no significant difference was also found between the frequencies of *GSTT1, GSTM1* and *CYP2E1* null genotypes among the farm workers and non-farmers diagnosed with leukaemia. In contrast, null polymorphism in *GSTT1* and *GSTM1* genes has been linked with an increased risk for different types of leukaemia among the diverse group of populations across the regions in India (Joseph et al., 2004). Similarly, other case-control studies have also suggested the contribution of CYP2E1 null genotype towards the genetic susceptibility to acute leukaemia (Aydin Sayitoglu et al., 2006).

Agricultural occupational risk has also been associated with a higher incidence rate of lymphoma in multiple studies (Merhi et al., 2007: Leon et al., 2019). In the present study, farm workers with GSTT1 polymorphism exhibited a five-fold risk for lymphoma as compared to non-farm workers. The findings from the present study were similar to the results from Human Genome Epidemiology (HuGE) review that showed association between GSTT1 null genotype and increased risk for lymphoma (Bin and Luo, 2013). Further, there also found no significant association between GSTM1 and CYP2E1 null polymorphism and risk for lymphoma among farm workers in the present study.

Epidemiological studies conducted in India had suggested that exposure to organochlorine pesticides could be a contributory factor for an increased incidence of breast cancer among younger women (Kaur et al.,

2019). In the present study, the GSTM1 gene deletion was associated with a higher risk for breast cancer than GSTT1 among the farm workers. Similar results in the non-farming population of Mizoram, India suggested significant association of deletion of GSTT1 and M1 genes with breast cancer susceptibility (Kimi et al., 2016). Further, in the present study, CYP2E1 polymorphism among the female farm workers was not associated with risk for breast cancer. Similar to our findings, another study reported the non-association between the CYP2E1 Rsa1 genotypes and breast cancer risk (Choi et al., 2003).

Similar to the present study findings, the higher levels of serum 8-OHdG in the farm workers as compared to the non-farm workers was also observed in several other studies in which elevated levels of 8-OHdG was detected in plasma and urine of the farm workers engaged in pesticide spraying activities (Koureas et al., 2014; Mishra et al., 2015). It is further interesting to note that no significant association was found between the serum 8-OHdG levels and other confounding factors such as age, duration of exposure to pesticides, personal habits such as smoking, consumption of alcohol and tobacco. These findings were in contrast to other studies conducted elsewhere (Chen et al., 2007; Yano et al., 2009; Zanolin et al., 2015; Gan et al., 2018). In the present study, it was found that sex did not influence the serum 8-OHdG levels which were in line with other study findings (Andreoli et al., 2011).

Very few studies have explored the genetic polymorphisms of xenobiotic metabolizing enzymes and their association with DNA damage among the agricultural workers. To the best of our knowledge, there are hardly any studies of this kind to serve as a reference. In the present study, it is important to note the significant difference ($p<0.05$) between the levels of 8-OHdG and *CYP2E1* polymorphism among the farm workers diagnosed with leukaemia. However, no such significant differences were observed between 8-OHdG levels and all the three genes among the farm workers diagnosed with lymphoma and breast cancer. However, results from other study findings had suggested that the null deletion of *GSTM1* and *GSTT1* genotype can also modulate DNA damage through gene-environmental interactions among farm workers (da Silva et al., 2008; Singh et al., 2011).

Studies of individual susceptibility to toxicants and gene-environment interactions are now emerging as an important component of molecular epidemiology. The determination of polymorphisms is becoming an increasingly important aspect that may help in identifying the sensitive subgroups (da Silva et al., 2008). The possible limitations of the present study in establishing any clear cause and effect relationship are the smaller sample size and shorter duration of exposure to pesticides. In addition, by providing only p values, the study did not allow assessing the magnitude of the effect. Hence, the current study has the potential to serve as a reference point for future research that can consider more precise, selective, and reliable methods for determining the individual CYP and GSTM1/T1 enzyme activities in order to associate the cause-and-effect relationship.

In conclusion, the findings of the present study suggest that the polymorphisms in detoxifying genes among farm workers occupationally exposed to pesticides and the oxidative stress may likely be responsible for triggering the mechanism of malignancy among the subjects visiting one of the tertiary cancer care hospitals in Hyderabad, Telangana State, India. However, the results of the present study can be utilized to conduct longitudinal studies based on larger sample size to identify the SNPs in xenobiotic metabolizing genes to establish an association with increased oxidative stress and risk of leukaemia, lymphoma and breast cancers among the occupationally exposed population using advanced molecular techniques.

List of abbreviations

8-OHdG – 8-hydroxy-2'-deoxyguanosine
CYP – Cytochrome P
DNA – Deoxyribonucleic Acid
dNTP – Deoxynucleoside triphosphate
EDTA – Ethylene Diaamine Tetraacetic Acid
EFSA – European Food Safety Authority
ELISA – Enzyme Linked Immuno Sorbent Assay
EtBr – Ethidium Bromide
GSTM1 – Glutathione S-transferase μ1
GSTT1 – Glutathione S-transferase θ1
HuGE – Human Genome Epidemiology
MNJ – Mehdi Nawab Jung
NCI – National Cancer Institute
PCR – Polymerase Chain Reaction
RFLP – Restriction Fragment Length Polymorphism
ROS – Reactive Oxygen Species
SNP – Single Nucleotide Polymorphism

Author Contribution Statement

All authors contributed equally in this study.

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