Chapter 5

Conclusions

The harmful effects of the pesticides on human health have been documented in both national and international literature. The pesticide sprayers, farmers and farm labourers are exposed to a variety of pesticides or their combinations. The health issues related to chronic pesticide exposure include eye irritation, pulmonary, neurologic and kidney problems, cancers, mutagenesis, fetotoxic and teratogenic effects, immunological changes and effects on fertility (Antle and Pingali, 1994). The epidemiological studies suggest that exposure to the pesticides is a plausible environmental risk factor for Parkinson’s disease (PD) (Mandel et al., 2000). A study by Baldereschi et al. (2003) demonstrated that such exposures increased the risk of PD 1.5 - 7 fold.

Several enzymes, including glutathione-s-transferases (GSTs), cytochrome P-450 (CYP) family, esterases, flavin mono-oxygenase (FMO) and paraoxonases (PONs) are typically engaged in the initial metabolism of the pesticides i.e. activating or inactivating them (Ecobichon and Joy, 1994). Studies have demonstrated that pesticide exposures along with single nucleotide polymorphisms (SNPs) in genes encoding for these enzymes, lead to increased risk of PD (Patel et al., 2005). GST gene family encodes genes that are critical for certain life processes as well as for detoxification and toxification mechanisms via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceutical and environmental pollutants (Nebert and Vasiliou, 2004). A CYP family gene CYP2D6 is a toxin metabolizing regulatory gene involved in the detoxification of environmental chemicals and toxicants (Hodgson and Levi, 1996).
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Strong association between polymorphism of this gene due to the pesticide exposure and PD has been reported (Ecobichon and Joy, 1994).

Punjab is an agriculture dominating North West Indian state and the pesticides have become integral part of its village life. The agriculture workers of the state are therefore highly exposed to several types of pesticides of different chemical families (Arbuckle et al., 2001). Mathur et al. (2005) reported pesticide residues in the blood samples of the villagers of Punjab. Since strong relationship between the pesticide exposure and PD has been documented in the world populations and as agriculture workers of Punjab are widely affected by direct pesticide exposure, the present study was planned to investigate the distribution of various pesticide metabolizing and detoxification gene polymorphisms viz., rs1045642 (MDR1), rs3892097 (CYP2D6*4), rs16947 (CYP2D6*2), rs1695 (GSTP1), GSTM1 and GSTT1 in the rural population of the state.

In the present study, a total of 300 subjects, comprising 164 sprayers and 136 non-sprayers, inhabiting different villages of Punjab were enrolled. Along with the professional sprayers, a large number of farmers were also found to be engaged in pesticide spraying activity. The present demographic data showed that most of the sprayers were in the age group of 36 - 40 yr while most of the non-sprayers were in the age group of 21 - 25 yr. As for the life style habits of the subjects, such as healthy diet, alcohol consumption, smoking and tobacco chewing, statistically significant differences were found in healthy diet and alcohol consumption behaviours between the sprayers and non-sprayers as the non-sprayers were eating a healthy diet and the sprayers were invariably found to be consuming alcohol.
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Most of the sprayers were found to be spraying on the wheat and rice, the two major crops cultivated by the farmers of Punjab. Both the insecticides and fungicides were found to be the widely used pesticides in the Punjab. Most of the sprayers enrolled in the present study had pesticide exposure of 1 - 10 yr and almost two thirds of the sprayers were spraying 1 - 30 l of pesticide in a year. Highest amount of the pesticide was found to be sprayed on the cotton crop, followed by vegetables as these crops are mostly under the attack of the pests.

As for the genetic analyses, barring CYP2D6*4, the remaining three SNPs studied were found to be in Hardy Weinberg Equilibrium indicating that the present rural population is in genetic equilibrium for these markers. No statistically significant difference was found between the genotypes of the sprayers and non-sprayers by the Chi square test. The mutant allele frequencies of the four studied SNPs and null genotype percentages of the studied gene polymorphisms were found to be in the ranges of the reported allele frequencies/genotype percentages of North India. The linkage disequilibrium (LD) found between the two SNPs viz., rs16947 (CYP2D6*2) and rs3892097 (CYP2D6*4) may be attributed to their close proximity on CYP2D6 gene.

In the world populations there are several gene polymorphism studies documenting the role of the pesticide exposure in the risk of PD. Dutheil et al. (2010) found a statistically significant association between the mutant allele T of rs1045642 (MDRI) polymorphism and organochlorine pesticide exposure in the risk of PD. Similarly, a study by Zschiedrich et al. (2009) documented a statistically significant association between the pesticide exposure and the T allele. Drożdżik et al. (2003) reported that the carriers (i.e. homozygous and heterozygous) carrying at least one copy of T allele of rs1045642
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ds to the pesticides. Elbaz et al. (2004) first provided evidence that $CYP2D6^{*4}$ mutant homozygote AA interacts with pesticide exposure in the risk of developing PD. Similarly, Deng et al. (2004) also found that the poor metabolizers of $CYP2D6^{*4}$ (genotype AA) were at increased risk of PD in highly pesticide exposed subjects. The risk of PD by joint effects of $GST$ polymorphisms and pesticide exposure was first investigated by Menegon et al. (1998) in Australian population. This was followed by a study by Wilk et al. (2006) on SNPs of $GSTP1$ gene, including rs1695 polymorphism, revealing a joint effect of $GSTP1$ with pesticide exposure in the age of PD onset.

Thus, there is scientific evidence for the possibility of role of pesticide exposure in risk of developing PD by interacting with polymorphisms in pesticide detoxification and metabolising genes. To investigate such a possibility in the sprayer and non-sprayer rural inhabitants of Punjab, DNA polymorphisms of $MDR1$, $CYP2D6$ and $GST$s genes were studied. Although in the present study no sprayer was found to have Parkinson’s disease (PD), a non-sprayer subject was found to have the disease. The female subject who showed the symptoms of PD was occupationally labourer who had worked in farms where she might have been exposed to the pesticides. The other environmental or genetic factors might also have played role in developing the PD in her. The absence of any PD subject in the sprayer group in this study may be attributed to a low prevalence of PD in India.

The present study, nonetheless, has provided baseline data on four SNPs viz., rs1045642 ($MDR1$), rs3892097 ($CYP2D6^{*4}$), rs16947 ($CYP2D6^{*2}$) and rs1695 ($GSTP1$)
and two gene polymorphisms viz., \textit{GSTM1} and \textit{GSTT1} in people of Punjab and filled the void on the genetic map of India.

Further studies based on large sample sizes in the agriculture dominating regions of North India such as the state of Punjab and Haryana and other such states of India are desirable to fully appreciate the role of the pesticide metabolizing and detoxification genes and pesticide exposure in the development of PD under Indian conditions.
Summary

Introduction

The pesticides are widely available agrochemicals which are simple to apply and enhance the crop production and safe storage of the agriculture produce including the grains, thereby providing efficient economic returns to the farmer (Mathur et al., 2005). The pesticides comprise insecticides, herbicides and fungicides and are primarily designed to be toxic and poisonous to kill the pests of various types of crops. Because of their toxic properties they may also be harmful to the humans and animals. Nonetheless, they are popular among the agriculture workers including farmers. They may be exposed to the agrochemicals through the direct or indirect routes; the direct exposure occurs to individuals who spray the pesticides in farms and orchards etc. while the indirect exposure occurs through the intake of water, air, dust and food contaminated with the pesticides (Alavanja et al., 2004).

Health risks have been found to be significantly associated with those who are environmentally or occupationally exposed to pesticides (Lebailby et al., 1998). The health problems associated with the pesticide exposure include both acute and chronic effects. These include eye irritation, pulmonary, neurologic and kidney problems, cancers, mutagenesis, fetotoxic and teratogenic effects, immunological changes and effects on fertility (Antle and Pingali, 1994).

An epidemiological study by Fleming et al. (1994) showed relationship between exposure to organochlorine pesticide such as dieldrin, a mitochondrial poison, and Parkinsonism. A study by Baldereschi et al. (2003) demonstrated that such exposures
increased the risk of PD 1.5 - 7 fold than the unexposed population. Thus, the epidemiological data suggest that exposure to the pesticides is a plausible environmental risk factor for PD (Mandel et al., 2000). In another study the residual levels of the pesticide were diagnosed in the brain tissue samples of one third of Parkinson’s disease (PD) patients compared with the controls (Sanchez-Ramos et al., 1998).

Several pesticide detoxification and metabolizing genes such as GSTs, CYPs, MDR1 and PONs, among others, and their enzymes including glutathione-s-transferases (GSTs), cytochrome P-450 family (CYP), esterases, flavin mono-oxygenase (FMO) and paraoxonases (PONs), among others, are typically engaged in the initial metabolism of the pesticides i.e. activating or inactivating them (Ecobichon and Joy, 1994).

Punjab is an agriculture dominating North West Indian state and the pesticides have become an integral part of its village life. The agriculture workers of the state are highly exposed to several types of pesticides of different chemical families (Arbuckle et al., 2001). The farm workers suffer chronic health effects such as neurotoxicity and PD due to prolonged exposure to the pesticides (Antle and Pingali, 1994). Mathur et al. (2005) reported pesticide residues in the blood samples of the villagers of Punjab.

The relationship between the polymorphisms in the pesticide metabolizing and detoxification genes and PD has been documented in the various world populations exposed to the pesticides. As agriculture workers including the pesticide sprayers of Punjab are widely affected by direct pesticide exposure, the present study was conducted to investigate the distribution of such gene polymorphisms including rs1045642 (MDR1), rs3892097 (CYP2D6*4), rs16947 (CYP2D6*2), rs1695 (GSTP1), GSTM1 and GSTT1 in the rural population of the state.
Aims and Objectives

The present study aimed, first, to investigate the effect of direct pesticide exposure in combination with selected DNA polymorphisms of the pesticide detoxification and metabolism genes viz., *MDR1*, *CYP2D6* and *GSTs* in the rural population of Punjab and to validate association, if any, of the present polymorphisms with PD. Second, it aimed to provide the baseline data on the investigated DNA markers in the rural population of Punjab.

Subjects, Materials and Methods

A total of 300 adult subjects of various districts of Punjab were included in the present study after written informed consent. The subjects were categorized into two groups viz., the sprayers (n = 164) and non-sprayers (n = 136).

From each subject, the general information was collected using a general information schedule regarding the pesticide exposure, including types of chemicals used, number of times the pesticide was applied in a crop season and the duration of the pesticide usage in year (yr) along with the presence or absence of PD. For genetic analysis about 5 ml intravenous blood samples was collected from the subjects with the help of a trained technician. For conducting the present study prior permission was obtained from the Institutional Ethics Committee (IEC) of Punjabi University, Patiala.

The DNA from the collected blood samples were extracted by using the salting out method of Miller et al. (1988) with some modifications. Genotyping for the four SNPs viz., rs1045642 (*MDR1*), rs3892097 (*CYP2D6*4), rs16947 (*CYP2D6*2) and rs1695
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(*GSTP1*) was done using PCR-RFLP and that of the two gene polymorphisms viz., *GSTM1* and *GSTT1* was done using multiplex PCR.

The frequencies of the various demographic variables and genotypes were obtained and t test was performed using PSPP statistical software (Stover, 2010). The genetic equilibrium of the studied markers was tested using Hardy-Weinberg equilibrium. The allele frequencies were calculated using the gene counting method. Linkage disequilibrium was tested using Haploview software of Broad Institute of MIT and Harvard (Barrett et al., 2005).

**Results and Discussion**

In the present study more non-sprayers were found to consume healthy diet than the sprayers and the trend was reverse for the alcohol consumption, and the comparison using the t test revealed statistically significant differences between them for these two lifestyle habits of these two groups of rural population of Punjab.

Barring rs3892097 (*CYP2D6*<sup>4</sup>) SNP, the three remaining SNPs were found to be in Hardy Weinberg Equilibrium in the studied groups. No statistically significant difference was found in the genotypes of the sprayers and non-sprayers in Chi square test, suggesting homogeneous distribution of the studied markers. The mutant allele frequencies of the four SNPs and null genotype percentages of the two gene polymorphisms were found to be in the range of reported frequencies/genotypes of the North Indian region populations. The linkage disequilibrium (LD) found between the two SNPs viz., rs16947 (*CYP2D6*<sup>2</sup>) and rs3892097 (*CYP2D6*<sup>4</sup>) may be attributed to their close proximity on *CYP2D6* gene.
Conclusion

There is scientific evidence for the possibility of role of the pesticide exposure in risk of developing Parkinson’s disease (PD) by interacting with polymorphisms in the pesticide detoxification and metabolising genes. To investigate such a possibility in the sprayer and non-sprayer rural inhabitants of Punjab, DNA polymorphisms of three pesticide detoxification and metabolism genes viz., \textit{MDR1}, \textit{CYP2D6} and \textit{GSTs} were studied.

However, in the present study no sprayer was found to have PD, but surprisingly a female non-sprayer subject showed its presence. The absence of any PD subject in the sprayers of the present study might be attributed to a low prevalence of PD in India. The global prevalence of PD ranges from 60 to 261 cases per 100,000 while in North India the incidence reported was 14.1 per 100,000, suggesting a comparative low occurrence of the disease in people of India. As for the second aim of the present study, it has provided baseline data on four SNPs viz., rs1045642 (\textit{MDR1}), rs3892097 (\textit{CYP2D6}*4), rs16947 (\textit{CYP2D6}*2), and rs1695 (\textit{GSTP1}) and two gene polymorphisms viz., \textit{GSTM1} and \textit{GSTT1} in people of Punjab and filled the void on the genetic map of India.

The results of this study are based on data obtained from the state of Punjab. Further studies utilizing large sample sizes in the agriculture dominating regions of India such as Haryana, Western Uttar Pradesh and other states of North India, including Punjab, are required to fully appreciate the interaction of the pesticide exposure, pesticide metabolizing and detoxification genes and life style habits in the development of PD under the rural Indian environmental conditions.