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 \textit{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.
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. Drozdzik et al. (2003) reported that the carriers (i.e. homozygous and heterozygous) carrying at least one copy of T allele of rs1045642
(MDR1) polymorphism were at a significant five-fold increased risk of PD when exposed 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 GSTs 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 occupational 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 rs16947
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(GSTP1) and two gene polymorphisms viz., GSTM1 and 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.