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Beta hexachlorocyclohexane (β-HCH) and Risk of Alzheimer's Disease and Parkinson's Disease

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Abstract. Background: Pesticides have been considered as likely environmental risk factors to play a major role in neurodegenerative disease pathogenesis either through interaction with genes or direct effects. With this background we have tried to find out the association of organochlorine pesticides with Alzheimer's & Parkinson's disease. Methods: Total of 180 subjects (70-Alzheimer's disease (AD), 60- Parkinson's disease (PD) & 50-healthy patients), attending out patients department in tertiary care hospital were screened. Their blood samples were analysed for different organochlorine pesticides (α-HCH, β-HCH, γ-HCH, aldrin, dieldrin, α-endosulfan, β-endosulfan, *pp'*-DDE, p,p'-*pp'*-DDT and *op'* DDT)levels by gas chromatography. Results: Mann Whitney U test revealed significant difference of β-HCH levels between the control and Alzheimer's disease (U = 845, P = 0.000). Also significant β-HCH levels were seen in Parkinson's disease group as compared to control (U = 823, P = 0.00). β-HCH levels increases risk of AD six times [C.I=95%,O.R=6 (2.58-13.9)] & PD five times [C.I=95%,O.R= 4.9 (2.07- 11.54)] ($p<0.001$) respectively. Conclusion: This study clearly indicates that increased level of β-HCH in blood are associated with the presence of AD or PD.

Keywords: Alzheimer's disease, Parkinson's disease, Organochlorine pesticides

1. Introduction

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most prevalent disorders that account for the global burden of neurodegenerative diseases. There are number of genetic and environmental factors which are responsible for both AD and PD. As most of the cases are sporadic, environmental factors definitely play a major role in disease pathogenesis either through interaction with genes or direct effects.

Pesticides are one such environmental factor which is used extensively throughout the world including India. There are studies which suggest the role of organochlorine pesticides (OCPs) in AD and PD [1-3]. Two of them have reported increased level of dieldrin and γ- hexachlorocyclohexane (γ- HCH) in brain tissue of PD as compared to control [3-4]. Although there are few studies on relationship between pesticides and AD, a study on post-mortem brain samples by Fleming and co-workers found p,p'-dichlorodiphenyldichloroethylene (pp'-DDE) (a long-lasting residue of dichlorodiphenyltrichloroethane (DDT)) more significantly in AD than the PD cases [3]. They also reported significant relationship between dieldrin and PD [3]. Also, work by Richardson and group revealed increased level of β-HCH in the blood of PD patients as compared to AD and control. They also found higher level of *pp'*-DDE in AD patients as compared to PD and controls [5].

Dichlorodiphenyltrichloroethane (DDT) and HCH have been banned in India for use in agriculture since 1998 and 1989, respectively, but are still used for control of vectors in public health [6]. However, OCPs not only tend to accumulate in adipose tissue but also biomagnify through food chain due to their lipophilic nature and long half-lives [7]. Significant levels of many OCPs have been found in human body tissues including blood, thus making blood a good medium to study its levels [8,9]. OCPs can induce endocrine

dysfunctions, immunological changes, oxidative stress and DNA damage [10, 11]. There are evidences that long term exposure to pesticides may have toxic effects on CNS [12, 13] as many pesticides exert their killing effects through neurotoxic mechanisms.

Many epidemiological studies have strengthened the association between OCPs and AD and PD. Study on blood levels of pesticides have also supported the role of pesticides in AD and PD. Although a few studies have reported the association of pesticides with presence of PD, data for AD is lacking. We have tried to identify the association between specific OCPs in blood and diagnosis of AD and PD patients in north Indian population with adequate sample size and it does not encompass the duration of exposure of pesticides.

2. Material and Methods

2.1. Selection Criteria

70 AD patients, 60 PD patients and 50 controls attending the out patients department of Institute of Human Behaviour and Allied Sciences (IHBAS), Delhi, from February 2010 to January 2011were included in the study after written informed consent. The subjects were 50 to 85 years old. Patients with diagnosis of AD defined by the NINCDS-ADRDA [14], and diffuse cerebral atrophy on MRI/CT/PET brain scan were included in AD group. Additional inclusion criteria were a score of zero to 23 on the Mini-Mental State Examination (MMSE), a Clinical Dementia Rating (CDR) score of > 0.5 and Global Deterioration Scale (GDS) of > 4.

Similarly, patients with diagnosis of PD defined by United Kingdom Parkinson's Disease Society Brain Bank Criteria [15] constituted PD group. Additional inclusion criteria for PD group were a score of > 23 on MMSE, a CDR score of < 0.5, and GDS of < 2. Control group comprised age-matched healthy volunteers who attended the hospital OPD for routine health checkup. Exclusion criteria in both case and control groups included: i) lack of consent to participate in the study, ii) history of cerebral stroke, epilepsy, head trauma, and other concomitant disease potentially associated with dementia, iii) chronic intake of drugs affecting cognitive processes, iv) moderate to severe depressive episode and v) familial history of any kind of cognitive/behavioral abnormality. Patients with nutritional deficiency, metabolic abnormalities and central nervous system infections were also excluded. The study was approved by the Institutional ethical committee.

2.2. Sample Collection and Estimation of OCP Levels

Blood sample was drawn using standard venepuncture techniques and stored at -80^0C until analysis. The OCPs that were assayed were α - HCH, β - HCH, ɣ - HCH, aldrin, dieldrin, α-endosulfan, β-endosulfan, pp'-DDE, and DDT. The compounds selected for assay were based on previous findings [16] that those were the pesticides most commonly found in human samples and based on the compounds that the Environmental Protection Agency used to assay organochlorine pesticides in biological samples. Samples were analysed on Perkin Elmer Gas Chromatograph equipped with 63Ni electron capture detector under standard operating procedure. [17]

Quantitative analysis of organochlorine residues in each sample were analysed by comparing the peak area with those obtained from a chromatogram of a mixed OCPs standard (Supelco, Sigma-Aldrich) of known concentration. The detection limit of the detector was <0.05 pg per chloroethylene with nitrogen as a carrier gas. The detection limit of the method was 4pg/ml for each OCP. For quality control process, five blood samples in triplicate were spiked with a mixed standard of OCPs at 5 and 25ng/ml. The average recoveries of prepared samples exceeded 95 %. The case and control samples were run in the same analytical batches and further, a quality check sample was run always with each set of samples for pesticide analysis to maintain accuracy.

2.3. Statistical Analysis

Chi square test were used for analysis of categorical variables, including sex and pesticide detection with presence of AD & PD. The distribution of independent variables i.e. β-HCH, Aldrin and pp'DDE levels were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Non parametric Kruskal wallis tests were applied to compare β-HCH levels with presence of Parkinson's disease and Alzheimer's disease. Mann Whitney tests were applied for post hoc comparisons. Similarly unadjusted odds ratios with 95% confidence intervals were estimated with β-HCH as predictor variables for Alzheimer's disease and Parkinson's disease status. The data was analysed using SPSS version 17 ® on windows 7® platform.

3. Results

Results in Table 1 demonstrating the demographic characteristics of the participants reveal no significant difference between the three groups viz. patients with AD, patients with PD and control.

Distribution of organochlorine pesticide levels were seen among three groups (Table -2), showing that β-HCH levels were detected above 50% in study subjects (AD & PD).

A Kruskal Wallis test revealed a statistically significant difference in β-HCH levels across the three groups (group 1, n = 70: patients with AD, group 2, n = 60: patients with PD, group 3, n = 50: Controls), X^2 (3, n = 180) = 31.353, p = 0.00. Patients with AD had a higher mean rank of 107, than the other two groups; patients with PD recorded mean rank of 97.23, whereas control group recorded mean rank of 58.86. (Table-3)

Post hoc test done by Mann Whitney U test revealed significant difference between the control and AD group's median β-HCH concentration (U = 845, P = 0.000), higher being in AD group. Also, it was significantly more in PD group as compared to control (U = 823, P = 0.00). However, difference was not significant between AD group and PD group (U = 1826, P = 0.184)

The diagnosis of AD was significantly more when β-HCH was present in blood (80%) than when it was absent (41%), $\chi^2(1.\ N = 120) = 19.95$, $_p = .000$, odds ratio =6, $CI_{.95}$ = 2.585, 13.925. Also, presence of PD was more likely to be found when β-HCH was present in blood (76%) than when it was absent (40%) $\chi^2(1.\ N = 110) = 14.5$, $_p = .000$, odds ratio =4.9, $CI_{.95}$ = 2.070, 11.548).

4. Discussion

This is the first study in north Indian population which shows the association of β-HCH with presence of PD and AD. The results are in accordance with other studies reported erstwhile, on relationship between pesticides and incidence of PD and AD [3-5]. Richardson and colleagues have found a positive relationship between serum levels of β-HCH and diagnosis of PD [5]. Pesticide exposure is also related to neurobehavioral deterioration like cognitive decline, dementia etc. Few studies have shown a direct relationship between pesticide exposure and AD [3-5].

According to results, we found six times more chances to having risk of AD and in similar fashion risk of PD increases five times when β-HCH is present in blood.

The presence of detectable β-HCH levels in control group only support the fact that other factors, like cigarette smoking or genetic polymorphism, may interact with β-HCH exposure to increase the risk development of AD or PD. Cigarette smoking is associated with decreased incidence of PD as nicotine has a neuroprotective effect on dopaminergic neurons in nigrostriatal tract [18]. The positive relation between pesticide exposure and PD has been reported to be independent of cigarette smoking [19]. There may be a possible role of enzymes that regulate the xenobiotic transport and metabolism [20, 21]. Cytochrome P450 2D6 (CYP2D6) is one such enzyme involved in the metabolism of environmental toxins including OCPs. The activity of CYP2D6 is genetically determined. Poor metabolizers have undetectable CYP2D6 activity [22]. TERRE study in France has revealed a stronger relationship between pesticide exposure and PD among poor metabolizers [23]. Data from TERRE study also found that PD patients have low P-glycoprotein function which is involved in transport of OCPs from brain side to blood side of the blood-brain barrier, thus increasing the brain concentration of OCPs in PD patients [24].

We also found a significantly high level of dieldrin and ppDDE in serum of AD and PD patients as compared to controls. Study by Weisskopf et al revealed an increased risk of PD with exposure to dieldrin [25]. Dieldrin contributes to neurotoxicity through mitochondrial apoptosis by inducing oxidative stress and GSH depletion [26,27]. Dieldrin is in particular toxic to dopaminergic neurons as it causes aggregation and fibrillation of α-synuclein and intracellular depletion of dopamine by inducing dopamine release [28,29].*pp′*-DDE is found more persistent than DDT. Fleming and co-workers found that DDT and its metabolites were more likely to be found in brain tissue of AD than PD patients or of control subjects [3]. Also, Jason et al. [5] reported significant elevated levels of *pp′*-DDE in patients with AD vs. patients with PD and controls. *pp′*-DDE induce neural death by apoptosis through the activation of mitogen activated protein kinases (MAPKAs) [30].

It is evident that neurodegenerative diseases have common pathways leading to disease state; AD and PD also share common molecular pathways like neuronal loss by apoptosis, oxidative damage and disruption of synapse integrity [31]. But why some people develop AD and some PD may be explained by individual

genetic susceptibility. Thus it may be said that conglomeration of environmental and genetic factors decides the disease development in susceptible individuals.

In conclusion the results obtained from this study clearly indicate that increased levels of β-HCH in blood are positively associated with the development of AD or PD. Our results support the epidemiology based studies that associate exposure to pesticides with increased risk of AD and PD. Small sample size and non-observance of the effect of genetic polymorphism were the limitations of our study.

5. Acknowledgement

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Table 1: Demographic characteristics of AD, PD and Controls

Variables	**AD (n = 70)**	**PD (n = 60)**	**Controls (n = 50)**
Age (Years), Mean ± SD	64.2 ± 9.05	58.48 ± 8.75	59.32 ± 8.81
Sex (M/F), No.	39/31	30/30	29/21

Table 2: Distribution and comparison of OCPs levels in the AD, PD and Controls

OCPs	**AD (n= 70)**		**PD (n=60)**		**Control (n=50)**	
	Detected in Patients (%)	Detectable range (ng/ml)	Detected in Patients (%)	Detectable range (ng/ml)	Detected in Patients (%)	Detectable range (ng/ml)
β- HCH	60.0	2.12 – 24.13	55.0	1.49 – 23.6	20.0	0.67 – 2.75
Dieldrin	50.0	1.20– 25.52	66.33	2.1 – 28.63	12.0	0.96 – 4.02
pp'-DDE	41.43	2.04 – 26.63	43.33	1.4 – 12.5	24.0	0.67 – 10.68
α- HCH	8.57	1.70 – 6.10	23.33	1.3 – 12.6	4.0	1.3 – 1.45
γ- HCH	10.0	1.25 – 10.2	13.33	0.9 – 4.8	6.0	0.42 – 2.69
Aldrin	8.57	1.2 – 4.2	13.33	1.4 – 6.8	4.0	1.23 – 2.19
α-endosulfan	10.0	1.4 – 4.0	10.0	0.91 – 4.2	8.0	0.62 – 1.99
β-endosulfan	4.28	1.49 – 2.82	6.66	1.6 – 4.2	4.0	0.12 – 1.7
DDT	10.0	1.9 – 10.4	16.66	1.2 – 10.0	6.0	0.99 – 3.8

Table 3: Comparison of β- HCH among AD, PD and Controls

	MEAN ±S.E.M		
	Alzheimer's disease (AD) * (n=70)	Parkinson's Disease (PD) # (n=60)	Control Group † (n=50)
β- HCH (ng/ml)	4.16±1.4	3.32±0.31	0.29±0.30

* † Highly significant (AD v/s Control) ($p<0.001$)
# † Highly significant (PD v/s Control) ($p<0.001$)
* # NS (AD v/s PD)