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Research Article

Study on Effect of Environmental and Occupational Pesticide Exposure on Marker Enzymes of Malignancies in Cancer Patients

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Abstract

Cancer is one of the top leading causes of death and increasing globally on daily basis. Environmental exposure to pesticides causes disposition to various types of cancer in humans. The prime objective of the current research was to analyze the synergistic effect of pesticides in carcinogenesis through measuring the oxidative stress marker enzymes in serum/plasma of the cancer patients as compared to the control sample. We have found a significant decrease in the level of monooxygenase, Glutathione S-transferase, acetylcholine esterase and, total protein along with a significant increase in β-glucuronidase activity in serum sample of cancer patients as compared to control. However we have not found synergistic role of pesticide in carcinogenesis. To validate the above results further studies on large sample size are required.

Keywords: Pesticides, Mono-oxygenase, Glutathione S-transferase, Acetylcholine esterase, β-Glucuronidase

INTRODUCTION

Cancer is one of the top growing public health burdens leading to a substantial increase in the number of cases that are unexplained by known risk factors. Available literature suggests pesticide exposure as a potential environmental factor related to different types of cancers. Residential use and proximity to the agricultural area is a non-occupational pesticide exposure route (Deziel NC et al., 2015). Mono-Oxygense mediated detoxification activates pesticides which involved in several biochemical changes and induce carcinogenesis in exposed individuals (Jin X et al., 2014). Pesticide induced reactive oxygen species (ROS) damages biomolecules and may initiate the process of carcinogenesis depending on the patient's age and antioxidant defense systems (Prasad S et al., 2017) (Silva JF et al., 2016). Pesticides undergo bio activation after conjugation to glutathione by glutathione **S**-transferases (**GST**s) that are responsible for the metabolism and detoxification of xenobiotic (Xie X et al., 2020). Glutathione (GSH) plays an important role in a multitude of cellular processes involved in the etiology, progression of cancer and the drug resistance of tumor cells (Hadem KL et al., 2014). Acetylcholine esterase (ACHE), a potential marker of organophosphate pesticides poisoning degrades neurotransmitter acetylcholine to acetyl and choline and acts as a growth factor for the development of various types of cancers (Friedman JR et al., 2019). There is increasing evidence supporting the involvement of cholinesterase in tumor genesis, but their role in human cancers is not very well defined. The serum β-glucuronidase (heparanase) enzyme contributes to the growth, angiogenesis, and metastasis of cancerous cells

and significantly differentiates cancer patients from healthy individuals (Waszkiewicz N et al., 2015). In compared to the use and demand of pesticides, throughout the world the studies on its potential to cause carcinogenesis are still relatively scarce.

Articles suggested the involvement of pesticide exposure in promotion of various types of cancer but not able to explain the possible biochemical mechanism induced by them in cancer promotion. It is still not clear that, is the oxidative stress induced by pesticide exposure can directly initiate the carcinogenesis process or any other alternative mechanism is involved. Depth research is needed to conclude the possible mechanism of carcinogenesis induced by chronic exposure to pesticides. The prime objective of the current research was to analyze the synergistic effect of pesticides in carcinogenesis through oxidative stress measurement in cancer patients. Present study will help to perform a correlational study between pesticide exposure and cancer onset in exposed patients.

MATERIAL AND METHODS

Patient's recruitment

Biopsy-proven confirmed cancer cases, without chemotherapy were included in the present study. Our study group consisted of 168 subjects divided into 3 groups. Group I, consists of 51 randomly selected healthy volunteers with no history of previous disease, drug, or alcohol consumption served as control. Group II, consists of 61 histopathologically confirmed cancer patients (pesticides unexposed) and, group III consists of 56 histopathologically confirmed cancer patients (pesticides exposed) involved in agricultural activities. Patients were chosen from Hanuman Prasad Poddar Cancer Hospital and Research Centre, Gorakhpur, who visited the hospital during 8 January 2021 to 14 February 2021. The study was approved by the Ethics Committee of the hospital and before blood collection informed written consent was obtained from each patient. Socio-demographic characteristics, such as age, sex, height, weight and, diet pattern were also collected using a standard questionnaire.

The extent of pesticide exposure was calculated by using the equation mentioned below (No. of treatments per year) X (Total year of pesticide application) X (cultivation area (ha))

Cancer patients, who have not worked within the agriculture sector as their main or secondary occupation and lived in urban and suburban regions, were included in the unexposed category.

Blood collection

The venous blood was collected through the needle and was transferred into plain yellow and EDTA containing vial (red cap) immediately, and stored on the ice at 4°C. The plasma and serum were separated by centrifugation at 1,300 × g for 20 min at room temperature and were transferred to sterile test tubes and re-centrifuged at 3,000 × g for 15 min at room temperature to remove residual cellular components. The plasma and serum were transferred to cryo-tubes and stored at –20°C until the day of analysis.

Biochemical estimation of monooxygenases

The activity of monooxygenases was determined by following the method described by Vulule et al based on the total amount of heme content in the plasma (Vulule JM et al., 1999). A standard curve of purified cytochrome C was prepared in 0.625 M potassium phosphate buffer (pH 7.2) by adding varying concentrations (0.01, 0.02, 0.05 µg, and so on) of cytochrome C containing heme protein (Horse heart origin, Sigma Aldrich) in a fixed volume of tetramethylbenzidine solution. Reaction mixture comprised of 20µl of plasma, 200µl of 3,3, 5′,5′- Tetramethyl benzidine (TMBZ) solution [TMBZ solution was made by dissolving 0.01g TMBZ in 5ml ethanol and 15ml of 0.25M sodium acetate buffer (pH 5.0)], 100µl of 0.625M potassium phosphate buffer (PPB) at pH 7.0 and 30µl of 3% hydrogen peroxide. Reference solution contained 120µl TMBZ, 600µl of 0.625M potassium phosphate buffer (PPB) at pH 7.0, and 30µl of 3% hydrogen peroxide. For each sample, triplicate was used. The tubes were incubated in dark for 5 min and endpoint absorbance was measured at 650 nm. The quantities of monooxygenases were calculated from a standard curve of cytochrome C and were expressed as n moles equivalent of cytochrome P450/mg of protein.

Biochemical estimation of glutathione S-transferases (GST)

The activity of glutathione S-transferases towards 1-chloro-2, 4-dinitrobenzene (CDNB) was estimated according to the method of Habig (Habig WH et al., 1974). The total volume of the mixture was comprised of 40µl of 1.0mM reduced Glutathione, 20µl 1.0mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 200µl phosphate buffer (100mM, pH 7.0) and 20µl of serum sample. Reference solution for reaction mixture contained 20µl 1.0mM CDNB, 220µl phosphate buffers (100mM, pH 7.0) and 40µl 1.0mM reduced glutathione. The absorbance was measured at 340 nm after five minutes of the reaction. Absorbance values were converted to units of concentration using a molar extinction coefficient (ε) of 9.6mM cm-1 for CDNB-GSH conjugate. The enzyme activity was calculated as:

Unit/ml= (Delta OD_{340} standard/sample - Delta OD_{340} blank) X 3/0.1 X 9.6

Biochemical estimation of serum Acetyl Cholinesterase (ACHE)

The acetylcholine esterase activity in serum was measured by following the protocol of Ellman (Ellman GL et al., 1961). Acetylthiocholine was hydrolyzed by AChE to corresponding fatty acid and thiocholine. The rate of thiocholine formation

was monitored by continuous reaction of the thiol group with 5, 5'-dithiol-bis- (nitrobenzoic acid) to form a yellow anion that was measured spectrophotometrically at 410 nm. Enzyme activity was calculated by molar absorption coefficient of the product of the chemical reaction, 5-to-2-nitrobenzoate ($1.36 \times mmol^{-1} \times min^{-1} \times cm^{-1}$), and activity were expressed in µmoles/min/ml of serum sample.

Biochemical estimation of the β-glucuronidase (b-D glucuronide glucuronohydrolase)

β-Glucuronidase was determined by the method of Szasz, using substrates, p-nitrophenyl glucuronide (Szasz G et al., 1967). The system for the assay of β-glucuronidase consisted of 0.2 ml of acetate buffer (0.2M, pH 5.0), 1.0 ml of glycine (0.6M, pH 11.7), 0.1 ml serum, and 0.1 ml of p-nitrophenyl glucuronide (50 mM) as substrate with appropriate serum and reagent blanks. After incubation at 37^0C for 2 hours, absorbance at 546 nm was read in a spectrophotometer against water blank. The activity was expressed in milli units (mU=0.001 IU) using the equation.

mU = Ax-(Asb + Arb) X10^6/E X TV/SV X1/t

Where Ax = absorbance of unknown sample, Asb = serum blank absorbance, t = incubation time, TV = total volume, SV = sample volume and E = molar absorptivity. Molar absorptivity of p-nitrophenyl glucuronide is 18,500. The amount of p-nitro phenol liberated was calculated using the above formula.

Total Protein estimation

Total protein concentration in the blood samples was determined by Lowry's method using bovine serum albumin as a standard (Lowry OH et al., 1951).

Statistical analysis

Results were expressed as mean±S.E. of all the observations. Statistical analyses were performed using a one-way analysis of variance with post hoc Bonferroni's multiple comparison test applied across the control and cancer groups (SPSS statistical software, Version 11.5, Chicago, IL, USA). The correlation between different variables was calculated using Pearson's linear correlation coefficient. Statistical significance was set at a p-value ≤ 0.5.

RESULTS

Clinical characteristic of patients

In the present study the mean age of group I, II and group III was found to be 41.52±1.8, 48.97±1.99 and, 51.80±1.46 respectively and, the difference in mean age were statistically significant as compared to control. BMI of group I, II and group III was found to be 21.38±0.58, 21.41±0.53 and, 20.68±0.63 respectively. Each group has more female participants. In terms of diet, only 9-12% was vegetarian and 76-85% of the recruited control and cancer patients were non-vegetarian (Table 1).

Types of Cancer

Oral, gall bladder and cervix cancer constituted 62-75% in studied patients. In the patients of group II oral, gall bladder and cervix cancer constituted 24.59%, 21.31% and, 16.39% respectively while in group III, it was 26.79%, 25% and 23.21% respectively. Breast, blood, lung and other types of cancer range from 25-38% in cancer patients group (Table 2).

Activity of Mono-Oxygenase

The results of the present study clearly showed 39-55% statistically significant decrease in monooxygenase activity, in cancer patients of group II and, III as compared to control ($P<0.05$) whereas the correlation study of monooxygenase activity among the cancer group was found to be not statistically significant ($P<0.05$) (Table 3).

Activity of GST

In the present study the levels of GST in serum of the control, and cancer patients (group II and III) were found to be not much changed as compared to control and is not statistically significant ($P<0.05$) **Table 3.**

Activity of ACHE

In the present study we have found a 44-48% decrease in the activity of AChE in cancer patients as compared to control ($P<0.05$). The levels of ACHE in the serum of control, and cancer patients (group II and III) were significantly

Table 1. Clinical characteristics of control and cancer patients.

	Control	Cancer	Cancer and Pesticide
No. of Patients	51	61	56
Mean Age	41.52±1.8	48.97±1.99*	51.80±1.46**
Mean BMI	21.38±0.58	21.41±0.53	20.68±0.63
Sex			
Male	21(41.18%)	24(39.34%)	29(51.79%)
Female	30(58.82%)	37(60.66%)	27(48.21%)
Diet			
Non-Veg	39(76.47%)	52(85.24%)	47(83.92%)
Veg.	12(23.53%)	09(14.75%)	09(16.07%)

Results are mean ± S.E.

*Significance levels are based on P < 0.05.

*** Significance levels are based on P < 0.001 when compared with control.

Table 2. Types of cancer found in studied patients.

	Cancer	Cancer and Pesticide
Breast	09(14.75%)	05(8.93%)
Gall Bladder	13(21.31%)	14(25%)
Blood	06(9.84%)	03(5.36%)
Oral	15(24.59%)	15(26.79%)
Cervix	10(16.39%)	13(23.21%)
Lung	02(3.28%)	02(3.57%)
Ovary	02(3.28%)	02(3.57%)
Others	04(6.55%)	02(3.57%)

Table 3. Enzyme activity in serum and plasma of Control and Cancer patients.

	Control	Cancer	Cancer and Pesticide
Monooxygenase (nmoles)	0.115±0.0161	0.052±0.010**	0.070±0.013*
GST (U/mL)	0.041±0.001	0.041±0.007	0.040±0.001
ACHE (µmoles/ min/ml)	4.795±0.176	2.688±0.154***	2.473±0.148***
B-Glucuronidase (mU)	11.16±0.59	12.52±0.61	14.47±0.9**
Total Protein (mg/dL)	7.59±0.18	7.46±0.28	6.39±0.21*****

Results are mean ± S.E.

*Significance levels is based on P < 0.05.

*** Significance levels is based on P < 0.001 when compared with control.

decreased and found to be 4.8±0.1759, 2.69±0.15 and, 2.47±0.15 µmole/min/ml of serum, respectively (P<0.05) (Table 3).

Activity of β-Glucuronidase

The results of present study showed statistically significant increase of 12-30% in the β-Glucuronidase activity in the serum of the cancer patients group as compared to the control. The β-Glucuronidase activity was found to be 11.16±0.59, 12.52±0.61 and, 14.47±0.9, in group I, II and, III respectively (P<0.05) (**Table 3).**

Level of total proteins

The results of present study showed statistically significant decrease of 2-16% in the levels of total protein in the serum of cancer patients. In the present study we have found the total protein content in group I, II and, III were 7.59±0.18, 7.46±0.28 and, 6.39±0.21 g/dl, respectively (P<0.05) (Table 3). The mean of the total protein concentration group III cancer patients was found to be statistically significant as compared to healthy control individuals (P<0.05).

DISCUSSION

Cancer disease is caused by multiple factors associated with environmental toxicant exposure, lifestyle, and chronic illness. The substrate specificity of enzyme in the presence of other biomolecules make them a safe, easy to use, consistent, less-invasive, and cost-effective tool for early diagnosis of various types of cancer. In the present study, significant decrease in monooxygenase activity represents either increased metabolism of pesticides or decreased expression of CYP enzymes. It is reported that the metabolism of environmental carcinogens through CYP enzymes and hormonal deregulation causes activation and induction of high CYP1B1 expression in tumor cells (Docea AO et al., 2017). The decrease in GST activity makes the cells more susceptible to the attack by toxic compounds (Lu Y et al., 2019). Varieties of literature are available on the GST polymorphism occurring in various types of cancer studies in human (Didziapetriene J et al., 2020)

AChE inhibition has been used as a biomarker of pesticide exposure and cancer (Zhang XJ et al., 2012). The drop in cholinesterase activity is said to have a crucial non classical pathophysiological role in tumor generation (Barman SM et al., 2016). β-glucuronidase is an acid hydrolase released by inflammatory cells (the only mammalian endoglycosidase) that cleaves heparan sulfate and is used as a screening test for malignancies. The increase in β-glucuronidase may be associated with the severity of inflammation occurring in cancerous cells. Increased level of serum β-glucuronidase is reported in patients of diabetes mellitus atherosclerotic, coronary artery disease, and Gaucher's (Simeonovic CJ et al., 2020). Organophosphate compounds are known to cause a selective increase of β-glucuronidase activity in rat serum (Rasouli M et al., 2005).The decrease in serum proteins profile may be partially explained by the inflammation, oxidative stress reported in the progression of cancer development (Singh N et al., 2019) (Sideras K et al., 2014). The serum concentration of albumin and globulin varies under various stress conditions. Various environmental toxicants are reported to have the capacity to inhibit the immune system and thus may be responsible for the decrease in total protein in cancer patients as compared to control (Winans B et al., 2011). Additional investigation is required to define the specific functions of various serum/plasma biomarkers, both for diagnosis and progression of cancer.

CONCLUSION

A significant change in the activity of biochemical marker enzymes like monooxygenase, GST, ACHE and, β-glucuronidase was observed in both groups of cancer patients. Results strongly indicate that these cancer patients have a high risk of oxidative stress that may responsible for progression of carcinogenesis. In the present study it seems that pesticides do not have synergistic effect on cancer progression. To validate the above results further studies on large sample size are required.

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