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Cancer incidence of digestive system with reference to the assessment of selected polychlorinated biphenyl congeners

Mohammad Attaullah[*1], Masarrat J. Yousuf[1], Islam Dad[1], Muhammad Amin[1], Syed Ishtiaq Anjum[2], Rooh Ullah[3]

[1]Department of Zoology, University of Karachi, Karachi, Pakistan

[2]Department of Zoology, Kohat University of Science and Technology, KPK, Pakistan

[3]Department of Zoology, SBBU, Sheringal, Dir Upper, Pakistan

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Abstract

The present study was aimed to ascertain the levels of seven polychlorinated biphenyls (PCBs) in patients with cancers of the digestive system in comparison to normal healthy subjects. Fasting blood samples were collected from the donors with informed consent and the isolated sera of each sample was extracted with organic solvents and analyzed for the residues of PCBs with Gas Chromatograph coupled with Electron Capture Detector (GC-ECD). Mean level of total PCBs (ΣPCBs) was found significantly elevated in the cancer patients (2.555 mg/kg) compared with the healthy subjects (0.361 mg/kg). PCB 52 was detected in high concentrations compared with other PCB congeners with a level of 1.629 mg/kg in the cancer patients and 0.12 mg/kg in the healthy subjects. All of the cancer samples (100 %) and 91.42 % of the healthy subjects were detected positive for the presence of PCBs. Highest mean level of ΣPCBs (7.259 mg/kg) was found in cancer cases of the gall bladder while lowest mean level (0.44 mg/kg) was found in cases of the small intestine. Concentrations and frequencies of the studied individual PCB congeners (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 and PCB-209) as well as ΣPCBs were found significantly elevated in the cancer cases compared with the healthy subjects. It can be concluded from the obtained results that PCBs have a positive association with the risk of cancer of the digestive system. These persistent chemicals which circulate in various environmental compartments including humans seem to have a role in the chemical basis of carcinogenesis.

* **Corresponding Author:** Mohammad Attaullah ✉ attaullah.ms@gmail.com

Introduction

Polychlorinated biphenyls are synthetic chlorinated hydrocarbon compounds consisting of two benzene rings linked by a single carbon-carbon bond. PCBs have been produced commercially since 1929 and are widely used in many industrial, commercial and household products. These chemicals resist acids and alkalis, relatively heat-stable and are very persistent and therefore circulates in all of the environmental compartments including human beings. PCBs come into the human body through contaminated food, water, air, dust or soil and are absorbed through the skin by inhalation or accidental ingestion (Covaci *et al.,* 2002). Exposure to PCBs has been associated with increase in the risk of cancers of the digestive system, particularly of the liver and malignant melanoma (WHO, 2003). The association between polychlorinated biphenyls and risk of cancer has been reported in a number of previous studies (Dharmani and Jaga, 2005; Clark and Snedeker, 2005; Attaullah *et al*., 2016).

Cancer incidence is on the rise worldwide which affects the life expectancy of humans. The risk of disease involves a number of factors like smoking, contaminated food, chemicals, life style, obesity, lack of exercise, inactivity and a number of unknown factors PCBs are in use for a long time in Pakistan but there is no regulatory framework to control and monitor these chemicals although Pakistan is signatory of the Stockholm convention on persistent organic pollutants (Malik and Nadeem, 2011). Cancer incidence has increased manifolds particularly in the industrial and densely populated cities like Karachi, Pakistan where residues of PCBs have been detected in human milk, serum and other matrices of the body (Khawaja *et al.,* 2010; Sobia *et al.,* 2012, 2013). The Government medical authorities such as ACSP (Agha Khan University Cancer Surveillance for Pakistan), KCR (Karachi Cancer Registry) and PMRC (Pakistan Medical Research Council) provide only the country profile of cancer. The epidemiological pattern of cancer incidence with reference to the assessment of persistent organic pollutants like PCBs has not given due share of studies.

Mortalities and cancer incidence in populations exposed to PCBs has studied extensively in a number of studies as reviewed by Agency for Toxic Substances and Disease Registry (ATSDR, 2000). The present study was performed in non-occupational people with no previous history of exposure to PCBs. In this way, the levels of PCBs detected in the studied cohort particularly in the cancer patients is a matter of concern.

The aim of the present study was to evaluate the levels of PCBs in diagnosed cancer patients of the digestive system and normal healthy humans with no previous history of exposure to these chemicals. The concentrations of PCBs detected in the present study will through light on a possible association of these chemicals with the risk of cancers of the digestive system. Further research is recommended to know the exact role of these chemicals in the carcinogenesis of humans. This will enhance awareness in the general public about the health hazards of PCBs. Proper regulatory and control measures for the usage and safe disposal of PCBs should be taken to mitigate the health risks associated with these persistent chemicals.

Materials and methods

Sample collection

Collection of fasting blood samples (8 ml each) was carried out with informed consent from the donors at various hospitals and health care units of Karachi City. The studied population consisted of diagnosed cancer patients of the digestive system and healthy residents. Collection of fasting blood samples enabled us to calculate the results in mg/kg serum without further adjustment for lipid contents. The whole blood samples were centrifuged within two hours of collection for the isolation of serum. The isolated serum samples were then transferred into new plain red top tubes, labeled and stored at minus 20°C for further analysis.

Extraction and clean-up of the organic phase from serum

The whole analytical procedure starting form extraction, cleanup and final quantification of PCBs through GC-ECD was carried out in light of the previously described method by Atuma and Aune (1999).

Protocols of USEPA were followed for the analysis of PCBs. Organic solvents viz. Methanol, n-Hexane and Diethyl Ether were used for the extraction of 2ml aliquot of serum samples. The final dried out organic phase was mixed with 1ml n-Hexane and then cleaned by mixing with H_2SO_4. Clean up of the final extract (1ml) was carried out in a Florisil column containing 1 gram of deactivated Florisil topped with 1 gram of Sodium Sulfate. The cleaned up residues were evaporated to dryness, dissolved each in 1ml of n-Hexane and then stored at -20°C.

Quantification of PCBs

Quantification of PCBs was carried by processing of 1µl of the cleaned up final organic extract of each sample with GC-17A Gas Chromatograph coupled with Ni63 Electron Capture Detector. Helium (He) was used as the carrier gas in a DB-5 column with dimensions of 30m×0.32mm×0.25µm. Chromatograms obtained after processing of each sample were compared with standard chromatograms of the seven studied PCB congeners (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 and PCB-209) for the identification and quantification of individual PCBs in all samples.

Results

Seven PCB congeners were tested in the serum samples of 41 cancer patients of the digestive system and 35 healthy humans (Table 1). The studied sub sites of the digestive system cancers included esophagus, stomach, liver, gall bladder, small intestine, pancreas, colon and rectum. Elevated concentrations of mean total PCBs were detected in the cancer group (2.555 mg/kg) as compared with the normal group (0.361 mg/kg) (Table 1, Fig. 1). The studied cancer cases were 100 % positive for the presence of PCBs while detection of PCBs in the normal samples was 91.42 % (Table 1, Fig. 2). Elevated levels of PCB 52 were detected in the cancer group (1.629 mg/kg) compared with the normal group (0.12 mg/kg). The second highest detected congener was PCB 180 with a mean concentration of (0.693 mg/kg) in the cancer cases and (0.096 mg/kg) in the normal samples. The third PCB congener with higher mean levels was PCB 101 while detection of PCB 138, PCB 153 and PCB 209 was very low and insignificant.

Table 1. Detected concentrations of PCBs in the Cancer and Normal samples.

Type of Test Sample	Name of PCB Congener	Positive Samples	Positive Test %	ΣX (mg/kg)	Mean (mg/kg)	SD
CANCER CASES	PCB-28	32	78.04%	3.71	0.09	0.095
	PCB-52	40	97.56%	66.803	1.629	3.262
	PCB-101	7	17.07%	3.818	0.093	0.276
	PCB-138	4	9.75%	0.607	0.014	0.066
	PCB-153	5	12.19%	0.908	0.022	0.022
	PCB-180	21	51.21%	28.44	0.693	2.119
	PCB-209	16	39.02%	0.502	0.012	0.023
	TOTAL	41	100%	104.788	2.555	0.837
NORMAL SAMPLES	PCB-28	19	54.28%	0.471	0.013	0.024
	PCB-52	25	71.42%	4.435	0.12	0.442
	PCB-101	14	40.00%	1.886	0.053	0.112
	PCB-138	4	11.42%	1.282	0.036	0.153
	PCB-153	6	17.14%	1.31	0.037	0.1
	PCB-180	4	11.42%	3.365	0.096	0.551
	PCB-209	9	25.71%	0.217	0.006	0.015
	TOTAL	35	91.42%	12.956	0.361	0.695

Legend: ΣX (sum of a PCB congener in all studied samples); SD (standard deviation); mg/kg (milligrams of PCBs per kilogram of body weight); % (percentage or frequency of detection).

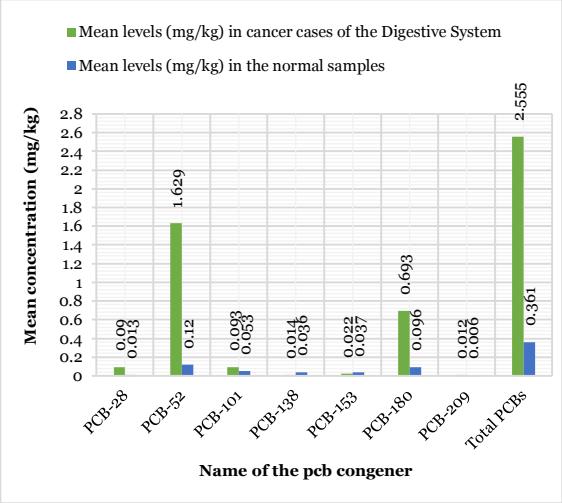

Fig. 1. Mean concentrations of the seven PCB congeners in the normal and cancer samples.

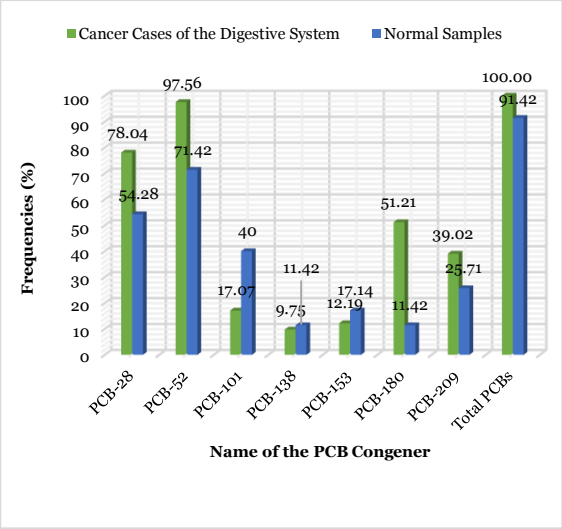

Fig. 2. Frequencies of the analyzed PCB congeners in the normal and cancer samples.

The highest detected congener in respect of frequency was PCB 52 with a frequency of (97.56 %) in the cancer cases and (71.42 %) in the normal subjects. The second and third most frequently detected PCB congeners were PCB 28 and PCB 101 respectively (Table 1, Fig. 2). Frequencies of the highly chlorinated congeners PCB 180 and PCB 209 were found elevated in the cancer cases compared with the normal samples.

When compared with the normal samples, all of the studied sub sites of cancer cases were having higher mean concentrations of total PCBs (Table 2, Fig. 3).

The sub sites of digestive system cancers in respect of the increasing mean total PCB concentrations (mg/kg) were found in the order of: small intestine (0.44) < liver (1.262) < rectum (1.496) < pancreas (1.789) < stomach (2.173) < colon (2.227) < esophagus (3.2) < gall bladder (7.259).

Amongst the analyzed sub sites of cancer cases of the digestive system, highest deposition of ΣPCBs was detected in cases of the gall bladder while lowest was detected in cases of the small intestine (Table 2, Fig. 3).

Table 2. Mean concentrations of Total PCBs in Cancer Sub Sites of the Digestive System.

Sub Sites of the Cancer Cases of Digestive System	Total PCBs		
	ΣX (mg/kg)	Mean (mg/kg)	SD
Oesophagus	22.465	3.2	4.687
Stomach	6.519	2.173	2.729
Liver	6.31	1.262	0.801
Gall Bladder	36.295	7.259	5.518
Small Instestine	1.761	0.44	0.474
Pancreas	5.368	1.789	0.349
Colon	15.592	2.227	3.636
Rectum	10.478	1.496	0.099

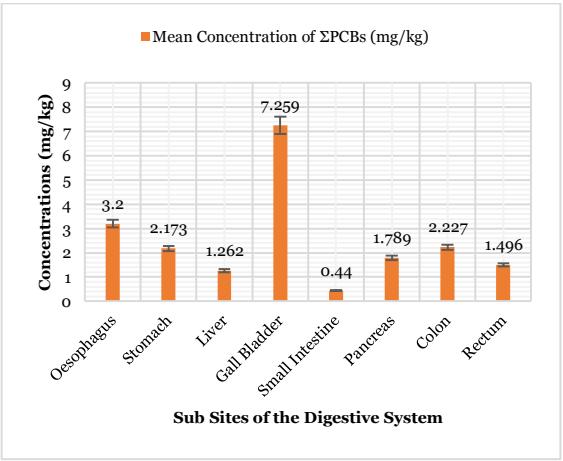

Fig. 3. Mean concentrations of ΣPCBs in the analyzed sub sites of cancer cases of the digestive system.

Discussion

Serum was used for residues determination of PCBs which is a homogenous matrix and does not coagulate during freezing. Serum and adipose tissues are reported as mirror images in respect of the accumulation pattern of the residues of PCBs (Pauwels *et al.*, 2000).

In this way our findings are validated in the serum analysis. On the basis of enough evidence of carcinogenicity in experimental animals and humans, PCBs are classified as Group 1 Carcinogens by the International Agency for Research on Cancer (ATSDR, 2014). PCBs have been demonstrated to have a positive role in the development of cancer (USEPA, 2015).

Mean ages of the subjects in the cancer and control groups were 41 years and 33.4 years respectively. The lower mean ages of the cancer cases in the present cohort compared with other developed countries of the world is indicative of the early onset of cancer and lower life expectancy in the residents of Karachi. This can be attributed to the presence of high body burdens of PCBs along with other confounding factors.

Elevated concentrations of PCBs in the cancer cases compared with normal healthy subjects has positively associated with various malignancies in the body, as reported in pancreatic cancer (Hoppin *et al.,* 2000); endometrial cancer (Weiderpass *et al.,* 2000); breast cancer (Romieu, 2000; Stellman, 2000; Ward *et al.*, 2000) etc. In the present study, the subjects belonged to the general population with no past history of occupational exposure to PCBs and the elevated levels of PCBs indicate prevalence of these contaminants in the environment and human matrices. This trend is evident from the detected frequencies of these contaminants in the studied cohort (Table 1, Fig. 2). The levels of PCBs in the cancer cases were found significantly elevated compared with the normal healthy individuals (Table 1, Fig. 1). This indicates a possible association of these chemicals with the development of cancer. The detected concentrations of PCBs in various organs of the digestive system indicate susceptibility of a particular organ more to these contaminants with ultimate risk of cancer (Table 2).

In the present study, PCB 52 emerged as most strongly associated with the risk of cancer of the digestive system as indicated by its high concentrations.

Lower chlorinated biphenyls like PCB 52 has found to be associated with oxidative DNA damage in the human breast cancer tissue by producing free radicals (Oakley *et al.,* 1996). PCB-52 in such high concentrations in the cancer cases may possibly have a role in DNA damage and the ultimate development of cancer.

PCBs have been associated with apoptosis of cells in the human body by damaging DNA and resulting in cancer (Ghosh *et al.,* 2010). This shows that a correlation exists between cellular PCB concentrations and cell death. PCBs are lipophilic and very persistent and so bio-accumulate in various biological compartments of the human body. The elevated levels of PCBs which are detected in the present study indicate that they might have caused apoptosis in the cells and ultimately may have resulted in cancer.

Reports from occupational workers of industries have shown a strong positive correlation between incidence of cancer and accumulation of the industrial wastes like PCBs and Dioxins (Demers *et al.,* 2002). In the residents of Karachi, the higher concentrations of PCBs reflect the residential impact near the industrial areas. Accumulation of higher concentrations of industrial wastes like PCBs from various environmental compartments into the bodies of humans occurs via contaminated food, air or water. With the consideration of the scientific literature to the subject, no such report is available to the best of our knowledge in Pakistan on the determination of PCB congeners in people who have no occupational exposure to such chemicals. About rural people of Pakistan, a report was published (Ejaz *et al.,* 2004) on hormone disruption due to endocrine disrupting effects of pesticides. The report showed the evidence of chronic health effects due to occupational exposure.

The increasing trend of cancer in Pakistan is alarming. This study may provide a leading role in prevention of health hazards due to PCBs. By taking preventive measures for the usage and safe disposal of PCBs, the concentrations of PCBs may be brought down to lower levels where they may not be so

harmful for humans. Moreover, there should be joint venture research planning between toxicologists and medical practitioners to investigate the exact mechanism and role of PCBs in the carcinogenicity of humans.

It has been concluded from the results obtained that polychlorinated biphenyls have a positive association with cancer of the digestive system. PCB 52 has a significant and more robust association with cancers of the digestive system compared with other PCB congeners. High body burdens of persistent polychlorinated biphenyls can trigger an early onset of cancer in the target individuals and make them more susceptible to the development of cancers of the digestive system compared with those individuals having low concentrations. PCBs may have a role in the chemical basis of carcinogenesis in humans.

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