T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

INVESTIGATION OF ORGANOCHLORINATED POLLUTANT LEVELS IN HUMAN BREAST MILK AND THEIR GENOTOXIC EFFECTS ON MAMMARY EPITHELIAL CELLS

MASTER THESIS

Hajer Atya EL-AZZUZI

Istanbul-2021

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 10.10.2021 and numbered 2021/10-03.

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

01.10.2021

Hajer Atya EL-AZZUZI

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LIST OF SYMBOLS AND ABBREVIATIONS

POPs	Persistent Organic Pollutants
OCPs	Organochlorine Pesticides
ER	Estrogen Receptor
PCBs	Polychlorinated Biphenyls
dl-PCBs	Dioxin-like Polychlorinated Biphenyls
ndl-PCBs	Non-dioxin-like Polychlorinated Biphenyls
AhR	Aryl Hydrocarbon Receptor
PXR	Pregnane X receptor
CAR	Constitutive Active Receptor
ROS	Reactive Oxygen Species
ROCK	Rho-Associated Kinase
γ-H2AX	Phosphorylated Histone H2AX
8-OHdG	8-hydroxy-2′-deoxyguanosine
t-HCH	Technical Hexachlorocyclohexane
α-HCH	Alpha Benzenehexachloride
ß-HCH	Beta Benzenehexachloride
γ-HCH	Gamma Benzenehexachloride
HCB	Hexachlorobenzene
o,p-DDE	2,4′-dichlorodiphenyldichloroethylene
p,p-DDE	4,4′-dichlorodiphenyldichloroethylene
o,p-DDT	2,4′-dichlorodiphenyltrichloroethane
p,p-DDT	4,4′-dichlorodiphenyltrichloroethane
o,p-DDD	2,4′-dichlorodiphenyldichloroethane
p,p-DDD	4,4′- dichlorodiphenyldichloroethane

ABSTRACT

ELAZUZZI, H. A. (2022), Investigation of Organocglorinated Pollutant Levels in Human Breast Milk and Their Genotoxic Effects on Mammary Epithelial Cells. Yeditepe University, Institute of Health Science, Department of Physiology, Master Thesis. Istanbul.

Persistent organic pollutants (POPs) pose a threat for the environment and human health. In this study, we investigated concentrations of organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) accumulated in the fat of the human breast milk. Lactating mothers were asked to fill the questionnaire form including established risk factors associated with breast cancer development. In addition, potential DNA damage in exfoliated epithelial cells was analyzed by using Comet Assay. A total of 62 healthy lactating mothers living in Istanbul volunteered to participate in this study. Yeditepe University Clinical Research Ethics Committee approved the study protocol, and written consent was obtained from the volunteers. The genotoxicity level of the DNA isolated from human breast milk was determined by Comet Assay. The amount of OCPs and PCB congeners accumulated in human breast milk was analyzed by gas chromatography. These indicator PCB congeners were analyzed: PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180 and PCB 202. The OCPs investigated in the present study were α-benzenehexachloride, β-benzenehexachloride, δ-benzenehexachloride, hexachlorobenzene (HCB), 2,4′-dichlorodiphenyldichloroethylene (o,p-DDE), 4,4′-dichlorodiphenyldichloroethylene (p,p-DDE), 2,4′-dichlorodiphenyltrichloroethane (o,p-DDT), 4,4′-dichlorodiphenyltrichloroethane (p,p-DDT), 2,4′-dichlorodiphenyldichloroethane (o,p-DDD), and 4,4′- dichlorodiphenyldichloroethane (p,p-DDD). Demographic, dietary and reproductive health information was obtained by filling a questionnaire from each lactating mother. The analysis showed that highest levels of PCB congeners were PCB 153 (1983±2050 ng/g lipid), PCB 101 (274.4±292.7 ng/g lipid) and PCB 180 (96.21±156.2 ng/g lipid) detected in human breast milk. The lowest PCB congener was determined as PCB 202 (7.51±7.78 2 ng/g lipid) in the milk samples. The highest OCPs detected were p,p-DDT (139.8±271.1 ng/g lipid) and remained metabolites of DDTs had nearly the same levels. The lowest OCP was determined as HCB (1.44±0.69 ng/g lipid) in fat fraction of the human breast milk. The highest level of HCH was detected as

Gamma-HCH (91.62±207.3 ng/g lipid) which is also known as lindane. Our findings showed that there was a significantly positive association between age and PCB 180 ($p<0.01$) and p,p-DDD ($p<0.05$) levels. However, significantly negative correlation was also detected between age and o,p-DDE ($p<0.05$). A significantly positive relationship was observed between number of nursed children and delta-HCH ($p<0.05$) and DDTs ($p<0.05$). Significantly positive association between age of first birth was seen in p,p-DDD ($p<0.001$), whereas significantly negative correlation was seen in o,p-DDE ($p<0.01$). Significantly higher amount of p,p-DDE ($p<0.02$) and o,p-DDE ($p<0.01$) were detected in breastfeeding mothers who had lived in İstanbul less than five years. PCB 52 ($p<0.05$) and p,p-DDT ($p<0.01$) had significantly positively correlated with weekly dairy consumption. There was a significantly positive association between tail moment (TM) and PCB 202 ($p=0.042$), whereas the significantly negative correlation was observed for gamma-HCH ($p<0.001$). Our findings show that traces of OCPs and PCBs are still detectable in human breast milk of lactating women living in Istanbul. Detection of these pollutants showed an age-dependent pattern in the breast milk samples and declined in multipara women compared to primipara mothers. We also confirm that breast milk is a useful and important biological tool for monitoring the burden of POPs in human.

Keywords: Breast milk, Comet assay, Fish, poultry and beef consumption, Polychlorinated biphenyls, Organochlorinated pesticides.

ÖZET

ELAZUZZI, H. A. (2022), İnsan Anne Sütündeki Organoklorlanmış Kirletici Düzeylerinin ve Meme Epitel Hücreleri Üzerindeki Genotoksik Etkilerinin Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi. İstanbul.

Kalıcı organik kirleticiler (KOK) çevre ve insan sağlığı için tehdit oluşturmaktadır. Bu çalışmada, anne sütünün yağında biriken organoklorlu pestisitlerin (OCP'ler) ve poliklorlu bifenillerin (PCB'ler) konsantrasyonları araştırıldı. Emziren annelerden, meme kanseri risk faktörlerini içeren anket formunu doldurmaları istendi. Ek olarak, dökülmüş epitel hücrelerindeki potansiyel DNA hasarı, Comet Assay kullanılarak analiz edildi. İstanbul'da yaşayan toplam 62 sağlıklı emziren anne bu çalışmaya gönüllü olarak katılmıştır. Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu, çalışma protokolünü onayladı ve gönüllülerden yazılı onam alındı. Süt örneklerinden izole edilen DNA'nın genotoksisite düzeyi Comet Assay ile belirlendi. Anne sütünde biriken OCP'lerin ve PCB türevlerinin (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180 ve PCB 202) miktarı gaz kromatografisi ile analiz edildi. Bu çalışmada araştırılan OCP'ler, α-benzenhekzaklorür, β-benzenhekzaklorür, δ-benzenhekzaklorür, hekzaklorobenzen (HCB), 2,4'-diklorodifenildikloroetilen (o,p-DDE), 4,4'-diklorodifenildikloroetilen (p,p-DDE), 2,4'-diklorodifeniltrikloroetan (o,p-DDT), 4,4'-diklorodifeniltrikloroetan (p,p-DDT), 2,4'-diklorodifenildikloroetan (o,p-DDD) ve 4,4' -diklorodifenildikloroetan (p,p-DDD) olarak sıralanabilir. Her emziren anneden bir anket doldurularak demografik, beslenme ve üreme sağlığı bilgileri elde edildi. Analizde anne sütünde tespit edilen en yüksek PCB türevleri PCB 153 (1983±2050 ng/g lipid), PCB 101 (274,4±292,7 ng/g lipid) ve PCB 180 (96,21±156,2 ng/g lipid) olarak tespit edildi. Süt örneklerinde en düşük PCB türevi PCB 202 (7,51±7,78 2 ng/g lipid) olarak belirlendi. Tespit edilen en yüksek OCP, p,p-DDT (139.8±271.1 ng/g lipid) olarak belirlendi ve DDT metabolitleri hemen hemen aynı seviyede olduğu görüldü. Anne sütünün yağ fraksiyonunda bulunan en düşük OCP, HCB (1,44±0,69 ng/g lipid) olarak belirlendi. HCH'nin en yüksek düzeyi, lindan olarak da bilinen Gamma-HCH (91,62±207,3 ng/g lipid) olarak saptandı. Bulgularımız yaş ile PCB 180 ($p<0.01$) ve p,p-DDD ($p<0.05$) düzeyleri arasında anlamlı pozitif bir ilişki olduğunu göstermiştir. Ancak yaş ile o,p-DDE arasında da anlamlı negatif korelasyon saptandı ($p<0.05$). Emzirilen

çocuk sayısı ile delta-HCH ($p<0.05$) ve DDT'ler ($p<0.05$) arasında anlamlı pozitif ilişki gözlendi. İlk doğum yaşı ile p,p-DDD'de anlamlı pozitif ilişki ($p<0.001$), o,p-DDE'de anlamlı negatif korelasyon görüldü ($p<0.01$). İstanbul'da beş yıldan az yaşamış emziren annelerde p,p-DDE ($p<0,02$) ve o,p-DDE ($p<0,01$) anlamlı olarak daha yüksek saptandı. PCB 52 ($p<0.05$) ve p,p-DDT ($p<0.01$) haftalık süt ürünleri tüketimi ile anlamlı pozitif korelasyon göstermiştir. Kuyruk momenti (TM) ile PCB 202 arasında anlamlı derecede pozitif bir ilişki bulunurken ($p<0,05$), gamma-HCH için anlamlı negatif korelasyon gözlendi ($p<0,001$). Bulgularımız, İstanbul'da yaşayan annelerden alınan anne sütü örneklerinde OCP ve PCB'lerin kalıntılarının hala tespit edilebildiğini göstermektedir. Bu kirleticiler, anne sütünde yaşa bağlı bir eğilim göstermiştir ve bunların miktarının primipar annelere kıyasla multipar kadınlarda daha az olduğu görülmüştür. Ayrıca, çalışmamız anne sütünün insandaki KOK miktarının izlenmesi için yararlı ve önemli bir biyolojik araç olduğunu göstermiştir.

Anahtar kelimeler: Anne sütü, Balık, tavuk ve kırmızı et tüketimi, Comet assay, poliklorlu bifeniller, organoklorlu pestisitler,

1. INTRODUCTION

There has been an enormous increase in the production of different types of chemicals during the last several decades throughout the World. Although these chemicals have proven to be useful for many aspects of modern life, it is increasingly recognized that they may pollute the environment and cause harm to human beings to varying extent. Of the thousands of manufactured chemicals, it is estimated that about 1000 may have endocrine-acting properties [1,2]. Persistent Organic Pollutants (POPs) are particularly important. POPs may persist a long time in the environment and pose a high degree of toxicity for human health [3,4]. They are lipophilic substances that can bioaccumulate in the food change, particularly in fatty tissues [5]. Thus, these chemicals may ultimately reach concentrations that may have harmful effects on human health and the environment.

Adverse effects of POPs on the environmental ecosystem and human health have been studied intensively, and there are hundreds of reports in the literature [2]. Several organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were classified under "Dirty Dozen" at the Stockholm Convention in 2001 organized by the World Health Organization (WHO) [3]. PCBs and OCPs tend to bio-accumulate in the adipose tissue once they enter into the organism [1,2,6]. The most important route of exposure of humans is through consumption of contaminated food, fish and water.

PCBs are mixtures of different congeners having various numbers and positions of chlorine atoms around biphenyl rings [1,7]. They began to be produced for industrial use in the 1930s were widely used as insulators in capacitors and transformers because of their anti-inflammable and insulating properties, as hydraulic fluids, and in paints and related products [1,6]. Because of their lipophilic nature and persistent properties, they remain global contaminants in both the environment and the human body [8] in spite of the fact that their production was banned in most countries in the late 1970s [1]. PCB contamination areas exist in many countries, including Türkiye. Although the extent of the PCB compounds in Turkey is not fully known, there are findings that Marmara Region, Izmir, Izmit Gulf, Kahramanmaraş, Mersin, Antalya and other regions of Türkiye also have polluted areas [9-16].

OCPs are a group of compounds with chlorine in their structure. These pesticides were used extensively in agriculture and forestry in the second half of the 20th

century. OCPs were also extensively used in agriculture in Türkiye for a long time [17]. After 1985, the use of OCPs was banned, except for endosulfan and toxaphene [18]. Because of their persistent features, they did not decompose in the environment for a long time. OCPs tend to bioaccumulate in biological samples that have high fat content [19]. These chemicals pollute rivers, lakes, seas and ground waters. Detection of hexacholorobenzene (HCB) in air samples in the North Arctic Region is taken to indicate long-range contamination of the World with OCPs [20]. Despite limitations and decreases, it is thought that OCPs continue to pollute the environment in Türkiye to some extent.

Several PCB and OCP compounds have been reported to have carcinogenic effects in various animal and in vitro models [2,21,22]. Primary route of exposure of humans to these environmental pollutants appears to be the ingestion of contaminated food such as fish [2,6]. Absorption through the skin is another important way of exposure. In addition, it has been shown that PCBs can be transferred to fetus by placenta and to the newborn through breastfeeding [23]. As mentioned above, inhalation of airborne persistent organic particles has also been reported.

Mechanism(s) of carcinogenic effects of PCBs and pesticides have still not been well understood. It has been suggested that these substances may have xenohormone (endocrine disruptor) properties; that they mimic or antagonize the effects of endogenous hormones of the organism [4,24]. It is thought that PCBs and OCPs exert carcinogenic effects by stimulating estrogen receptors (ER) [25]. However, some pesticides also produce estrogenic effects in MCF-7 breast cancer cell lines by ER-independent mechanisms [26]. It has been demonstrated that a xenobiotic response element is present on the DNA that may be stimulated by endocrine disruptors [27].

It has been reported that genotoxic changes in mammary epithelial cells and fat tissue do not affect viability of epithelial cells. It has previously been shown that DNA strand breaks detected by Comet Assay may occur in epithelial cells without losing cell viability and mitotic potential [28]. It is thought that lipophilic toxic substances-related genotoxic damage may accumulate in the breast tissue and trigger carcinogenic process. Although breast feeding is known to protect against breast cancer, underlying mechanism(s) are not known. Therefore, elimination of damaged mammary epithelial cells by breast feeding during lactation may provide a protective mechanism against

formation of breast cancer [29]. Exfoliated mammary epithelial cells in the breast milk are probably destroyed in the digestive tract of the new born. Besides, determination of POPs in the breast milk is of importance in order to estimate possible harmful effects of these environmental pollutants to the infant.

Aim: Environmental pollution and adverse effects on the human health is a very important public health problem in the World at large, also in Türkiye. It is important to undertake epidemiological studies to bio-monitor concentrations of POPs in human samples. We have aimed to investigate levels of PCBs and OCPs in breast milk and whether there is a correlation with damaged exfoliated epithelial cells in 62 breast-feeding mothers living in Istanbul. Concentrations of PCBs and OCPs were determined in the breast milk samples. Potential DNA damage in mammary epithelial cells was analyzed by using Comet Assay. In addition, a questionnaire was obtained from the study participants about their demographic, dietary and health status and compare with the levels of PCBs, OCPs and Comet Assay findings on genotoxicity.

Biopsy of the breast tissue is an invasive sampling method. Nipple aspiration and ductal gavage are not convenient for large scale screening studies and often number of mammary cells obtained are insufficient. In addition, such methods are uncomfortable and not preferred by mothers. Breast milk appears to be a very good biological sample for isolation and analysis of exfoliated mammary epithelial cells [28-30]. Breast milk samples collected during the first eight weeks of lactation contain large amounts of exfoliated epithelial cells that represent all lobes of the breast tissue.

2. LITERATURE REVIEW

2.1. Categorization of Persistent Organic Pollutants (POPs) and Organochlorines (OCPs)

POPs have generally been divided into two distinct categories which are unintentional POPs and intentional POPs [31]. Unintentional POPs are unwanted products or generated as a byproducts of chlorine compounds with combustion and burning. These POPs include polyaromatic hydrocarbons (PAHs), furans and dioxin compounds. Intentional POPs are produced by chemical reactions and wanted products with higher lipophilic and neurotoxic nature. Intentional POPs can be further divided into two subcategories which are organochlorine pesticides (OCPs) and industrial chemicals named as polychlorinated biphenyls (PCBs) [31].

OCPs are generally known as pesticides composed of chlorine, carbon, and hydrogen. They are organic compounds with five or more chlorine atoms. These pesticides generally have a steady chemical structure and often accumulate and persist in the environment [32,33]. They are also defined as chlorinated insecticides, chlorinated hydrocarbons and chlorinated synthetics. OCPs can be divided into four distinct categories which are dichlorodiphenyltrichloroethane (DDT) and methoxychlor, hexachlorocyclohexane (HCHs) and cyclodienes including endosulfan, heptachlor, aldrin, dieldrin, endrin, chlordecone and chlordane [34]. Previous studies implied that the mechanism of POPs-induced toxicity is exerted by binding of organic pollutants to Aryl Hydrocarbon Receptor (AhR) and formation of reactive oxygen species (ROS) [35,36].

2.2. Polychlorinated biphenyls (PCBs)

PCBs are described as polyhalogenated aromatic hydrocarbons that are composed of biphenyl ring with chlorine atoms [1,5]. These polyhalogenated aromatic hydrocarbons have high boiling points, insulating properties, chemically stable and non-flammability properties. Therefore, they were used as insulators in capacitors and hydraulic fluids, paints, carbonless copy paper and in other industrial products. High thermodynamic characteristics of the PCBs have caused them to pollute the environment and bioaccumulate in food chain. PCBs are generally accumulated and stored in the human adipose tissue and longer than two decades are needed for elimination of the half-life of the compounds [37]. PCBs have different biological and

chemical properties depending on the position and chlorine atom numbers. They are categorized into eleven homologous groups depending on the number of the chlorine which has been shown in table 2.1 [38,39].

Table 2.1. PCB homolog groups.

Chlorine numbers	Chemical formula	Molecular weight
1	$C_{12}H_{10}$	154.1
2	$C_{12}H_9Cl$	188.0
3	$C_{12}H_8Cl_2$	222.0
4	$C_{12}H_7Cl_3$	256.0
5	$C_{12}H_6Cl_4$	289.9
6	$C_{12}H_5Cl_5$	323.9
7	$C_{12}H_4Cl_6$	357.8
8	$C_{12}H_3Cl_7$	391.8
9	$C_{12}H_2Cl_8$	425.8
10	$C_{12}HCl_9$	459.7

PCBs are generally divided in to two categories as dioxin-like (dl-PCBs) and non-dioxin-like (ndl-PCBs). Dioxin-like PCBs have some similarities in terms of structure and toxic effects of polychlorinated dibenzo-p-dioxins. Chlorine configuration of the dioxin-like PCBs can be seen in none (non-) or only one (mono-) of the *ortho* positions and four of the lateral positions of biphenyl. Absence of the chlorine atom in ortho-positions cause dl-PCBs to show a coplanar configuration. Non-*ortho* substituted dl-PCBs are PCB 77, PCB 81, PCB 126, PCB 169. Mono-*ortho* substituted dl-PCBs include PCB 105, PCB 114, PCB 118, PCB 123, PCB 156, PCB 157, PCB 167, PCB 189. Ndl-PCBs are also described as PCB congeners. These congeners are known as six indicator of PCBs including PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180 and structurally referred as "*ortho*-substituted" or "non-coplanar" (WHO, 2001). They are summarised in table 2.1. Presence of one or more chlorine atoms are in ortho-positions cause the ndl-PCBs to decrease in planarity structure of molecule [40]. In the literature, several studies have reported effects of dl-PCBs, whereas health risk assessment and research for ndl-PCBs are relatively insufficient. The dl-PCBs cause biochemical activation by binding AhR and induction of several xenobiotic enzymes, such as

cytochrome P450 (CYP) enzymes 1A1, 1A2 and 1B1 [41]. CYP1A1 was assumed as one of the main sources of oxidative stress [42]. Also, PCBs may cause formation of ROS and secondary oxidative stress by induction antioxidant defence associated signalling pathways and enzymes [8,43,44]. The ndl-PCBs cause activation of pregnane X receptor (PXR) and constitutive active receptor (CAR) [45] resulting in the induction of CYP3A1 and CYP2B1 [46].

The negative influence of PCBs on the humans has been shown with many studies which are related with neurotoxicity, genotoxicity, disruption of homeostasis and endocrine system and immunotoxicity [47-49]. PCBs and their metabolites may result in DNA damage, autophagy, apoptosis, lipid peroxidation, imbalance of redox associated enzymes, metabolic dysfunction [50-53]. Even though all these cellular events have in common in terms of ROS dependency [54-58], the source of ROS changes depending on the PCB characteristics and its metabolites [59,60].

Studies showed that lower chlorinated biphenyls may be selectively accumulated in breast milk and tissue [61,62]. However, lower chlorinated PCBs are more prone to get metabolized especially for chlorine atoms with para and meta positions. Additionally, insoluble higher chlorinated PCBs have shown more resistant properties for metabolism and lend to accumulate in adipose tissue [40]. Recent studies showed that ndl-PCB 180 causes dose associated DNA damage response in the liver of female rats [63]. Metabolization of lower chlorinated PCBs have been resulted in arene oxides. Later, monohydroxylated or bihydroxylated PCBs have been obtained by hydroxylation activity of cytochrome P450. As the hydroxyl groups have para and ortho confirmation to each other, quinones are oxidated by peroxidases and prostaglandin H synthase [64-67]. Previous studies implied that binding of PCBs into RNA, protein and DNA was mediated by intermediated by arene oxide [68]. Recent studies presented that quinones or semiquinones are major contributors for DNA binding instead of arene oxide metabolites [65,69]. Additionally, these quinones appears to be selectively bind guanine residues of the DNA [69,70]. Also, PCB associated para-quinones had profound effect on DNA adduction than ortho-quinones [70]. Dreiem and colleagues showed that exposure of hydroxylated PCB caused elevation of ROS level at tenfold higher than control group [71]. Mariussen et al. reported that PCB 153 was also caused to increase ROS level [72].

Some recent investigations reported that PCB congeners have hazardous potential to promote breast cancer progression, high-grade tumours and contribute to poor prognosis in breast cancer [73,74]. It has been shown that PCBs increased the metastatic properties of breast cancer cell lines which are MCF7 and MDA-MB-231 by inducing ROS dependent rho-associated kinase (ROCK) signalling pathway [75]. It has been reported that plasma concentration of PCB 153 was related with increased breast cancer risk slightly and relative risk of axillary-lymph-node involvement [73]. Two studies showed that PCB 28 and 52 are related with increased risk for breast cancer in China and Spain [76,77]. Previously, exposure of PCB quinone caused ROS dependent DNA damage which were demonstrated with the elevation of the phosphorylated histone H2AX (γ-H2AX) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) [78]. Also, increase in the micronuclei frequencies and olive tail moment was shown by micronucleus and single cell gel electrophoresis assay after the PCB quinone exposure of the HepG2 cells [79]. PCB quinone-induced DNA adduct formation and chromosome and DNA strand breaks have been reported in vitro [80]. All these findings imply a potential genotoxic influence of PCB quinone.

Previous studies were carried out to evaluate the amount of PCB congeners accumulated in the lipid fraction of the human breast milk at several time intervals in various regions of Türkiye. These findings are shown in tables 2.2 and 2.3. Higher amount of the PCB is displayed in bold. The PCB levels detected in human breast milk changed depending on the location in the Anatolian part of the Türkiye. However, PCB 138 and PCB 153 were detected at higher frequency as compared to other congeners and common in various cities [11,81-83] which were shown in table 2.2. The similar results were also evaluated for PCB 138 and PCB 153 in human breast milk collected in Istanbul at 2007 [11] that was summarised in table 2.3. However, a recent study from our laboratory has shown that there has been a shift among the level of the PCB congeners detected in human breast milk collected in Istanbul [84].

Table. 2.2. PCB profiles of human milk from several cities in Türkiye.

Location	**Kahramanmaraş** (42)		**Şanlıurfa and Adıyaman** (43)		**Mersin** (44)		**Afyon** (45)	
Number	37		100		47		15	
Year	2003		2013		2009		2007	
PCBs (ng/g Lipid)	Mean±SD	Range	Mean±SE	Range	Mean±SD	Range	Mean±SD	Range
PCB 28	0.03±0.03	ND–0.07	**1597.30**±125.44	91.66-7278.63	0.57±0.31	0.23-1.44	1.30±0.61	0.489–2.978
PCB 52			**822.16**±81.84	74.20-3663.77	0.33±0.28	1.44-1.44	0.19±0.90	0.09–0.371
PCB 101			206.75±153.12	53.63-359.87	0.55±0.47	0.08-2.58	0.28±0.12	0.121–0.466
PCB 118	0.16±0.10	0.03–0.29	39.77±11.38	12.07-78.54	1.61±0.83	0.20-1.96	1.74±0.78	0.83–3.56
PCB 138	**0.19**±0.09	0.04–0.32	176.99±60.63	4.15-805.50	**1.63**±0.78	0.41-3.71	**3.19**±1.41	1.553–6.478
PCB 153	**0.34**±0.19	0.08–0.66	26.43±7.83	3.22-117.32	**3.37**±1.52	0.78-7.99	**5.63**±2.43	2.567–11.052
PCB 180	**0.15**±0.08	0.04–0.27	33.62±8.57	0.76-84.68	1.61±0.83	0.39-4.67	2.35±1.05	0.995–4.535

Table. 2.3. PCB profiles of human breast milk in Istanbul at several time intervals.

Location	**Istanbul** (45)		**Istanbul** (46)	
Number	13		48	
Year	2007		2013	
PCBs (ng/g Lipid)	Mean±SD	Range	Mean±SE	Range
PCB 28	1.42±1.13	0.57-4.63	11.44±5.16	LOD-236.14
PCB 52	0.29±1.00	0.09-1.00	23.0± 8.78	LOD-327.47
PCB 101	0.43±0.32	0.16-1.26	12.22±7.80	LOD-358.2
PCB 118	2.26± 1.78	0.78-6.55	0.45±0.32	LOD-11.28
PCB 138	4.35±3.57	1.32-14.76	<LOD	LOD
PCB 153	8.56±6.93	2.37-28.07	1.70±0.74	LOD-25.09
PCB 180	4.40±3.56	0.85-14.18	0.58±0.22	LOD-7.89

2.3. Hexachlorocyclohexanes (HCHs) and Hexachlorobenzenes (HCBs)

Technical hexachlorocyclohexane (t-HCH) is one of the most commonly used OCP that has resulted in environmental contamination. T-HCH which is also named as 1,2,3,4,5,6-hexachlorocyclohexane is composed of five HCH stereoisomers in several

concentrations. These are approximately 60–70 percent of α-HCH, 5–12 percent of β-HCH, 10–12 percent of γ-HCH, 6–10 percent of δ-HCH and 2–3 percent of ε-HCH [85]. Only γ-HCH has been specifically named as lindane among all isomers. Lindane has been used in several developed countries due to its insecticidal properties [86]. Apart from other isomers, β-HCH has been chemically more stable. It has been reported that β-HCH has present mainly in the contaminated soil samples after the long span exposure whereas others do not [87]. Also, previous investigations implied that β-HCH has been detected in breast milk [88], human blood, soil and animal tissues. Additionally, it has mutagenic and carcinogenic impacts on human health [89]. It has been also reported that acute exposure of lindane results in disruption of respiratory functions, whereas chronic exposures have negative impacts on reproductive [90], endocrine, nervous [91-93], renal and hepatic functions [93].

Metabolization of HCHs has been achieved by cytochrome P450 system of smooth endoplasmic reticulum. Lipid peroxidation has been assumed as a main molecular mechanism seen in lindane-induced tissue injury [34]. Previous studies reported that lindane caused oxidative effect in several organs of mammals including chick liver [94], rat blood [95], testis [94,96], rat liver [97] and rat brain [97,98]. These oxidative effects have also shown age dependent characteristics [99,100]. Also, it has been proposed that inhibition of myometrial gap junctions and uterine contractility achieved by lindane resulted in higher levels of oxidative stress [101,102].

Previous studies were done to assess the level of HCHs found in the lipid fraction of the human breast milk at various time intervals in Turkey. These findings are summarized in table 2.4. Higher levels of the HCHs are indicated with bold. The HCH profile found in human breast milk changed depending on the location. However, ß-HCH was found at highest level ad compared to other HCHs [81,83] which were shown in table 2.4.

Table. 2.4. HCH profiles of human milk from several cities in Türkiye.

Location	**Mersin** (44)		**Şanlıurfa and Adıyaman** (43)		**Kahramanmaraş** (42)	
Number	47		100		37	
Year	2009		2013		2003	
HCHs (ng/g Lipid)	Mean±SD	Range	Mean±SE	Range	Mean±SD	Range
α-HCH	0.23±0.28	0.06-1.86	**403.57**±378.35	3.56-3430.13	LOD	LOD
ß-HCH	**36.30**±55.57	4.88-367.43	177.72±11.98	2.85-726.81	**2.08**±3.55	LOD–13.5
γ-HCH	0.34±0.36	0.09-1.94	42.06±9.89	10.36-542.67	0.38±0.28	LOD–14.0
HCB					0.30±0.28	LOD–1.13

2.4. Dichlorodiphenyltrichloroethanes (DDTs)

Previous studies were carried out to investigate accumulation of residues of DDTs in lipids of the human breast milk at different time intervals in Türkiye. These findings are illustrated in table 2.5. Higher amounts of the DDTs are shown with bold. The DDT levels found in human breast milk did not show large differences even though human breast milk samples were collected at years and location in Türkiye. Findings showed that p,p-DDE was detected at higher level as compared to other DDTs [81-83] which are shown in table 2.5.

Table. 2.5. DDT profiles of human milk from several cities in Türkiye.

Location	**Mersin** (44)		**Şanlıurfa and Adıyaman** (43)		**Kahramanmaraş** (42)		**Istanbul** (46)	
Number	47		100		37		48	
Year	2009		2013		2003		2013	
DDTs (ng/g Lipid)	Mean±SD	Range	Mean±SE	Range	Mean±SD	Range	Mean±SE	Range
p,p-DDT	10.54±12.32	0.41-63.66	58.50±5.13	13.77-245.31	0.16±0.13	LOD–0.48	**3.33**±2.05	LOD-97.43
o,p-DDT	1.39±4.72	0.07-32.37	47.28±20.39	4.60-427.32			0.29±0.27	LOD-12.76
p,p-DDD	0.93±1.20	LOD-5.06			0.07±0.03	LOD–0.11		
o,p-DDD	0.21±0.44	LOD-2.88						
p,p-DDE	**325.05**±242.0	16.78-107.2	**566.9**±90.30	69.94-5480.12	**31.3**±53.8	0.44–31.5	0.86±0.39	LOD-4.18
o,p-DDE	0.27±0.20	LOD-1.25	102.62±25.57	27.07-1049.62				

3. MATERIALS AND METHODS

3.1. Human Breast Milk Collection Procedure and Participant Profile

This study was approved by the Clinical Ethics Board of Yeditepe University Hospital in Istanbul. A total of 62 breast milk samples were collected from healthy lactating mothers. Volunteer women were asked to fill a questionnaire about their age, nursed children number, age of first birth, occupational background, consumption of milk and dairy products, height and weight. All milk samples were obtained by hand pumping manually and collected in glass bottles and later stored at -80°C. The collected milk samples were analyzed to determine DNA damage in Yeditepe University Medical School, Department of Physiology. Levels of PCBs and OCPs in human breast milk was determined at Çukurova University, Medical School, Department of Forensic Medicine laboratories.

The distribution rate of the age of participants was 18-25 years (39 %), 26-30 years (24 %), and 31-44 years (37 %). Frequency of the age of first birth of the participant was younger than 20 years (29 %), 20-25 years (43 %), 26-30 years (18 %), older than 30 years (10 %). The distribution rate of the nursed children number was 39 % for 1 child, 31 % for two children, 17 % for three children, 11 % for four children and 2 % for seven children. The frequency rate of the milk and dairy consumption of the participants was 85 % for daily and 15% for weekly consumption.

3.2. Comet Assay

Breast milk samples (1 ml) were transferred into Eppendorf tubes and mixed with Phosphate Buffer Saline (PBS), (1:1). The mixture was centrifuged at 1500 rpm for 10 min. After the centrifugation, the milk was sedimented into three layers where the fat in the upper part, the middle is serum and pellet include mammary epithelial cells. The serum and fat part were separated and epithelial cells were mixed with 500 ul of PBS.

Before the experiment, slides were covered with 0.65 % High Melting Agarose (HMA) and 0.65% Low Melting Agarose (LMA) solution was prepared at 37°C. Lysis solution was prepared including 2.5 uM NaCl, 100mM EDTA, 10 mM Tris, pH 10, with 1%TritonX-100 and 10% DMSO. Alkaline electrophoresis solution was prepared including 1 mM EDTA and 300 mM NaOH.

After the mammary epithelial cells were suspended in to PBS solution, it was mixed with 0.65% LMA. The cell suspension was spread on the slides, covered with coverslip and incubated at 4°C for 10 minutes to solidify the agarose. After the removal of coverslip, the slides were incubated at 4°C for 1 hour in lysis solution. After the lysis, the slides were placed on the electrophoresis tank and incubated within the electrophoresis solution for 20 min. Later, the electrophoresis run 300 mA for 25 min. In the end, the slides were incubated neutralizing buffer at 4°C and for 5 min. This procedure was repeated at three times. The slides were exposed to 20 µg/ml ethidium bromide. They were visualized by fluorescent microscope at 200 X magnification. DNA damage assessment was done by using a software (Comet Assay IV), and Tail Moment (TM) and Tail Intensity (TI) were the comet assay parameters recorded for our analysis.

3.3. Gas Chromatography

3.3.1. Sample Preparation

All collected milk samples were stored at -20°C until analyzed. An amount of 7.5 mL of breast milk samples was homogenized by the addition of 7.5 mL of pure water at 1000 rpm for 1 minute. Acetonitrile (15 mL) with (1%) acetic acid was added to the homogenate and incubated at -20°C for 2 hours. Two grams of NaCl was added onto samples and centrifuged at 3000 rpm for 5 minutes. Liquid (upper) phase was removed and mixed with 7 grams of Na_2SO_4. Later, upper phase was vortexed with the addition of 0,3 gram of PSA and 0,9 gr of $MgSO_4$. The mixture was centrifuged at 3000 rpm for 3 minutes. Upper phase was transferred into glass tube and evaporated under nitrogen gas. The evaporated samples are dissolved in 1 mL of acetonitrile and then applied onto the device for analysis.

3.3.2. Gas Chromatographic (GC) analysis parameters

Gas Chromatography (GC) analysis was performed using an Agilent 6890N model GC equipped with an ECD detector (UK). A silica capillary column (60 m X 0.32 mm D.I.) HP-5 (Agilent, USA) was used for chromatographic separation of PCBs and OCPs. Working conditions are injector temperature 250°C; detector temperature 300°C; column temperature was 70–280°C (Temperature program; 2 minutes at 70°C; 25°C/min., 70 to 150°C; 30°C/min., 150 to 2000°C; 80°C/min., 200 to 280°C; 10 minutes at 280°C). Helium was used as the carrier gas. Standard pesticide solutions

were prepared in methanol in order to determine the retention times of pesticides by GC. The excellent linearity was obtained in the concentration range that the correlation coefficients were between 0.9970 and 0.9999. The recoveries were between 60.2% and 109.2%. OCPs under investigation are summarized in table 3.1.

Table 3.1. Analysis parameters for GC-ECD analysis of OCPs and PCBs.

OCs	**Retention time**	**Calibration range (ng/mL)**
HCB	14.858	1-100 ng/mL
β-HCH	15.745	10-100 ng/mL
δ - HCH	15.989	5-100 ng/mL
γ - HCH	17.164	5-100 ng/mL
PCB-28	18.801	10-100 ng/mL
PCB-52	20.536	5-100 ng/mL
PCB-101	24.668	5-50 ng/mL
PCB-118	26.896	5-100 ng/mL
PCB-138	28.398	5-100 ng/mL
PCB-153	27.167	25-100 ng/mL
PCB-202	29.732	1-100 ng/mL
PCB-180	30.221	5-100 ng/mL
o,p'-DDE	24.419	10-100 ng/mL
p,p'-DDE	24.566	1-100 ng/mL
o,p'-DDD	25.766	5-100 ng/mL
p,p'-DDD	26.048	5-100 ng/mL
o,p'-DDT	27.235	5-100 ng/mL
p,p'-DDT	28.276	10-100 ng/mL

3.4. Statistical Analysis

Statistical analysis was done by using SPSS package program (SPSS Inc., Chicago, IL, USA). Kolmogorov Smirnov Test was used to evaluate normal distribution of the analyzed samples. Correlation of the normally distributed samples was performed by Pearson correlation tests. Non normal distributed samples were analyzed by Spearman correlation tests. Nonparametric analysis of the demographic data was carried out by using Mann-Whitney U test for comparison of the independent groups.

4. RESULTS

4.1. Organochlorine Pesticides Levels

Concentrations of each OCP are shown in figures 4.1, 4.2 and 4.3. The amount of Beta-HCH, Delta-HCH, Gamma-HCH and HCB were evaluated by GC-ECD system and shown in figure 1. Each HCH parameter had nearly the same level, however, the Gamma-HCH level was detected mostly in human breast milk as compared to others. Mean values and standard deviation of each HCHs were determined for Beta-HCH (67.64±41.42), Delta-HCH (40.87±59.98), Gamma-HCH (91.62±207.3) and HCB (1.44±0.69). HCB level was observed the least as compared to other parameters in human breast milk samples.

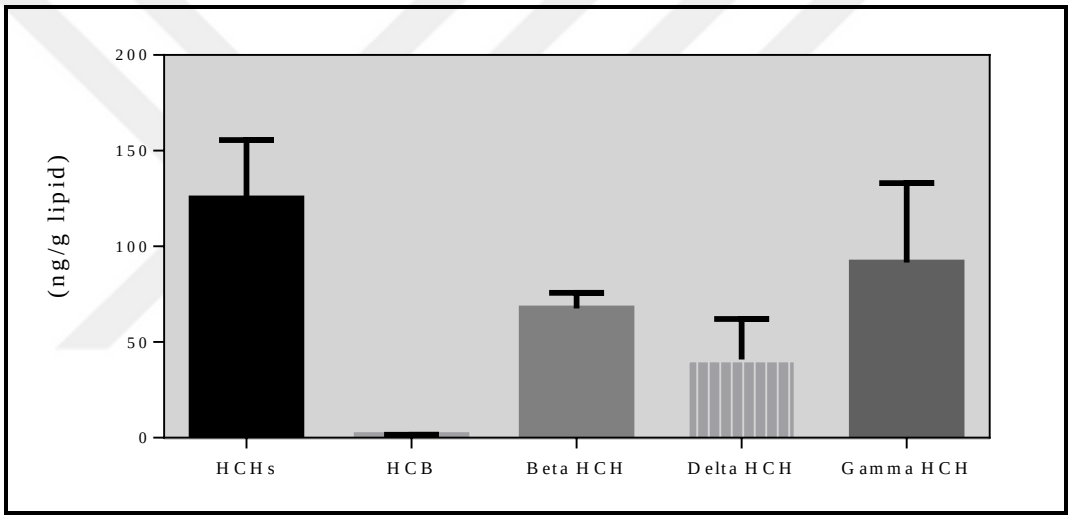

Figure 4.1. Summary of all HCHs and HCB levels detected in human breast milk.

Concentrations of the PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180, PCB 202) are shown in figure 2. The mostly seen PCB congeners was PCB 153, PCB 101 and PCB 180 in human breast milk. Mean values and standard deviation of each PCB congeners was determined for PCB 28 (17.16±8.23), PCB 52 (24.86±27.04), PCB 101 (274.4±292.7), PCB 118 (27.30±41.93), PCB 138 (32.55±19.66), PCB 153 (1983±2050), PCB 180 (96.21±156.2), PCB 202 (7.51±7.78). Depending on findings, PCBs were detected as a major contaminant in 62 human breast milk samples as compared to DDTs and HCHs.

The amount of residues of DDTs were also evaluated in collected breast milk samples including o,p-DDE, p,p-DDE, o,p-DDD, p,p-DDD, o,p-DDT, p,p-DDT. The DDTs and its metabolites are shown in figure 3. All metabolite of DDTs was found as

nearly the same level in human breast milk except for p,p-DDT which was the highest. Mean values and standard deviation of each DDT metabolite was calculated as o,p-DDE (59.77±37.49), p,p-DDE (48.54±52.86), o,p-DDD (46.33±136.2), p,p-DDD (31.10±46.05), o,p-DDT (33.86±28.64), p,p-DDT (139.8±271.1).

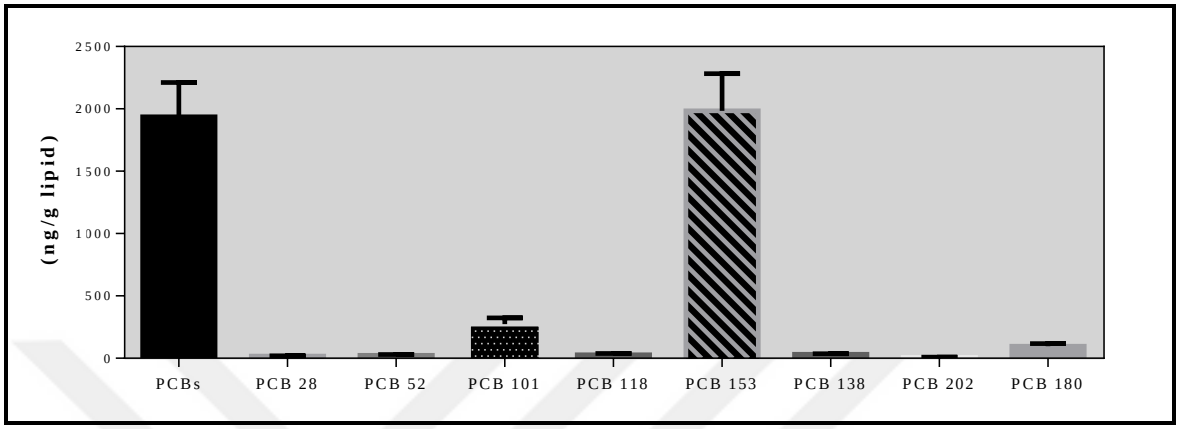

Figure 4.2. The amount of PCB congeners detected in breast milk.

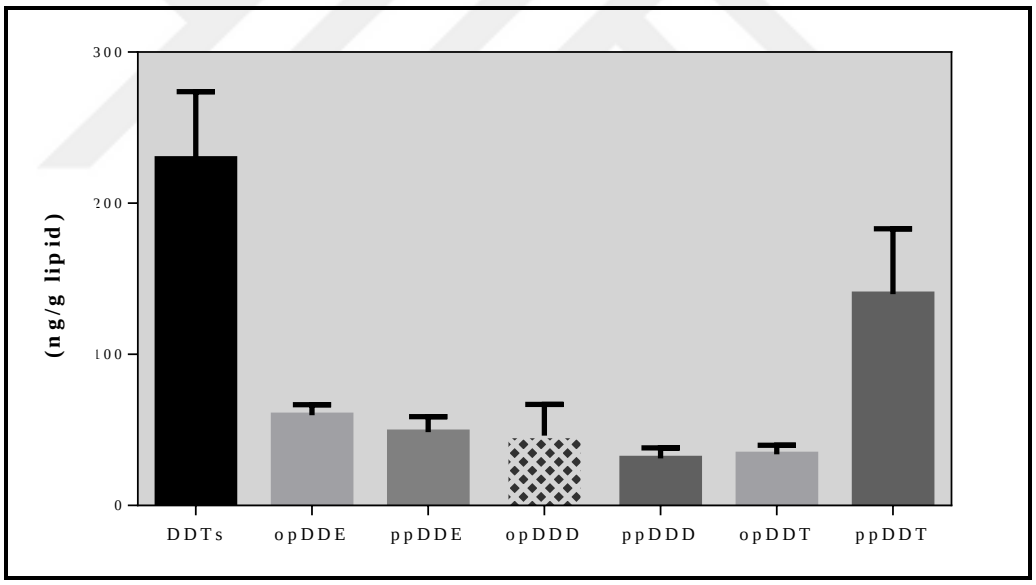

Figure 4.3. Concentrations of DDT detected in breast milk.

4.2. Age

Statistical analysis of the age of the participants is summarized in table 4.1 for HCHs, table 4.2 for PCBs and table 4.3 for DDTs. No significant correlation was found between age of the participants and HCHs. Even though significant association was not detected in many PCB congeners, a significantly positive correlation was seen in PCB 180 shown in figure 4.4. Only two metabolites of DDTs had significantly associated

with age of the participants, whereas other metabolites did not show correlation. A significantly positive association was found between age of the women and p,p-DDD level in human breast milk exhibited at figure 4.5. However, the amount of o,p-DDE was found as significantly decreasing in response to age that was shown in figure 7.

Table. 4.1. Correlation between age of the lactating women and level of HCHs in breast milk samples.

		HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
Age	Correlation Coefficient	0.135	-0.003	0.020	-0.143	0.077
	Sig. (2-tailed)	0.438	0.986	0.923	0.736	0.713
	N	35	39	26	8	25

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.2. Correlation between age of the lactating women and level of PCBs in breast milk samples.

	PCBs	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 202**	**PCB 180**
Correlation Coefficient	0.189	-0.263	-0.004	0.162	-0.075	0.113	0.281	0.175	0.404++
Sig. (2-tailed)	0.159	0.249	0.982	0.359	0.799	0.449	0.205	0.244	0.002
N	57	21	31	34	14	47	22	46	55

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.3. Correlation between age of the participants and level of DDTs in breast milk.

	DDTs	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**
Correlation Coefficient	0.353++	-0.441+	-0.154	0.216	0.340+	0.092	0.056
Sig. (2-tailed)	0.008	0.017	0.444	0.160	0.028	0.678	0.733
N	55	29	27	44	42	23	39

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

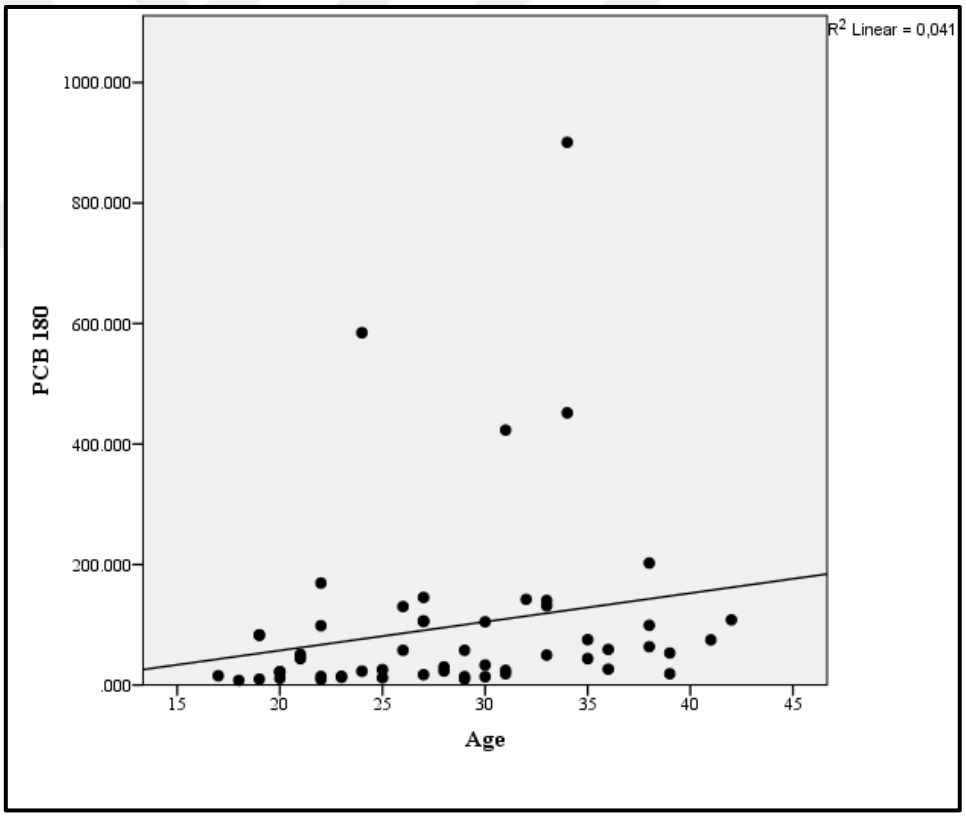

Figure 4.4. Association between age of the participants and PCB 180 amount detected in breast milk samples.

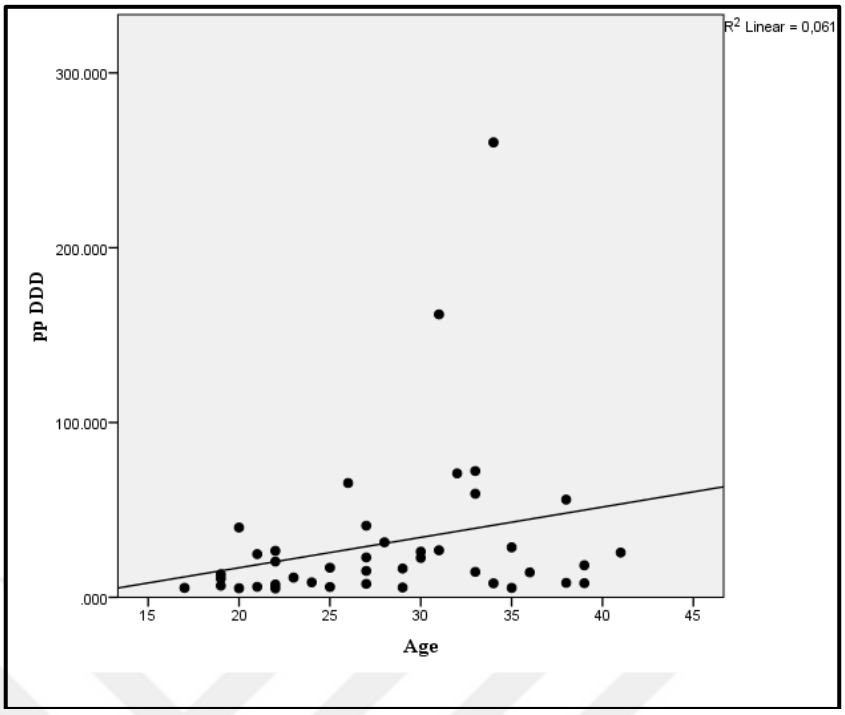

Figure 4.5. Association between age of the participants and p,p-DDD amount found in breast milk samples.

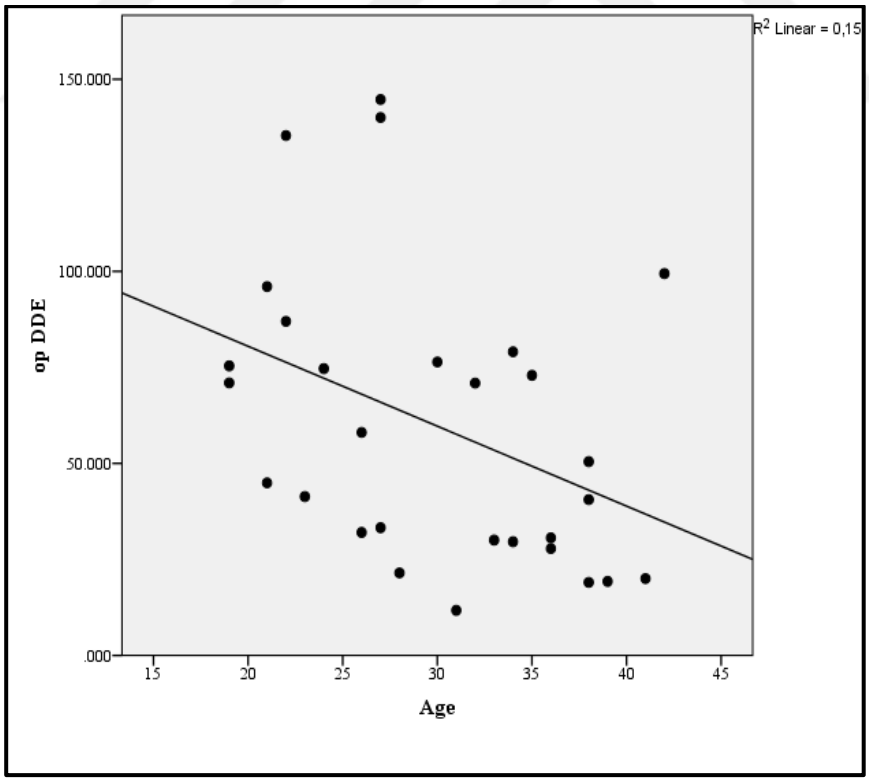

Figure 4.6. Association between age of the participants and o,p-DDE amount detected in breast milk samples.

4.3. Comet Assay

DNA damage level was assessed with alkaline comet assay within the mammary epithelial cells isolated from human breast milk samples. Tail Intensity (TI) and Tail Moment (TM), which are two parameters used for the evaluation of the DNA damage were determined and compared with OCPs. The DNA damage was evaluated in mammary epithelial cells isolated from human breast milk and shown in figure 4.7. Mean values and standard deviation of Comet Assay parameters were calculated for TI (20.41±13.05) and TM (0.66±0.77).

Statistical evaluation of TI is summarized in table 4.4 for HCHs, table 4.6 for PCBs and table 4.8 for DDTs. Also, statistical analysis of TM is displayed in table 4.5 for HCHs, table 4.5 for PCBs and table 4.9 for DDTs. Significantly negative relationship was found for Gamma HCH level with TI and TM parameters of the DNA of the mammary epithelial cells which were exhibited in figures 4.9 and 4.10. In addition, significantly negative correlation was determined between TI value and HCHs in human breast milk, shown in figure 4.8. Significantly positive correlation was evaluated between PCB 202 amount with TI and TM parameters (Figures 4.11 and 4.12).

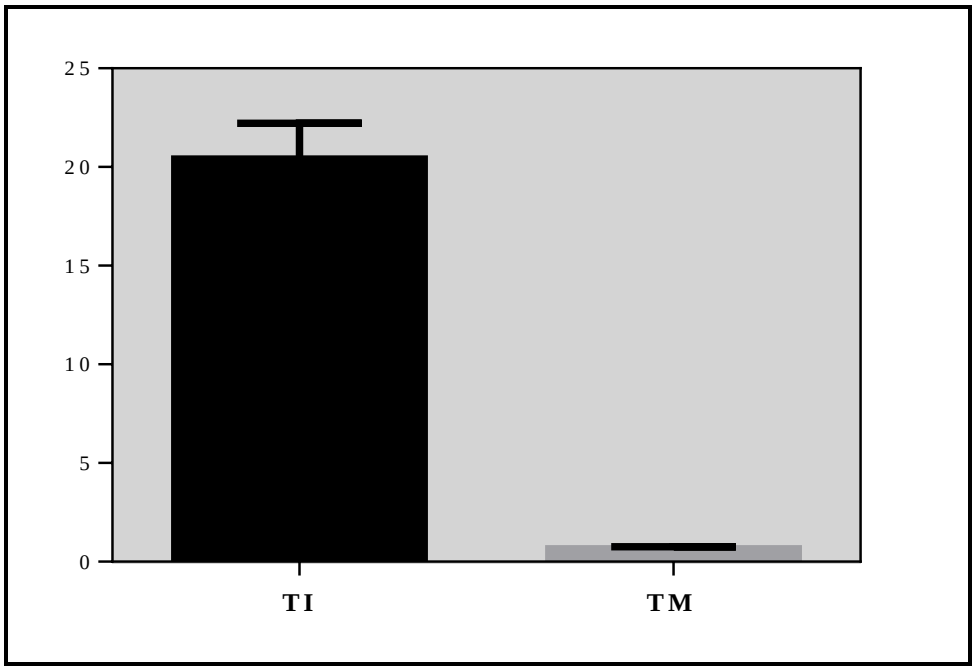

Figure 4.7. Comet Parameters found in breast milk samples.

Table. 4.4. Correlation between TI of the DNA of the mammary epithelial cells and level of HCHs in breast milk samples.

		HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
TI	Correlation Coefficient	-0.465+	-0.070	0.269	-0.216	-0.768++
	Sig. (2-tailed)	0.014	0.705	0.251	0.641	0.000
	N	27	32	20	7	19

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table 4.5. Correlation between TM of the DNA of the mammary epithelial cells and level of HCHs in breast milk.

		HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
TM	Correlation Coefficient	-0.331	-0.101	0.361	-0.179	-0.713++
	Sig. (2-tailed)	0.091	0.583	0.118	0.702	0.001
	N	27	32	20	7	19

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

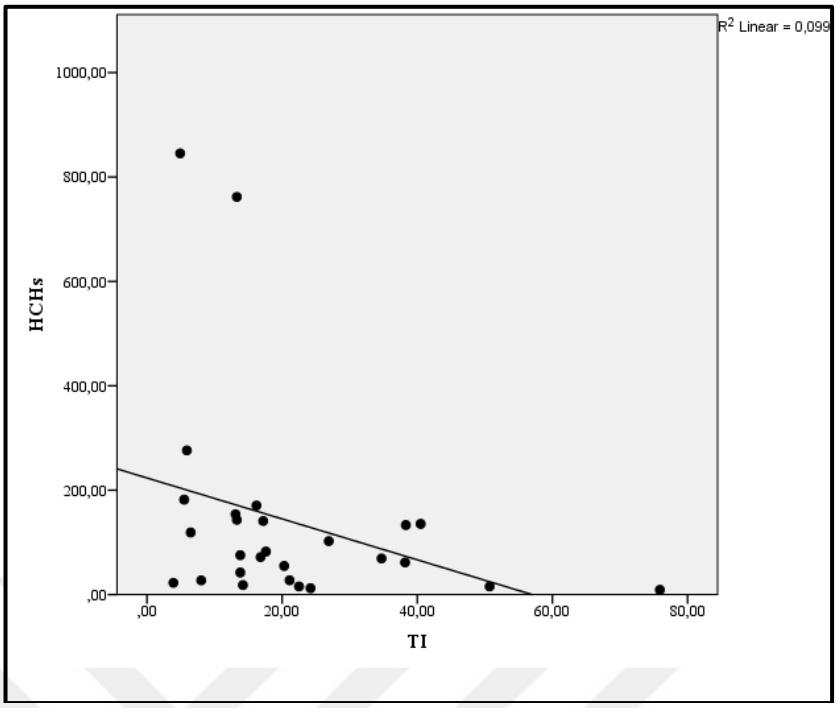

Figure 4.8. Negative association between TI and HCHs level detected in breast milk samples.

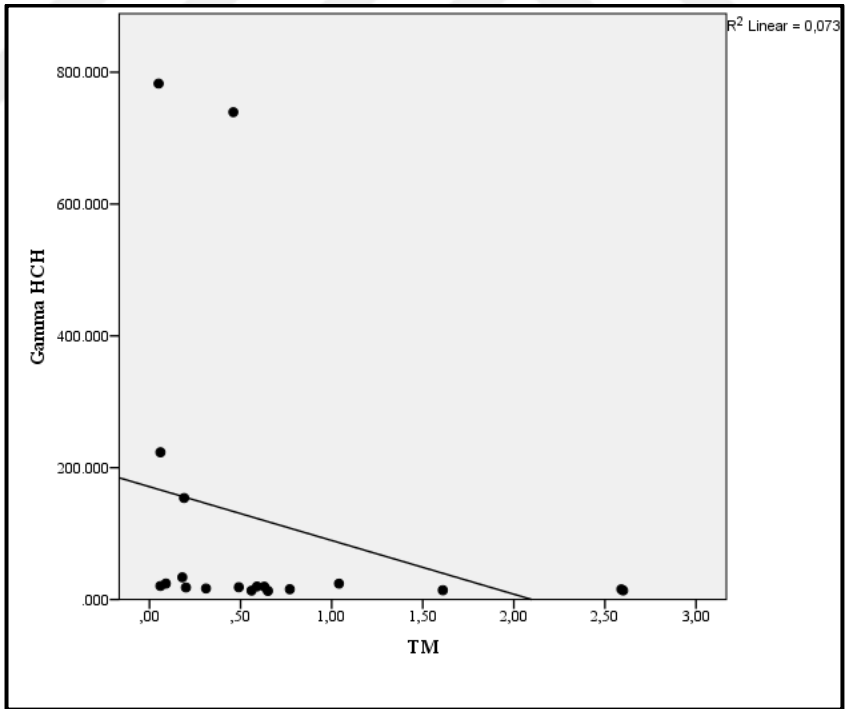

Figure 4.9. Negative association between TM and Gamma-HCH level found in breast milk samples.

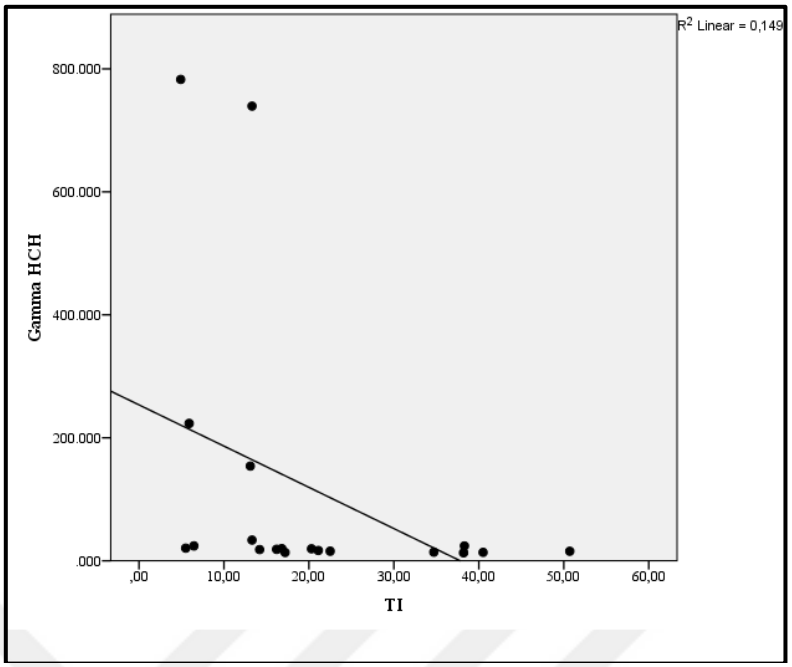

Figure 4.10. Negative association between TI and Gamma HCH level found in breast milk samples.

Table. 4.6. Correlation between TI of the DNA of the mammary epithelial cells and level of PCBs in breast milk samples.

		PCBs	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 202**	**PCB 180**
TI	Correlation Coefficient	0.170	-0.006	0.023	-0.048	-0.381	0.060	0.325	0.368+	0.009
	Sig. (2-tailed)	0.248	0.983	0.913	0.807	0.179	0.715	0.203	0.023	0.951
	N	48	16	25	29	14	40	17	38	46

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.7. Correlation between TM of DNA of the mammary epithelial cells and level of PCBs in breast milk samples.

		PCBs	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 202	PCB 180
TM	Correlation Coefficient	0.130	0.059	0.094	-0.040	-0.257	0.041	0.279	0.332+	-0.046
	Sig. (2-tailed)	0.377	0.829	0.655	0.837	0.374	0.803	0.277	0.042	0.761
	N	48	16	25	29	14	40	17	38	46

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

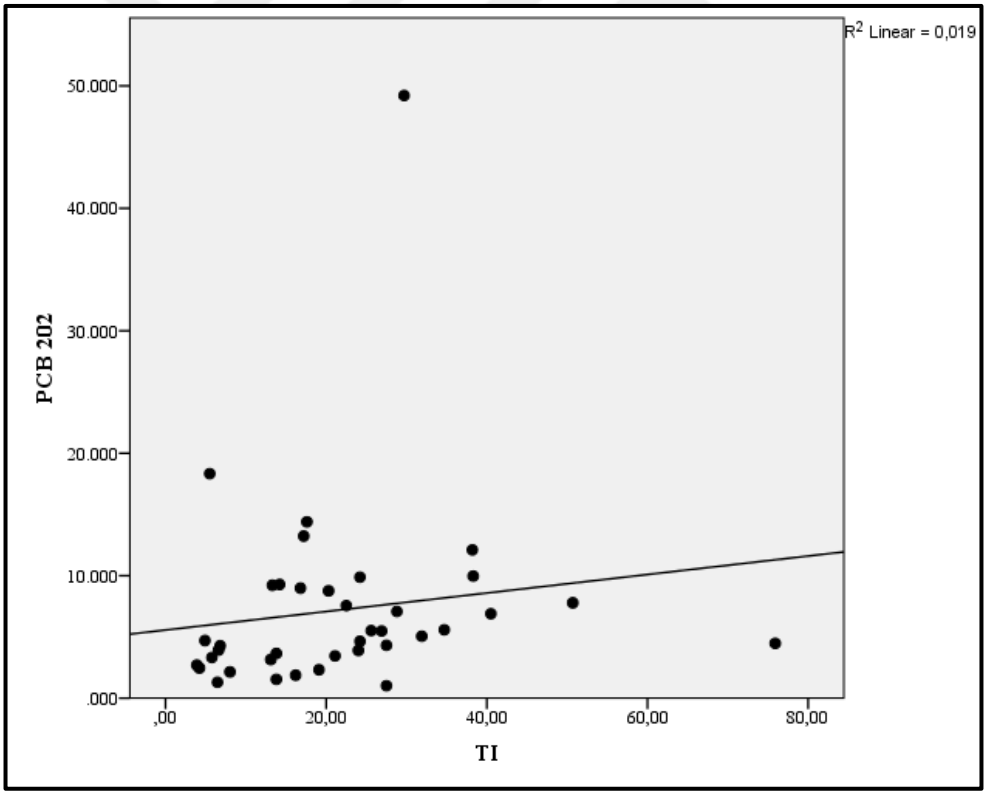

Figure 4.11. Positive association between TI and PCB 202 level detected in breast milk samples.

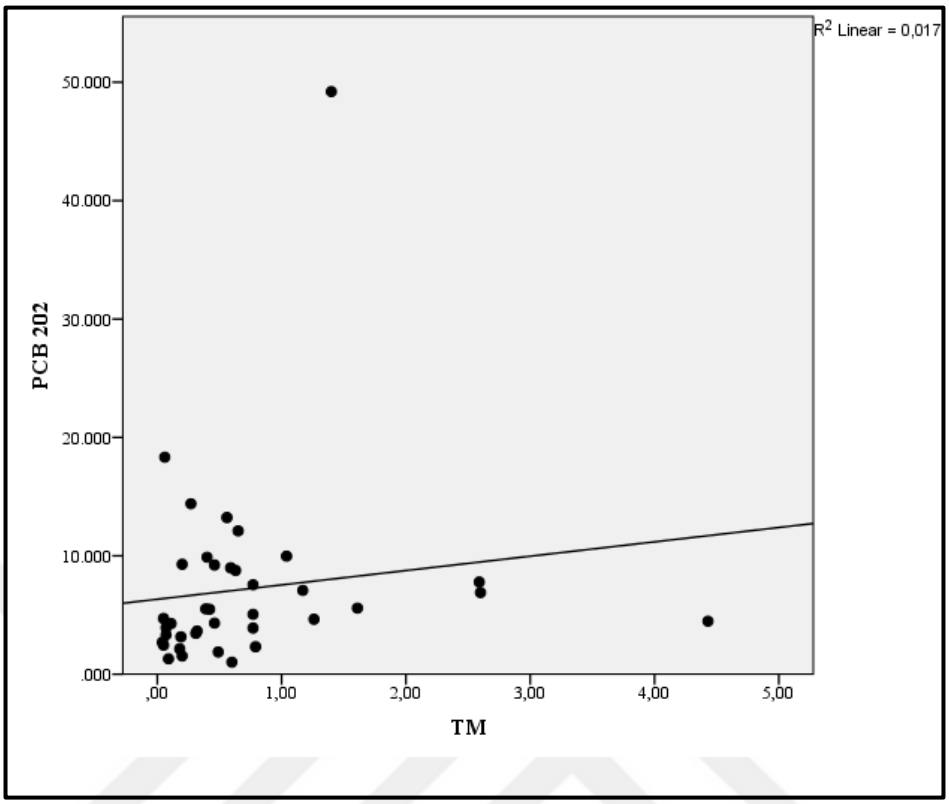

Figure 4.12. Positive correlation between TM and PCB 202 level found in breast milk samples.

Table. 4.8. Correlation between TI of DNA of the mammary epithelial cells and level of DDTs in breast milk samples.

		DDTs	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**
TI	Correlation Coefficient	0.008	0.341	-0.193	-0.048	0.158	-0.151	0.037
	Sig. (2-tailed)	0.960	0.103	0.390	0.779	0.357	0.513	0.837
	N	47	24	22	36	36	21	33

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.9. Correlation between TM of DNA of the mammary epithelial cells and level of DDTs in breast milk samples.

		DDTs	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT
TM	Correlation Coefficient	0.010	0.341	-0.246	-0.083	0.072	-0.146	0.181
	Sig. (2-tailed)	0.949	0.103	0.269	0.632	0.677	0.529	0.314
	N	47	24	22	36	36	21	33

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

4.4. Reproductive Information

Statistical evaluation of the nursed children number of the participants is summarized in table 4.10 for HCHs, table 4.11 for PCBs and table 4.12 for DDTs. No significant correlation was found between nursed children number of the participants and many of HCHs. Significantly positive correlation was detected just for Delta-HCH level which was shown in figure 4.13. No significant association was detected in PCB congeners with nursed children number of the participant. The metabolite of the DDTs did not show any significant change. However, significantly positive correlation was seen in overall level of DDTs and nursed children number of the women that was displayed in figure 4.14.

Table. 4.10. Correlation between nursed children number of the mothers and level of HCHs in breast milk samples.

	HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
Correlation Coefficient	0.294	0.070	0.092	0.781+	0.093
Sig. (2-tailed)	0.086	0.670	0.656	0.022	0.659
N	35	39	26	8	25

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.11. Correlation between nursed children number of the lactating women and PCB levels in breast milk samples.

	PCBs	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 202**	**PCB 180**
Correlation Coefficient	0.159	0.136	0.029	0.167	-0.398	0.003	0.090	0.235	0.199
Sig. (2-tailed)	0.237	0.557	0.878	0.346	0.158	0.983	0.690	0.116	0.145
N	57	21	31	34	14	47	22	46	55

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.12. Correlation between nursed children number of the lactating mothers and DDT levels in breast milk samples.

	DDTs	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**
Correlation Coefficient	0.302+	-0.083	-0.249	0.296	-0.061	0.206	0.047
Sig. (2-tailed)	0.025	0.667	0.211	0.051	0.702	0.346	0.776
N	55	29	27	44	42	23	39

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

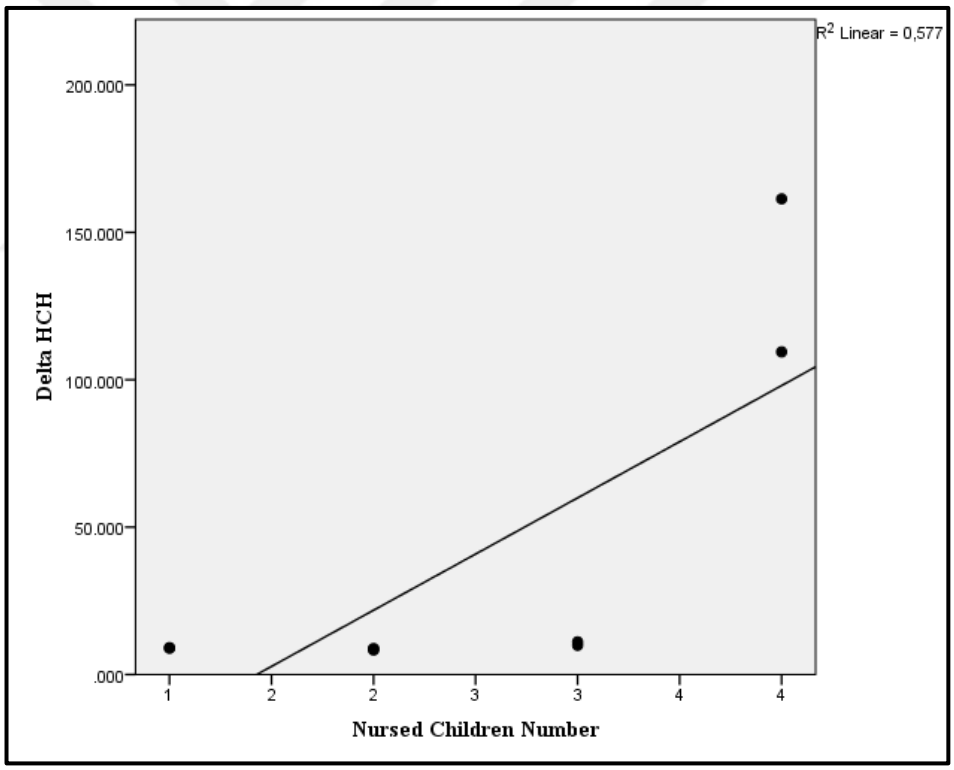

Figure 4.13. Association between nursed children number of the participant and Delta HCH level in breast milk samples.

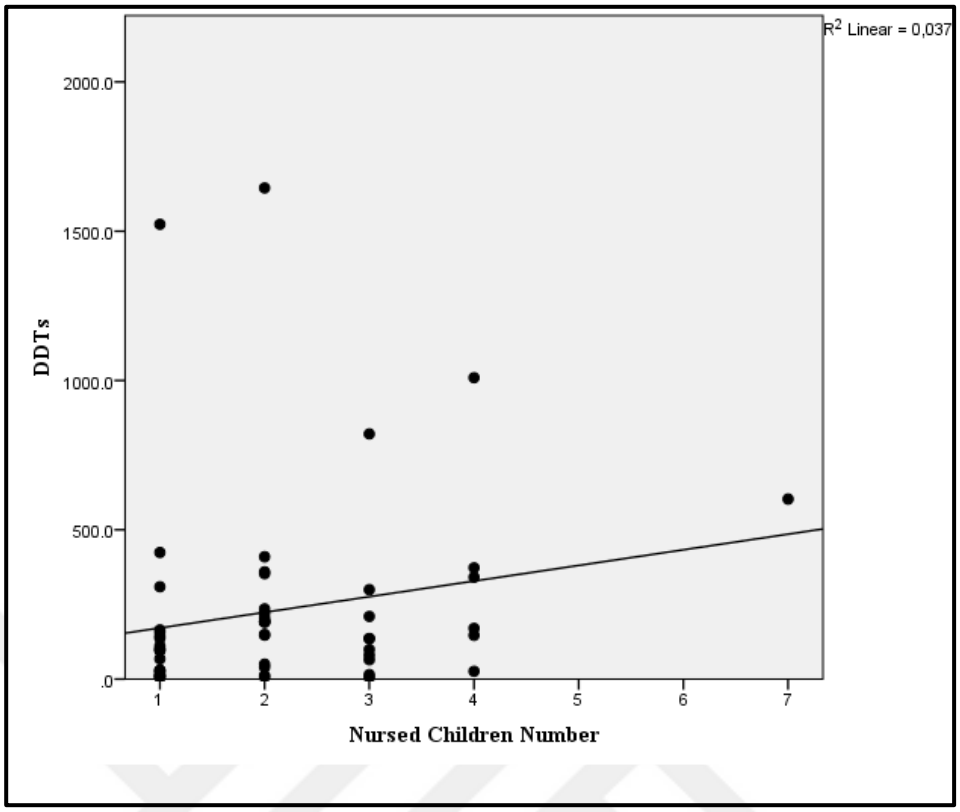

Figure 4.14. Association between nursed children number of the mothers and Delta HCH level in breast milk samples.

Statistical evaluation of the age of the first birth of the participants is summarized in table 4.13 for HCHs, table 4.14 for PCBs and table 4.15 for DDTs. No significant correlation was detected between age of first birth of the lactating women and level of HCHs, PCBs and most of the DDTs. Age of the first birth of the participant was found as significantly positive associated with p,p-DDD shown in figure 4.15. However, the significantly reverse association was detected between age of the first birth of the volunteers and o,p-DDE level (Figure 4.16).

Table. 4.13. Correlation between age of first birth of the lactating women and level of HCHs in breast milk samples.

	HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
Correlation Coefficient	-0.119	0.046	-0.143	-0.619	0.097
Sig. (2-tailed)	0.496	0.781	0.487	0.102	0.646
N	35	39	26	8	25

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.14. Correlation between age of first birth of the volunteer women and level of PCBs in breast milk samples.

	PCBs	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 202**	**PCB 180**
Correlation Coefficient	0.101	-0.388	-0.071	-0.101	0.152	0.150	0.146	0.000	0.214
Sig. (2-tailed)	0.453	0.082	0.705	0.570	0.603	0.315	0.516	0.998	0.117
N	57	21	31	34	14	47	22	46	55

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

Table. 4.15. Correlation between age of first birth of the lactating women and level of DDTs in breast milk samples.

	DDTs	**o,p-DDE**	**p,p-DDE**	**o,p-DDD**	**p,p-DDD**	**o,p-DDT**	**p,p-DDT**
Correlation Coefficient	0.065	-0.495^{++}	-0.026	0.108	0.497^{++}	-0.015	-0.056
Sig. (2-tailed)	0.635	0.006	0.896	0.484	0.001	0.946	0.736
N	55	29	27	44	42	23	39

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

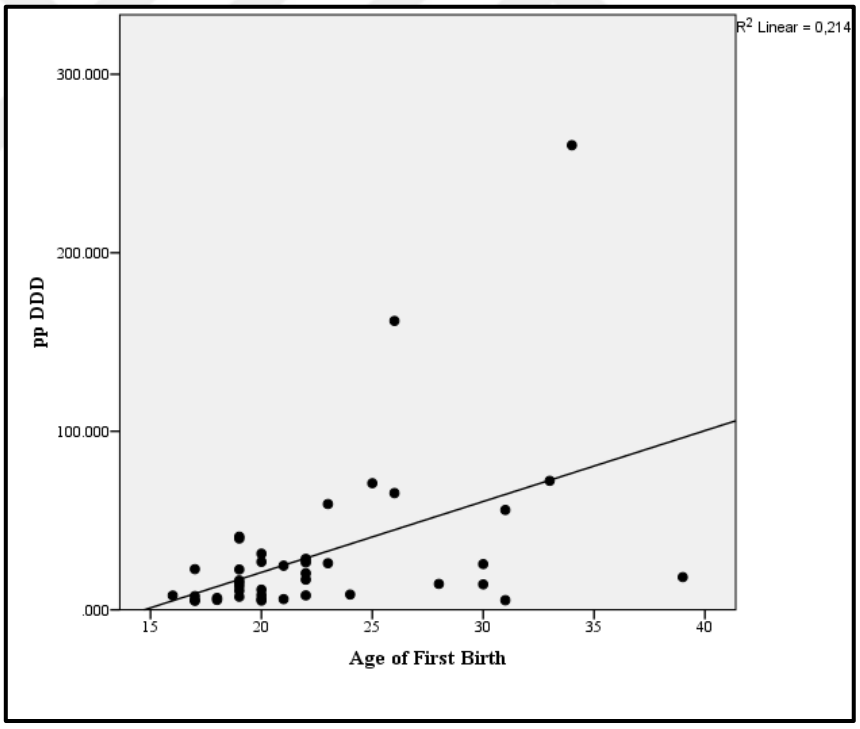

Figure 4.15. Association between age of first birth of the lactating women and p,p-DDD levels in breast milk samples.

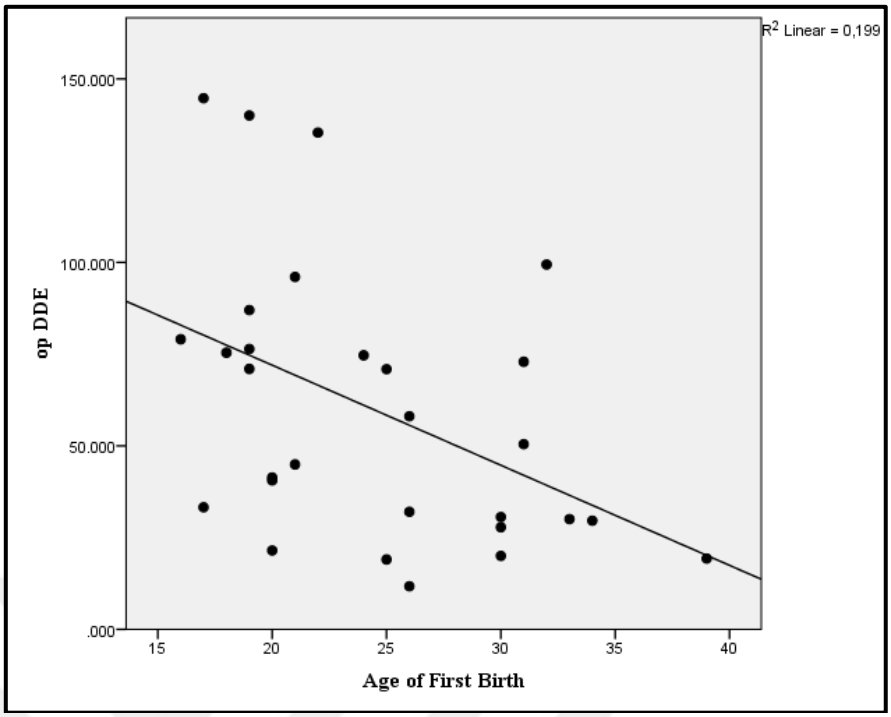

Figure 4.16. Association between age of first birth of the lactating women and o,p-DDE levels breast milk samples.

4.5. Location

Statistical assessment of the migratory behaviour of the lactating women is summarized in table 4.16 for HCHs, table 4.17 for PCBs and table 4.18 for DDTs. Volunteer participants who had been living in Istanbul was categorized according to their migratory characteristics. Participants who have lived less than five years were assumed as migrants in this study. Significant correlation was not detected between intercity migration and level of HCHs, PCBs and many of the DDTs. Migration information of the lactating women was found as significantly associated with p,p-DDE and o,p-DDE shown in figure 4.17 and 4.18. DDE metabolites were found at higher levels in the human breast milk of the migrated participants and who lived previously rural areas.

Table. 4.16. Correlation between migratory background of the participants and level of HCHs in breast milk samples.

	HCHs	HCB	Beta HCH	Delta HCH	Gamma HCH
Mann-Whitney U	117.0	106.0	53.0	1.0	70.0
Wilcoxon W	172.0	541.0	243.0	29.0	125.0
Z	-0.292	-1.254	-0.7	-1.091	-0.277
Sig. (2-tailed)	0.770	0.210	0.435	0.275	0.782
Number of Samples	35	39	26	8	25
Istanbul	25	29	19	7	15
Upstate	10	10	7	1	10

Table. 4.18. Correlation between migratory background of the participants and level of PCBs in breast milk samples.

	PCBs	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 202	PCB 180
Mann-Whitney U	278.0	28.0	66.0	73.0	10.0	189.0	39.0	173.0	231.0
Wilcoxon W	1224.0	119.0	342.0	398.0	88.0	280.0	67.0	239.0	1134.0
Z	-0.426	-1.738	-1.174	-1.542	-0.365	-0.761	-0.952	-0.502	-0.832
Sig. (2-tailed)	0.670	0.082	0.240	0.123	0.715	0.447	0.341	0.616	0.405
Number of Samples	57	21	31	34	14	47	22	46	55
Istanbul	43	13	23	25	12	34	15	35	42
Upstate	14	8	8	9	2	13	7	11	13

Table. 4.19. Correlation between migratory background of the participants and level of DDTs in breast milk samples.

	DDTs	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT
Mann-Whitney U	249.0	28.0	31.0	162.0	155.0	30.0	129.0
Wilcoxon W	1152.0	259.0	221.0	723.0	221.0	45.0	594.0
Z	-0.475	-2.733	-2.389	-0.529	-0.443	-1.118	-0.200
Sig. (2-tailed)	0.634	0.006	0.017	0.597	0.657	0.264	0.841
Number of Samples	55	29	27	44	42	23	39
Istanbul	42	21	19	33	31	18	30
Upstate	13	8	8	11	11	5	9

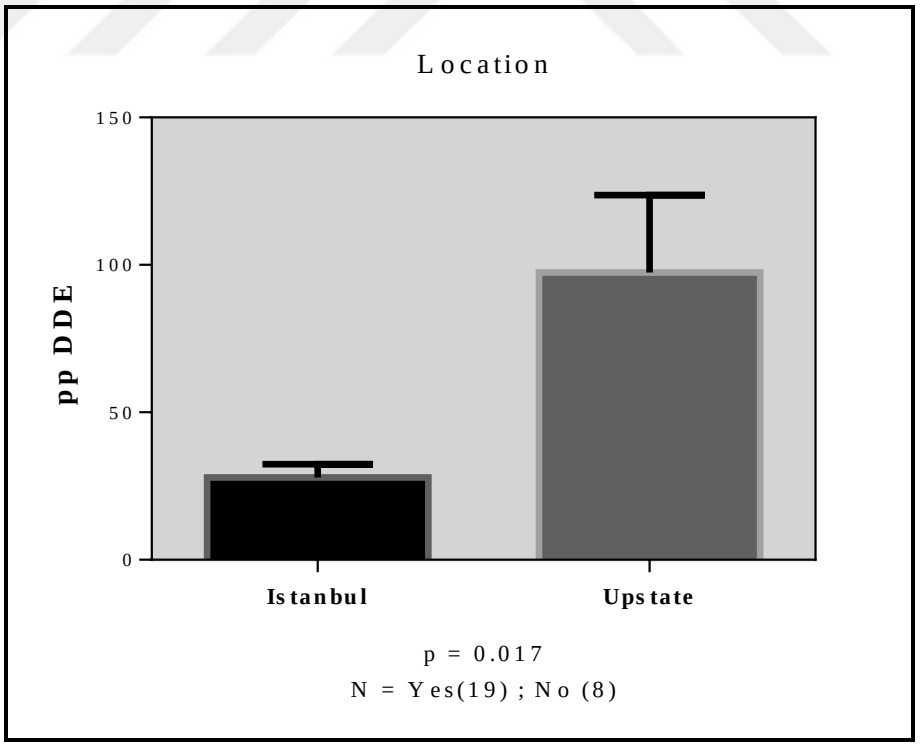

Figure 4.17. Comparison of participants living in Istanbul and migrants to Istanbul depending on p,p-DDE levels detected in breast milk samples.

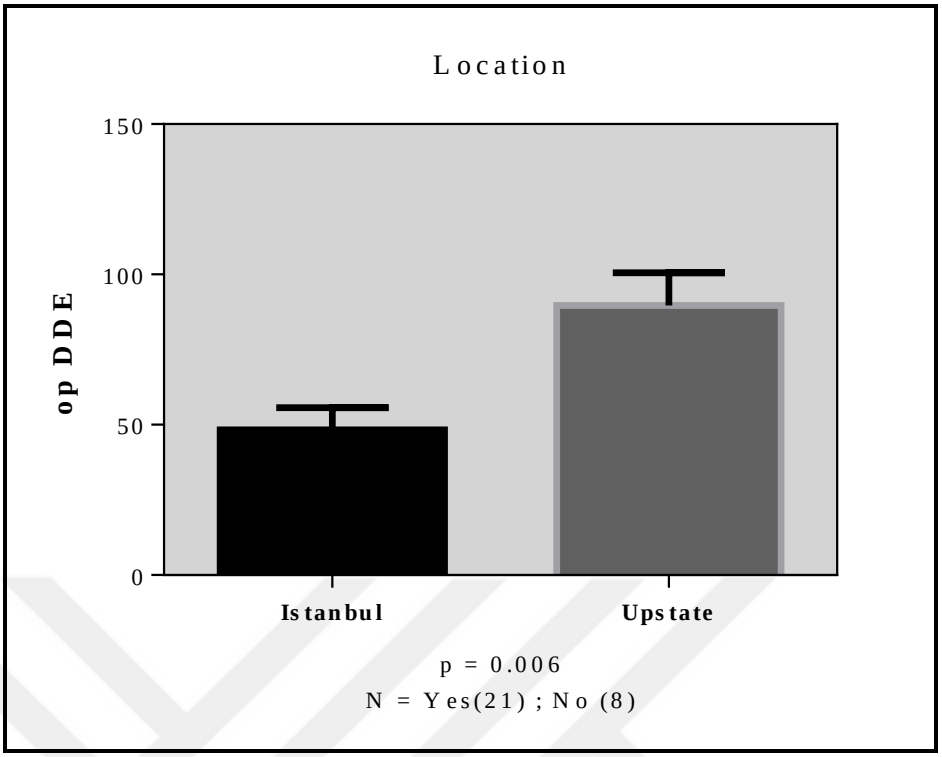

Figure 4.18. Comparison of participants living in Istanbul and migrants to Istanbul depending on p,p-DDE levels detected in breast milk samples.

4.6. Milk and Dairy Consumption Pattern

Statistical correlations of pattern of the milk and dairy consumption of the volunteer women is summarized in table 4.19 for HCHs, table 4.20 for PCBs and table 4.21 for DDTs. No significant relationship was detected between milk and dairy consumption and HCHs level in human breast milk. Only PCB 52 had significantly positively correlated with dairy consumption as compared with other PCB congeners. Also, higher amount of the PCB 52 was detected with increasing consumption behavior which was shown in figure 4.19. Only p,p-DDT was found as significantly positively associated with dairy consumption of the participants which was exhibited (Figure 4.20). Other metabolites of the DDTs were not correlated with dairy consumption behavior of the lactating women. Commonly, PCB 52 and p,p-DDT were found at higher level in the breast milk which consumed dairy products more frequently.

Table. 4.20. Relationship between milk and dairy consumption behavior of the lactating women and level of HCHs in breast milk samples.

	HCHs	**HCB**	**Beta HCH**	**Delta HCH**	**Gamma HCH**
Correlation Coefficient	0.120	0.055	0.000	0.252	0.063
Sig. (2-tailed)	0.558	0.779	1.000	0.547	0.799
N	26	29	19	8	19
+. Correlation is significant at the 0.05 level (2-tailed). ++. Correlation is significant at the 0.01 level (2-tailed).					

Table. 4.21. Relationship between milk and dairy consumption behavior of the lactating women and level of PCBs in breast milk samples.

	PCBs	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 153**	**PCB 138**	**PCB 202**	**PCB 180**
Correlation Coefficient	0.065	0.187	0.428+	-0.249	0.114	0.250	-0.222	0.267	-0.043
Sig. (2-tailed)	0.669	0.458	0.037	0.220	0.711	0.130	0.334	0.121	0.783
N	45	18	24	26	13	38	21	35	43
+. Correlation is significant at the 0.05 level (2-tailed). ++. Correlation is significant at the 0.01 level (2-tailed).									

Table. 4.22. Relationship between milk and dairy consumption behavior of the lactating women and level of DDTs in breast milk samples.

	DDTs	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT
Correlation Coefficient	0.092	0.022	0.082	0.084	-0.038	0.023	0.484++
Sig. (2-tailed)	0.558	0.923	0.704	0.638	0.831	0.927	0.006
N	43	21	24	34	35	18	31

+. Correlation is significant at the 0.05 level (2-tailed).

++. Correlation is significant at the 0.01 level (2-tailed).

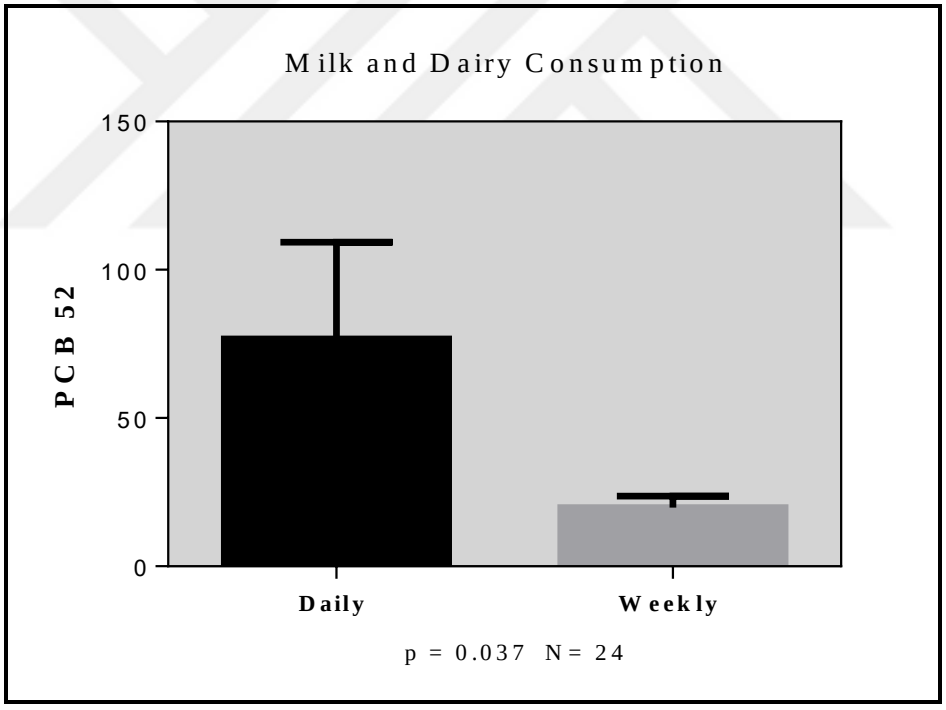

Figure 4.19. Correlation between dairy and milk consumption and PCB 52 levels in breast milk samples.

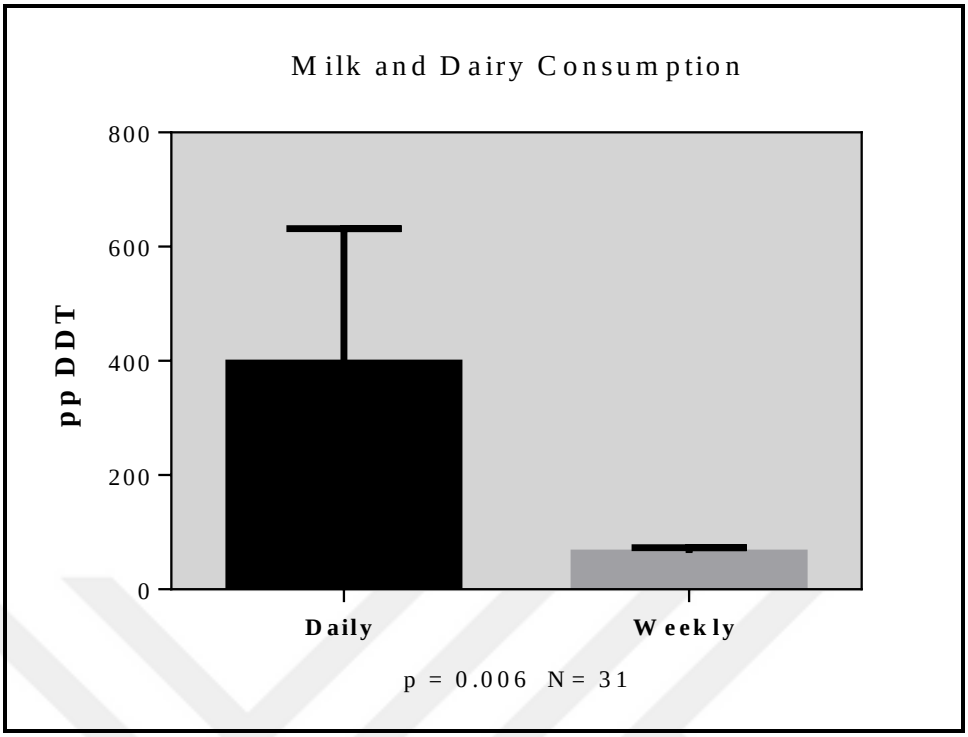

Figure 4.20. Correlation between dairy and milk consumption and p,p-DDT levels in breast milk samples.

5. DISCUSSION AND CONCLUSION

Human breast milk is a useful biological sample for monitoring environmental contaminants as POPS tend to bioaccumulate in lipid rich tissues in the body. Several epidemiological studies have utilized breast milk for monitoring POPs in various countries over the decades. Accumulation of OCPs in human breast milk can be affected by several epidemiological factors, such as diet, place of residence, occupation, age of first birth, maternal age and weight. However, their relationships are still under investigations by several research groups as different findings have been presented [103]. Studying correlations and adverse effects associated with a single EDC is complicated because humans are exposed to low doses of hundreds of chemicals starting at fetal life. In addition, human organisms are usually exposed to a mixture of chemicals, rather than a single contaminant. These features of exposure may generate a cocktail of endocrine disruptor effects with unknown consequences [104]. In the present study, we found that concentrations of PCBs and OCPs in human breast milk detected in the order of PCBs> DDTs> HCHs. We have shown that PCBs were the major contaminants in a total of 62 human breast milk samples obtained from volunteers living in Istanbul. Our findings

have indicated that PCB 153 was the majorly detected PCB, p,p-DDD was the most concentrated compound of DDTs and gamma-HCH was the mostly found HCHs in 62 of human breast milk samples.

The most establish risk factors that have contributed the breast cancer risk development are early menarche, late age at first pregnancy and birth, nulliparity, lactation, years of reproductive life, hormonal contraception and hormone replacement therapy [105]. Environmental factors are also associated with increased breast cancer incidence, these organochlorine compounds are agricultural pesticides (DDTs) and PCBs [106,107], even though consistent results have been also present [108,109]. PCBs and DDTs which are lipophilic organochlorines have been accumulated in fat and detected in human tissues and milk [109-111] showing direct exposure of the mammary tissues. Previous studies also showed their accumulation in adipose tissue [112], plasma lipids [112,113], fatty tissue of the breast [114,115]. Terminal end buds (TEBs) are one of the most important structures of the mammary gland as it is the most suitable part for the carcinogenic exposure [116]. It has been reported that PCBs may cause reduction in the TEB differentiation and delay in breast development. This results in presence of proliferating TEBs in long term interval that may elevate the susceptibility of the other carcinogens [117]. Infants have exposed to OCPs in prenatal period with placental transfusion and lactational period. As the infants have incomplete detoxification mechanisms and consumed higher food depending on their body weight, they may be exposed the elevated levels of OCPs [118,119]. In the light of this aspect, determination of the organochlorine pesticides in breast milk, provides us information not only breast cancer risk, but also health risk of infants in long term.

We found that PCB 153, PCB 101 and PCB 180 were detected at highest level among PCB congeners in human breast milk. These findings showed similarities with previous studies. Also, PCB 101, PCB 118, PCB 138 and PCB 153 have been specifically important congeners as they have been assumed as an indicator of the degree of the industrial contamination depending on Council for the Exploration of the Seas [120]. Therefore, presence of PCB 153, PCB 101 and PCB 180 in human breast milk were especially important in our study. Additionally, previous worldwide epidemiological studies associated with breast cancer showed that PCB 118, PCB 138, PCB 153 and PCB 180 have been the most frequently detected congeners [121-124]

especially in urine [122] and serum of blood [121-123]. Even though the rank of the abundance of the measured PCB congeners can also show variations depending on the selected region in the world [125,126], many studies revealed that PCB 153 was the most prevalently detected congener in human blood and tissues [127,128].

Previous studies claimed that half-lives of heavy congeners which are known as more chlorinated are higher than light congeners. Therefore, more chlorinated PCBs have been more toxic and associated with elevated breast cancer risk. This positive association of breast cancer risk was shown with previous investigations for PCB 118 [109,129-131], PCB 138 [126,130], PCB 180 [109,126,132].

Previously investigations implied that age was positively associated with PCB levels detected in breast tissue [133] and serum and adipose tissue of the breast cancer patients [134,135]. Concentrations of PCB-180 and ΣDDTs detected in human serum were positively correlated with age of the women [136]. It has also been shown that OCP and PCB amount in human breast milk was associated with age and number of children of the mothers [137]. Our findings were in agreement with previous investigations in which age has been positively correlated with OCs. We found that accumulated amount of PCB 180, DDTs and p,p-DDD in human breast milk were positively related with age of the lactating women. Additionally, age of the first birth of the women showed similar characteristics. We detected that p,p-DDD was positively associated with age of the first birth of the lactating women. Unexpectedly, only o,p-DDE was found as inversely correlated with age and age of the first birth of the participants. Also, we found that delta-HCH and DDTs amount were positively related with nursed children number.

Previous studies showed that PCB 153 exposure caused DNA damage [138]. In our study, we found that PCB 202 was positively associated with comet assay parameters which are TI and TM. However, negative correlation was also seen between comet assay parameters and HCHs and gamma-HCH.

Even though, the use of α-HCH, β-HCH, and lindane have banned and regulated by Stockholm Convention at 2010, these contents were shown in the human breast milk with several studies because of incorrect pesticide production. Also, HCH accumulation were displayed in the raw materials of foods such as milk, butter and cheese [139-142]. In our study, we found that significantly higher concentration of PCB 52 and p,p DDT

were detected in human breast milk for the participants who consumed dairy products more frequently.

We show that traces of OCPs and PCBs are still present in breast milk of lactating women residing in Istanbul. Our findings suggest that dietary factors contribute to accumulation of POPs in human breast milk of lactating women through milk and dairy consumption. It was also concluded that level of OCPs and PCBs increase in age. We also suggest that breast milk can be used as a representative and important biological tool for biomonitoring of environmental disrupter chemicals. As the PCBs and OCPs have lipophilic nature and accumulated in adipose tissue, therefore they have prolonged half-life in the body. The time interval between early exposure and emergence of disease may be long. Due to this reason, assessment of the full impact of POP exposure on human health is difficult and negative influences may develop at later ages. Time interval of the environmental toxicant exposure have been important issue as the neonates and fetuses are the most affected [143]. A possible strategy and prevention ways or reduction of exposures may be developed as the presence of these POPs have been proved in human biological samples in the light of the more and similar researches like our study. Possible prevention strategies and recommendations for protection from environmental POPs contamination have previously been suggested [2]. As the residues of PCBs and OCPs are still present in human breast milk samples, preventive and protective policies should be developed for the reduction of the usage and spread of these chemicals.

6. REFERENCES

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7. APPENDICES

7.1. Curriculum Vitae

NAME	Hajer Atya	SURNAME	Elazzuzi

EDUCATION

Degree	Department	The name of institution graduated from	Graduation Year
Bachelors	Medicine and surgery	Omar Almuktar univ.	2009-2010
High school	O levels	ALZAHRA SCHOOL	2000-2001

Language

Language	Grade	
ENGLISH	TOEFL-IBT, Proficiency	
TURKISH	A2	
ARABIC	Native speaker	

WORK EXPERIENCE

Position	Institute	Duration
Physician	7th October	2011-2014

Computer Skills

Program	Level	
MS office	Excellent	

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Articles published in other journals

Proceedings presented in international scientific meetings and published in proceedings book.

Journals in the proceedings book of the refereed conference / symposium

Others (Projects / Certificates / Rewards)
