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Effects of the interleukin-6 (IL-6) polymorphism on toxic metal and trace element levels in placental tissues

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ABSTRACT

The placenta is a crucial organ of fetal origin that functions in providing nutrients to the fetus from the mother. During pregnancy, the need for essential micronutrients, such as Fe and Zn, increases due to the requirements of the growing fetus. Maternal Fe deficiency induces an increase in Cu levels and can also affect cytokine levels in the placenta. On the other hand, Cu deficiency, although not as common, can also have destructive effects on the fetus. Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological activities, including such as immune responses, acute-phase reactions, and inflammation. The placenta produces a significant amount of IL-6 during pregnancy. The effects of the IL-6 −174 G/C single nucleotide polymorphism (SNP) on IL-6 gene transcription and on plasma cytokine levels were assessed in the present study. We investigated the association between the IL-6 −174 G/C polymorphism and trace element/toxic metal levels in placental tissues. For the purposes of this study, 95 healthy volunteers were evaluated. Presence of the IL-6 polymorphism was determined using the standard polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique, and metal levels were analyzed by atomic absorption spectrometry (AAS). Based on our data, there were no significant associations between the IL-6 −174 G/C polymorphism and Pb, Cd, Fe, or Zn levels in the placental tissues ($p>0.05$), but a statistically significant association was detected between the polymorphism and Cu levels ($p=0.016$). We determined that the mean Cu levels in the placental tissues from individuals with GG, GC and CC genotypes were 5.62 ± 1.98, 6.22 ± 3.22 and 8.00 ± 1.32 ppm, respectively, whereas the overall mean Cu level from the placental tissues was 5.98 ± 2.51 ppm.

1. Introduction

The placenta is the highly specialized organ maintained during pregnancy that, along with the fetal membranes and amniotic fluid, supports the normal growth and development of the fetus (Gude et al., 2004). The transfer of trace elements from the mother to the fetus via the placenta is also important for development of the human body (Honey et al., 1992). The placenta is the point of contact between maternal and fetal circulation and also serves as a barrier in preventing the passage of toxic substances, such as metals (Osman et al., 2000). Although the placenta does act as a barrier, it is unable to protect the fetus from exposure to certain toxic metals (Butler Walker et al., 2006). Studies on the transfer of these toxins from the mother's blood to the developing fetus have mostly been conducted in heavily-exposed individuals (Loiacono et al., 1992; Fagher et al., 1993). The placenta cannot completely prevent the fetus from exposure to lead and cadmium. Both of these metals, particularly cadmium (Osman et al., 2000), accumulate in the human placenta (Baghurst et al., 1991; Semczuk and Sikora, 2001) and may cause adverse perinatal effects, including reduced fetal growth and birth weight, fetal malformations, impaired transfer of essential micronutrients to the fetus and premature birth. Several studies have suggested that metal accumulation may interfere with the regulatory and nutritional functions of the placenta, potentially resulting in adverse effects on the fetus (Goyer, 1990). Currently, essential trace elements, specifically Fe, Zn, and Cu, have been studied extensively in the human placenta (Iyengar and Rapp, 2001). Iron deficiency during pregnancy is quite common (Fosset et al., 2004). Studies have shown that babies born to iron-deficient mothers are smaller in size than babies born to non-deficient mothers. Previous work has also demonstrated that these babies present with developmental problems, including neurological disorders (Beard et al., 2007), hypertension and other diseases (Zimmermann and Hurrell, 2007). Zinc participates in carbohydrate and protein metabolism, nucleic acid synthesis and other important functions. This element is required for cellular division and

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differentiation and is an essential nutrient for normal embryogenesis. The trace element copper is essential for several different enzyme systems, including the catalase, superoxide dismutase and cytochrome oxidase systems. Copper deficiency can lead to vascular disorders, emphasizing the importance of this trace element. Recently, the maternal-fetal status of certain essential trace elements in pregnant women during late gestation was assessed with respect to birth weight and placental weight (Al-Saleh et al., 2004), and elemental analysis of the human placenta was investigated with respect to neonate weight and maternal age (Carvalho et al., 2001).

Cytokines function to maintain homoeostasis during pregnancy, and they play a critical role in regulating placental formation. The placenta produces pro-inflammatory cytokines such as IL-6 (Robertson et al., 1994; Dudley et al., 1996; Griesinger et al., 2001), a glycoprotein with a molecular mass ranging from 21 to 28 kDa. The human IL-6 gene, located on chromosome 7p21 (Bowcock et al., 1988), is composed of five exons and four introns. IL-6 is a highly polymorphic gene in both the 5′ and 3′ flanking regions (Fishman et al., 1998), resulting in polymorphisms that can affect their rate of synthesis or degradation. One IL-6 polymorphism, the G/C base exchange located in the promoter region at position −174, has been shown to induce a biological function. The effects of −174 G/C polymorphism on IL-6 gene transcription and plasma cytokine levels have been demonstrated by several different genetic studies (Fishman et al., 1998; Olomolaiye et al., 1998). IL-6 functions in numerous biologic activities, including the regulation of immunocompetent cell growth and differentiation, induction of acute phase proteins, and stimulation or inhibition of cell growth depending on the target cell type. This cytokine also plays an active role in reproductive physiology by regulating ovarian steroid production and the early implantation event (Wu et al., 2004). The role of IL-6 expression during pregnancy, as well as its predictive significance for pregnancy outcome, is unclear. IL-6 also controls the induction and expression of metallothioneins (MTs), which function to maintain the homeostasis of zinc, copper and other trace elements and heavy metals (Mazzatti et al., 2008). MTs are low-molecular-weight, cysteine-rich proteins that have a high affinity for metals (Vasak, 2005). These proteins are induced by exposure to various heavy metals and are able to inhibit toxicity by binding to the metals (Miles et al., 2000). Increased IL-6 expression and production result in a strong upregulation of MT gene expression (Giacconi et al., 2004). The aims of the present study were to measure Cd, Pb, Fe, Zn and Cu levels in placental tissues and to determine whether the levels of these metals were associated with the G/C single nucleotide polymorphism (SNP) at position −174 in the promoter region of IL-6.

2. Materials and methods

2.1. Study subjects

In this study, the IL-6 −174 G/C SNP was examined in 95 healthy females (mean ages 29.73 ± 5.01 years; range 18 to 41 years), and levels of Pb, Cd, Fe, Zn and Cu levels were assessed in their placental tissues. Whole placentas were collected from the mothers who delivered at term neonates (mean gestational ages 272.12 ± 7.46 days) with normal birth weights (mean 3.32 ± 0.46 kg), birth length (mean 49.75 ± 1.97 cm), head circumferences (mean 35.28 ± 1.14 cm) and placental weights (mean 632.84 ± 152.98 g). Inclusion criteria included young, healthy, non-smoking and non-anemic pregnant mothers with normal pregnancies and without a history of alcohol or drug use. All mothers were asked to fill out a questionnaire, which included medical and dietary history, as well as data on occupational and potential environmental sources of metal exposure. Exclusion criteria for the subjects included a medical history of renal failure, carcinoma or diagnosed hepatic or cardiovascular diseases that may be related to possible heavy metal accumulation from environmental or occupational exposures. A small questionnaire used to gather demographic information was also given to individuals. Only Turkish subjects were included in the study. Placental and blood samples were handled in accordance with the principles of The Declaration of Helsinki. The present study was approved by the institutional ethics committee (approval no: 152–4828 in 2009).

2.2. Determination of metal levels

To avoid external metal contamination, each placenta was placed in a plastic bag immediately after delivery. Each bag was marked with the subject's identification code, placed on ice in a portable refrigerator and transported to the freezer in the Ankara University Analytical Toxicology Laboratory. In order to prevent any contamination originating from maternal blood and mucus, all placental samples were washed prior to analysis. Each sample was washed with 0.01% Triton X-100 solution and then 3 times with distilled water. Six representative samples were cut from each placenta using titanium tools, excluding the chorionic plate and decidua basalis, for the metal analysis (Pb, Cd, Fe, Zn and Cu). Two samples were taken from the center, avoiding the umbilical cord insertion, and four samples were taken from within 3 cm of the outer placental margin between the central region and the periphery. All tissue samples were stored at −20 °C in a refrigerator before analysis. Each sample was dried for 24 h at 75 °C and weighed. Samples were then dissolved in 10 ml of nitric acid in Teflon microwave tubes and digested at 800 W and 220 °C for 20 min in a CEM Mars Xpress microwave oven. Prior to analysis with dual atomic absorption spectrometry (AAS), the solutions were diluted with 25 ml deionized water in 50-ml polypropylene tubes.

Pb and Cd levels were quantified using Varian AA 240 Z Zeeman Graphite Atomic Absorption Spectrometry (GFAAS), whereas Fe, Zn and Cu levels were evaluated with Varian AA 240 FS Fast Sequential Atomic Absorption Spectrometry (FSAAS). The metal levels were given as ppm for Fe, Zn, Cu and as ppb for Cd and Pb. Additionally, the AAS method was validated by evaluating certified reference materials (Seronorm™ Trace Elements Whole Blood Level-2; Ref Number: 201605) with known values.

2.3. Determination of the IL-6 −174 G/C SNP by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method

Genomic DNA was isolated from 100-μl whole blood samples using a Qiagen QIAamp DNA Mini Kit, according to the manufacturer's instructions.

The −174 G/C SNP located in the promoter region of the IL-6 gene (SNP rs1800795; Gene access number: NM_000600; Gene ID: 3569) was genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method previously reported by Kayaaltı et al. (2011a). In order to screen for the IL-6 gene −174 G/C polymorphism, a 303-bp fragment containing the whole core promoter region was amplified by PCR with the following primers: forward: 5′-TTG TCA AGA CAT GCC AAA GTG CT-3′ and reverse: 5′-GCC TCA GAC ATC TCC AGT CC-3′. Amplification was conducted on a Techne Tc 512 PCR System in a 50-μl reaction mixture containing 200 μM of dNTPs, 10 pmol each of the forward (F) and reverse (R) primers, 1 U of Hot Star Taq DNA polymerase (Qiagen), 10× PCR buffer (Qiagen) and 50 ng of genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 5 min; 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min; and a final extension step at 72 °C for 5 min. The PCR product (303 bp) was then digested with *NlaIII* (New England Biolabs, Hertfordshire, UK) and incubated at 37 °C overnight. Digestion of the PCR product by *NlaIII* yields fragments that represent the presence of the C allele (122, 111, 57 and 13 bp fragments) and the G allele (233, 57 and 13 bp fragments). The undigested and digested polymerase chain reaction products were separated by gel electrophoresis on a 2.5%

agarose gel, visualized by ethidium bromide staining under an ultraviolet illuminator, and then scanned and photographed using the Syngene Monitoring System. Digested and undigested PCR products separated using agarose gel electrophoresis are indicated in Fig. 1.

2.4. Statistical analyses

Statistical analyses were performed using the SPSS Statistics 16 software package. Frequencies of IL-6 alleles and genotypes were obtained by direct count, and departure from the Hardy–Weinberg equilibrium was evaluated by the χ^2 test. In terms of metric variables, Student's *t*-tests were used in order to compare two independent groups, and the Kruskal–Wallis test was used to compare more than two groups. Correlations were assessed by determining the Pearson correlation coefficient. Throughout the analyses, 95% ($p<0.05$) and 99% ($p<0.01$) were selected as the minimum confidence levels.

3. Results

The aim of this study was to investigate the relationship between the IL-6 −174 G/C promoter region single nucleotide polymorphism and metal levels in placental tissues. This study assessed 95 healthy mothers. The mean age of these women was 29.62 ± 4.97 years (ranging from 18 to 41). Regarding the genotypes, a total of 56 pregnant women were homozygote-typical (GG), 33 were heterozygote (GC) and 6 were homozygote-atypical (CC) for the IL-6 polymorphism. The genotype frequencies in the 95 subjects were 58.95% for GG, 34.74% for GC and 6.31% for CC. The observed frequencies for the G and C alleles were 76.3% and 23.7%, respectively. The genotype and allele frequencies were consistent with Hardy–Weinberg equilibrium ($p>0.05$).

The IL-6 polymorphism was evaluated statistically for its association with placental weight, gestation age at delivery, birth weight, birth length, and head circumference; however, no significant associations were detected ($p>0.05$). The average Pb, Cd, Fe, Zn and Cu levels within the placental tissues were 8.16 ± 3.54 ppb, 19.42 ± 19.10 ppb, 537.98 ± 192.61 ppm, 49.96 ± 9.85 ppm and 5.98 ± 2.51 ppm, respectively (Table 1). Significant associations were not detected between the IL-6 −174 G/C polymorphism and Pb, Cd, Fe, or Zn levels within the placental samples. Alternatively, a statistically significant association was observed between the polymorphism and the Cu levels ($p=0.016$). The mean Cu levels in the placental tissues from individuals with GG, GC and CC genotypes were 5.62 ± 1.98 ppm, 6.22 ± 3.22 ppm and 8.00 ± 1.32 ppm, respectively, whereas the overall mean Cu level from all placental tissues was 5.98 ± 2.51 ppm.

Pb, Cd, Fe, Zn and Cu levels in placenta tissues were also analyzed according to IL-6 C- (GG genotype) and C+ (GC + CC genotypes) carriers. Pb and Cd levels were detected at 8.32 ± 4.21 and 20.82 ± 19.15 ppb, respectively, for individuals with the C- genotype and 7.92 ± 2.31 and 17.40 ± 19.08 ppb for individuals with the C+ genotype. Conversely, Fe, Zn and Cu levels were quantified as 520.19 ± 173.56, 49.69 ± 10.10, and 5.62 ± 1.99 ppm, respectively, for C− carriers and 563.53 ± 216.88, 50.34 ± 9.58, and 6.50 ± 3.06 ppm for C+ carriers. Based on these results, we can conclude that while both Pb and Cd levels in C- carriers were higher than in C+ carriers, Fe, Zn and Cu levels in C− carriers were lower than in C+ carriers. These results, however, were not statistically significant ($p>0.05$). Additionally, statistically significant correlations were detected between the metal levels. The highest positive correlation coefficients were found between Pb and Cd levels within placental tissues ($r=0.265$, $p<0.01$), whereas negative correlations were detected between Fe and Zn levels ($r=-0.225$, $p<0.05$). Significant correlation coefficients were not detected between levels of any of the metals and gestational age, birth weight, birth length, head circumference or placental weight.

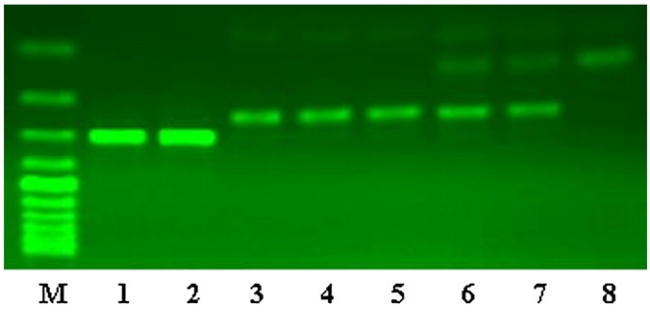

Fig. 1. A representative agarose gel image of undigested PCR products and PCR products digested with NlaIII: (M: 100-bp ladder, Lanes 1 and 2: undigested PCR product, Lanes 3, 4 and 5: homozygote-typical genotype (GG), Lanes 6 and 7: heterozygote genotype (GC), Lane 8: homozygote-atypical genotype (CC).

4. Discussion

IL-6 is a multifunctional cytokine produced by many different cell types, including immune cells, fibroblasts, endothelial cells, adipocytes and myocytes (Papanicolaou et al., 1998). Many studies have indicated a relationship between the IL-6 polymorphism and pre-term birth (Moura et al., 2009). Currently, the role of IL-6 expression during pregnancy, as well as its predictive significance for pregnancy outcome, remains unclear (Raghupathy et al., 1999). The placenta serves as an interface between fetal and maternal circulation and functions to mediate the exchange of gases and the transport of nutrients and waste products. This tissue has been widely regarded as an indicator organ following exposure to metals. Accumulation of toxic metals within placental tissue may result in abnormal placental function, leading to impaired nutrient transport (Zadorozhnaja et al., 2000). Previous work conducted in experimental studies has suggested an increased risk of miscarriage, fetal malformation, placental insufficiency and premature birth as results of metal exposure (Fagher et al., 1993; Laudanski et al., 1991).

Expression levels of inflammatory cytokines from trophoblasts and other cells in the normal placenta were previously reported (Kameda et al., 1990; Kauma et al., 1993). IL-6 levels in maternal serum (Arntzen et al., 1997), amniotic fluid (Olah et al., 1996), vaginal fluid (Imseis et al., 1997) and the placenta (Steinborn et al., 1996) were found to increase during the process of normal labor relative to the non-labor state. Previous work indicating a significant positive correlation between MT gene expression and IL-6 suggests that increased IL-6 expression is strictly related to an enhanced production of MTs (Giacconi et al., 2007). MTs are cysteine-rich proteins that detoxify heavy metals by binding to the metals, protecting cells and tissues from their toxic effects. The major functions of MTs include the maintenance of Zn homeostasis and protection of cells from the toxicity of metals (e.g., Cd and Pb). Additionally, transgenic mice that constantly overexpress metallothionein genes are considered tolerant to Cd, whereas knockout mice with defective metallothionein genes are more sensitive to cadmium toxicity than wild-type mice (Palmiter et al., 1993).

MT expression can be induced by certain elements (e.g., metals) (Kita et al., 2006), interleukins (Cousins and Leinart, 1988), ionizing radiation (Cai et al., 1999), ethanol (Kershaw et al., 1990), paraquat (Bauman et al., 1992), interferon (Sciavolino and Vilcek, 1995), tumor necrosis factor-alpha (Ebadi et al., 1996) and glucocorticoid hormones (Karin et al., 1984). A human study conducted by Kita and co-workers, however, demonstrated that the MT2A gene polymorphism can limit the expression of MT. As metal toxicity appears to be associated with low MT expression, low MT expression could potentially result in a predisposition for increased metal accumulation in people (Kita et al., 2006). Previous studies conducted by our group determined the distribution of the MT2A gene polymorphism in a Turkish population (Kayaalti and Söylemezoğlu, 2010) and found that individuals having the MT2A −5 GG and AG genotypes

Table 1
IL-6 polymorphism and Pb, Cd, Fe, Zn and Cu levels in placenta tissues.

Metals		IL-6 Polymorphism					Total
		GG n = 56	GC n = 33	CC n = 6	C− (GG) n = 56	C + (GC + CC) n = 39	n = 95
Pb(ppb)	Mean ± S.D.	8.32 ± 4.21	7.69 ± 2.29	9.20 ± 2.36	8.32 ± 4.21	7.92 ± 2.31	8.16 ± 3.54
	Median	7.65	7.41	10.56	7.65	7.45	7.48
	Min	3.31	3.51	6.12	3.31	3.51	3.31
	Max	28.53	12.38	11.04	28.53	12.38	28.53
	p	p = 0.471			p = 0.594		
Cd(ppb)	Mean ± S.D.	20.82 ± 19.15	15.53 ± 16.50	27.63 ± 29.62	20.82 ± 19.15	17.40 ± 19.08	19.42 ± 19.10
	Median	13.53	8.59	18.77	13.53	11.54	12.15
	Min	2.85	1.67	3.74	2.85	1.67	1.67
	Max	79.30	78.84	82.89	79.30	82.89	82.89
	p	p = 0.263			p = 0.392		
Fe(ppm)	Mean ± S.D.	520.19 ± 173.56	561.31 ± 225.04	575.69 ± 182.03	520.19 ± 173.56	563.53 ± 216.88	537.98 ± 192.61
	Median	477.56	523.19	540.00	477.56	523.19	493.01
	Min	94.94	288.49	407.23	94.94	288.49	94.94
	Max	1315.76	1414.55	888.39	1315.76	1414.55	1414.55
	p	p = 0.650			p = 0.283		
Zn(ppm)	Mean ± S.D.	49.69 ± 10.10	50.12 ± 9.80	51.54 ± 9.04	49.69 ± 10.10	50.34 ± 9.58	49.96 ± 9.85
	Median	50.55	49.75	51.14	50.55	49.75	49.97
	Min	22.98	27.51	40.08	22.98	27.51	22.98
	Max	74.93	72.26	61.43	74.93	72.26	74.93
	p	p = 0.899			p = 0.755		
Cu(ppm)	Mean ± S.D.	5.62 ± 1.98	6.22 ± 3.22	8.00 ± 1.32	5.62 ± 1.98	6.50 ± 3.06	5.98 ± 2.51
	Median	5.09	5.15	7.55	5.09	5.42	5.15
	Min	2.45	2.98	6.56	2.45	2.98	2.45
	Max	12.55	16.23	10.20	12.55	16.23	16.23
	p	**0.016***			p = 0.094		

* $p<0.05$.

have high levels of metal accumulation in the human renal cortex (Kayaalti et al., 2010) and in blood samples (Kayaaltı et al., 2011b). These studies also revealed the effects of the metallothionein 2A polymorphism on placental cadmium accumulation (Tekin et al., in press). Based on all these results, it is hypothesized that MT deficiency may enhance sensitivity to Cd and other toxic metals.

Additionally, interleukin-6 gene polymorphisms can indirectly cause low MT expression. The IL-6 polymorphism can affect the rate of IL-6 synthesis or degradation. As a result of IL-6 polymorphisms, we hypothesize that decreased IL-6 expression leads to reduced metallothionein expression, resulting in increased sensitivity to toxic metal accumulation by tissues.

In the present study, we assessed whether expression of the IL-6 −174 G/C polymorphism was associated with placental Pb, Cd, Fe, Zn and Cu levels and determined that the only statistically significant association was with placental Cu levels. Distribution of the IL-6 −174 CC genotype was observed at a very low frequency (only six individuals), and the C allele was rare in this gene polymorphism. Higher placental metal levels were detected in individuals with the CC genotype relative to individuals with the other IL-6 −174 GC and GG genotypes. One potential explanation for this observed effect is that the IL-6 CC genotypes result in decreased cytokine production, which leads to reduced MT expression. Because decreased MT expression results in reduced metal-binding capacity, this situation causes higher metal accumulation in placental tissues.

Based on the statistical analyses, a statistically significant positive correlation was detected between Pb and Cd levels ($r = 0.265$, $p<0.01$), and a significant negative correlation was detected between Fe and Zn levels ($r = -0.225$, $p<0.05$) in the placental tissues. Cu deficiency can induce Fe deficiency, and excessive Pb accumulation can lead to excessive Cd accumulation. Based on these results, we conclude that trace elements (Fe and Cu) and toxic metals (Pb and Cd) may utilize similar metabolic pathways for their transport and accumulation.

Currently, there are numerous studies focusing on the distribution of IL-6 gene polymorphisms in different populations and their association with various diseases, such as diabetes and atherosclerosis (Giacconi et al., 2005; Papanicolaou et al., 1998). In addition, we were able to demonstrate an association between the interleukin-6 −174 G/C promoter polymorphism and trace metal levels in autopsied kidney and liver tissues in a previous study (Yalçın et al., 2011). Although several studies have reported on toxic and trace elements in the placenta, maternal blood and umbilical cord blood (Rudge et al., 2009; Tekin et al., in press), this study is the first report evaluating the association between the IL-6 polymorphism and Pb, Cd, Fe, Zn and Cu levels in the placental tissues. The transport mechanisms for Pb, Cd, Fe, Zn and Cu appear to be different from each other. Because more work is needed to clarify the roles of these pathways, future studies should examine possible associations between the effects of various gene polymorphisms with respect to the transport of trace elements and accumulation of toxic metals in cord and maternal blood samples, as well as pathological findings and gene expression levels.

Conflict of interest

The authors declare that we have no conflict of interest.

References

Al-Saleh E, Nandakumaran M, Al-Shammari M, Al-Falah F, Al-Harouny A. Assesment of maternal-fetal status of some essential trace elements in pregnant women in late gestation: relationship with birth weight and placental weight. J Matern Fetal Neonatal Med 2004;16:9-14.

Arntzen KJ, Lien E, Austgulen R. Maternal serum levels of interleukin-6 and clinical characteristics of normal delivery at term. Acta Obstet Gynecol Scand 1997;76:55–60.

Baghurst PA, Robertson EF, Oldfield RK, King BM, McMichael AJ, Vimpani GV, et al. Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. Environ Health Perspect 1991;90:315–20.

Bauman JW, Madhu C, McKim Jr JM, Liu Y, Klaassen CD. Induction of hepatic metallothionein by paraquat. Toxicol Appl Pharmacol 1992;117:233–41.

Beard J, Ungar EL, Bianco LE, Paul T, Rundle SE, Jones BC. Early postnatal iron repletion overcomes lasting effects of gestation iron deficiency in rats. J Nutr 2007;137:1176–82.

Bowcock AM, Kidd JR, Lathrop GM, Daneshvar L, May LT, Ray A, et al. The human "interferon-beta 2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 1988;3:8-16.

Butler Walker J, Houseman J, Seddon L, McMullen E, Tofflemire K, Mills C, et al. Maternal and umbilical cord blood levels of mercury, lead, cadmium, and essential trace elements in Arctic Canada. Environ Res 2006;100:295–318.
Cai L, Satoh M, Tohyama C, Cherian MG. Metallothionein in radiation exposure: its induction and protective role. Toxicology 1999;132:85–98.
Carvalho ML, Custodio PJ, Reus U, Prange A. Elemental analysis of human amniotic fluid and placenta by total-reflection X-ray fluorescence and energy-dispersive X-ray fluorescence: child weight and maternal age dependence. Spectrochim Acta B 2001;56:2175–80.
Cousins RJ, Leinart AS. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 1988;2:2884–90.
Dudley DJ, Collmer D, Mitchell MD, Trautman MS. Inflammatory cytokine mRNA in human gestational tissues: implications for term and preterm labor. J Soc Gynecol Invest 1996;3:328–35.
Ebadi M, Leuschen MP, el Refaey H, Hamada FM, Rojas P. The antioxidant properties of zinc and metallothionein. Neurochem Int 1996;29:159–66.
Fagher U, Laudanski T, Schütz A, Sipowicz M, Akerlund M. The relationship between cadmium and lead burdens and preterm labor. Int J Gynaecol Obstet 1993;40: 109–14.
Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–76.
Fosset C, McGaw B, Abramovich D, McArdle HJ. Interrelations between ceruloplasmin and Fe status during pregnancy. Biol Trace Elem Res 2004;98:1-12.
Giacconi R, Cipriano C, Albanese F, Boccoli G, Saba V, Olivieri F, et al. The −174 G/C polymorphism of IL-6 is useful to screen old subjects at risk for atherosclerosis or to reach successful ageing. Exp Gerontol 2004;39:621–8.
Giacconi R, Cipriano C, Muti E, Costarelli L, Maurizio C, Saba V, et al. Novel -209A/G MT2A polymorphism in old patients with type 2 diabetes and atherosclerosis: relationship with inflammation (IL-6) and zinc. Biogerontology 2005;6:407–13.
Giacconi R, Caruso C, Lio D, Muti E, Cipriano C, Costarelli L, et al. CD14 C (−260)T polymorphism, atherosclerosis, elderly: role of cytokines and metallothioneins. Int J Cardiol 2007;120:45–51.
Goyer RA. Transplacental transport of lead. Environ Health Perspect 1990;89:101–5.
Griesinger G, Saleh L, Bauer S, Husslein P, Knöfler M. Production of pro- and anti-inflammatory cytokines of human placental trophoblasts in response to pathogenic bacteria. J Soc Gynecol Invest 2001;8:334–40.
Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. Thromb Res 2004;114:397–407.
Honey S, Dhall GI, Nath R. Distribution of zinc and copper in human placenta and fetal membranes at various periods of gestation. J Trace Elem Exp Med 1992;5:165–73.
Imseis HM, Greig PC, Livengood III CH, Shunior E, Durda P, Erikson M. Characterization of the inflammatory cytokines in the vagina during pregnancy and labor and with bacterial vaginosis. J Soc Gynecol Investig 1997;4:90–4.
Iyengar GV, Rapp A. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 2: essential minor, trace and other (non-essential) elements in human placenta. Sci Total Environ 2001;280:207–19.
Kameda T, Matsuzaki N, Sawai K, Okada T, Saji F, Matsuda T, et al. Production of interleukin-6 by normal human trophoblast. Placenta 1990;11:205–13.
Karin M, Haslinger A, Holtgreve H, Richards RI, Krauter P, Westphal HM, et al. Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIA gene. Nature 1984;308:513–9.
Kauma SW, Herman K, Wang Y, Walsh SW. Differential mRNA expression and production of interleukin-6 in placental trophoblast and villous core compartments. Am J Reprod Immunol 1993;30:131–5.
Kayaaltı Z, Şahiner L, Durakoğlugil E, Söylemezoğlu T. Distributions of interleukin-6 (IL-6) promoter and metallothionein 2A (MT2A) core promoter region gene polymorphisms and their associations with aging in Turkish population. Arch Gerontol Geriatr 2011a;53:354–8.
Kayaaltı Z, Aliyev V, Söylemezoğlu T. The potential effect of metallothionein 2A–5 A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. Toxicol Appl Pharmacol 2011b;256:1–7.
Kayaalti Z, Mergen G, Söylemezoğlu T. Effect of metallothionein core promoter region polymorphism on cadmium, zinc and copper levels in autopsy kidney tissues from a Turkish population. Toxicol Appl Pharmacol 2010;245:252–5.
Kayaalti Z, Söylemezoğlu T. The polymorphism of core promoter region on metallothionein 2A-metal binding protein in Turkish population. Mol Biol Rep 2010;37:185–90.
Kershaw WC, Iga T, Klaassen CD. Ethanol decreases cadmium hepatotoxicity in rats: possible role of hepatic metallothionein induction. Toxicol Appl Pharmacol 1990;106:448–55.
Kita K, Miura N, Yoshida M, Yamazaki K, Ohkubo T, Imai Y, et al. Potential effect on cellular response to cadmium of a single-nucleotide A→G polymorphism in the promoter of the human gene for metallothionein IIA. Hum Genet 2006;120:553–60.
Laudanski T, Sipowicz M, Modzelewski P, Bolinski J, Szamatowicz J, Razniewska G, et al. Influence of high lead and cadmium soil content on human reproductive outcome. Int J Gynaecol Obstet 1991;36:309–15.
Loiacono NJ, Graziano JH, Kline JK, Popovac D, Ahmedi X, Gashi E, et al. Placental cadmium and birthweight in women living near a lead smelter. Arch Environ Health 1992;47:250–5.
Mazzatti DJ, Malavolta M, White AJ, Costarelli L, Giacconi R, Muti E, et al. Effects of interleukin-6–174 C/G and metallothionein 1A +647A/C single-nucleotide polymorphisms on zinc-regulated gene expression in ageing. Exp Gerontol 2008;43: 423–32.
Miles AT, Hawksworth GM, Beattie JH, Rodilla V. Induction, regulation, degradation, and biological significance of mammalian metallothioneins. Crit Rev Biochem Mol Biol 2000;35:35–70.
Moura E, Mattar R, de Souza E, Torloni MR, Gonçalves-Primo A, Daher S. Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. J Reprod Immunol 2009;80:115–21.
Olah KS, Vince GS, Neilson JP, Deniz G, Johnson PM. Interleukin-6, interferon-gamma, interleukin-8, and granulocyte-macrophage colony stimulating factor levels in human amniotic fluid at term. J Reprod Immunol 1996;32:89–98.
Olomolaiye O, Wood NA, Bidwell JL. A novel NlaIII polymorphism in the human IL-6 promoter. Eur J Immunogenet 1998;25:267.
Osman K, Akesson A, Berglund M, Bremme K, Schütz A, Ask K, et al. Toxic and essential elements in placentas of Swedish women. Clin Biochem 2000;33:I31-I138.
Palmiter RD, Sandgren EP, Koeller DM, Brinster RL. Distal regulatory elements from the mouse metallothionein locus stimulate gene expression in transgenic mice. Mol Cell Biol 1993;3:5266–75.
Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 1998;128:127–37.
Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E. Maternal Th1- and Th2- type reactivity to placental antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. Cell Immunol 1999;196: 122–30.
Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The role of cytokines in gestation. Crit Rev Immunol 1994;14:239–92.
Rudge CV, Röllin HB, Nogueira CM, Thomassen Y, Rudge MC, Odland JØ. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. J Environ Monit 2009;11:1322–30.
Sciavolino PJ, Vilcek J. Regulation of metallothionein gene expression by TNF-alpha and IFN-beta in human fibroblasts. Cytokine 1995;7:242–50.
Semczuk M, Sikora A. New data on toxic metal intoxication (Cd, Pb, and Hg in particular) and Mg status during pregnancy. Med Sci Monit 2001;7:332–40.
Steinborn A, Gunes H, Roddiger S, Halberstadt E. Elevated placental cytokine release, a process associated with preterm labor in the absence of intrauterine infection. Obstet Gynecol 1996;88:534–9.
Tekin D, Kayaaltı Z, Aliyev V, Söylemezoğlu T. The effects of metallothionein 2A polymorphism on placental cadmium accumulation: is metallothionein a modifiying factor in transfer of micronutrients to the fetus? J Appl Toxicol in press. doi 10.1002/jat.1661.
Vasak M. Advances in metallothionein structure and functions. J Trace Elem Med Biol 2005;19:13–7.
Wu R, Van der Hoek KH, Ryan NK, Norman RJ, Robker RL. Macrophage contributions to ovarian function. Hum Reprod Update 2004;10:119–33.
Yalçın S, Kayaaltı Z, Söylemezoğlu T. Role of interleukin-6–174 G/C promoter polymorphism in trace metal levels of autopsy kidney and liver tissues. Int J Hyg Environ Health 2011;214:219–24.
Zadorozhnaja TD, Little RE, Miller RK, Mendel NA, Taylor RJ, Presley BJ, et al. Concentrations of arsenic, cadmium, copper, lead, mercury and zinc in human placentas from two cities in Ukraine. J Toxicol Environ Health A 2000;61:255–63.
Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet 2007;370:511–20.