Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile

Humberto Reyes[1,2], María E. Báez[3], Manuel C. González[1], Ismael Hernández[2], Joaquín Palma[1], José Ribalta[1], Lorena Sandoval[1] and Rodrigo Zapata[1]

[1]*Department of Medicine, Eastern Campus, Hospital del Salvador, and* [2]*Institute of Biomedical Sciences, Faculty of Medicine,* [3]*Department of Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago de Chile*

Background/Aims: **Low blood Se levels have been previously shown in normal pregnancies (third trimester) and significantly lower levels in patients with intrahepatic cholestasis of pregnancy (ICP), in Finland and in Chile, suggesting that a low or marginal dietary availability of Se may contribute to the pathogenesis of this disease. The aim of this study was to investigate whether a temporal change in plasma concentration of Se, and seasonal fluctuations in plasma concentrations of Se, Zn and Cu, could coincide with changes in the prevalence of ICP.**

Methods: **A cross-sectional cohort study was done including 21 ICP patients, 98 women in the third trimester of a normal pregnancy, 29 non-pregnant women, and also 13 individuals (seven non-pregnant women and six men) who had been studied 9 years before. Plasma Se, Zn and Cu were measured by atomic spectroscopy. Plasma Se levels in the present study were compared to the results obtained 5 to 7 years before, employing identical methodology in similar population samples.**

Results: **Plasma Se concentrations in non-pregnant women were higher than in the previous study: 1.43 ± 0.34 μmol/l *vs* 0.85 ± 0.13; $p<0.001$. In comparison to non-pregnant women, normal pregnancies near term had lower plasma levels of Se: 1.08 ± 0.25 μmol/l; $p<0.01$, and Zn: 17.90 ± 3.61 μmol/l *vs* 19.71 ± 3.21; $p<0.05$, but higher plasma levels of Cu: 34.35 ± 7.12 μmol/l *vs* 20.62 ± 3.34; $p<0.01$. In normal pregnancies, plasma Se concentration was significantly higher in summer (1.34 ± 0.19 μmol/l) than in the other seasons, while Zn and Cu diminished. Similar to previous studies, ICP patients had significantly lower Se plasma levels than normal pregnancies: 0.94 ± 0.12 μmol/l, $p<0.05$, and Cu levels were significantly higher: 50.80 ± 7.02 μmol/l, $p<0.01$. Cu plasma levels correlated with the biochemical severity of the disease. Zn did not change in ICP.**

Conclusions: The present study shows that the decrease in the prevalence of ICP in Chile during the last decade coincides with an increase in plasma Se levels. Its lower incidence during summer coincides with a higher plasma Se concentration in summer than in other seasons, as observed in normal pregnancies.

Key words: **Bile acids and salts; Cholestasis, intrahepatic; Copper; Pregnancy trimester, third; Selenium; Zinc.**

INTRAHEPATIC cholestasis of pregnancy (ICP) is a rare disorder of late pregnancy, characterized by skin pruritus and mild biochemical cholestasis appearing during the third trimester of pregnancy and disappearing shortly after delivery, with a tendency to recur in future pregnancies. Its clinical relevance is related to an increased risk of premature deliveries – with a subsequent increase in perinatal morbidity and mortality – and also to a high risk of stillbirths (1–3).

The cause of ICP is unknown. A genetic predisposition can explain familial aggregation of cases in two or three generations and the higher prevalence of the disease seen in some geographic locations (Scandinavian countries and in Chile) and in certain ethnic groups (i.e. Araucanian-indian admixed population, in Chile). However, some epidemiological features suggest the participation of environmental factors: ICP recurs

Correspondence: Humberto Reyes, Departamento de Medicina Oriente, Facultad de Medicina, Universidad de Chile, Casilla 16038, Santiago 9, Chile.
Tel: 56 2 2741560. Fax: 56 2 2741628.
e-mail: htoreyes@reuna.cl

only in 50–60% of pregnancies in multiparous patients and its severity varies in subsequent pregnancies; more cases are diagnosed in winter than in other seasons, as observed in Finland and in Chile; finally, its prevalence has diminished in the last decades, first in Sweden (where it reached a peak of about 2% of deliveries in the late fifties and currently is almost anecdotal) and afterwards in Chile (with a peak near 14% of deliveries in the late sixties, declining progressively to 6% in recent years) (1).

Currently, no evidence is available to incriminate any specific environmental factor in these epidemiological changes. Dietary components are primary subjects of research because changes in their local availability could influence bile secretion and composition during pregnancy and/or the hepatic metabolism of sex hormones (4–7).

In 1987, Kauppila et al. reported that ICP patients in Finland had significantly lower levels of selenium in serum and erythrocytes than normal pregnant women, correlating with a low activity of the selenium-dependent enzyme glutathione peroxidase (8). Blood levels of selenium in population samples in Finland were lower than in other groups in Europe and in North America, attributable to a low selenium content in soil determining a marginal dietary supply of this essential trace element (9–11). In a similar study in Chile, our group observed that:

1) The selenium concentration in plasma from 15 healthy men and 43 healthy non-pregnant women living in the Eastern District of Santiago was 0.83±0.11 (mean, SD) μmol/l. This figure was closer to the levels reported in the Finnish population (mean 0.57 μmol/l) than in other European countries or the USA, where the range of mean values reported vary from 1.77 to 2.66 μmol/l.

2) In 22 women with normal pregnancies, sampled weekly or biweekly in the third trimester, the selenium concentration in plasma and erythrocytes was lower than in non-pregnant women; these parameters diminished mildly but progressively during the third trimester, and in the final weeks (36 to 40) the selenium concentration in plasma was 0.60±0.08 μmol/l. Twenty four to 72 h after delivery plasma Se concentration increased almost to non-pregnant levels.

3) Ten patients with ICP had even lower plasma selenium levels (0.54±0.06 μmol/l) and, in contrast to normal pregnancies, it remained stable during the third trimester and did not recover in the early puerperium (12).

The reports from Kauppila et al., and our previous study, raised the question whether a low or marginal dietary supply of selenium could play a role in the pathogenesis of ICP, particularly in genetically predisposed individuals.

Encouraged by the decrease in the prevalence of ICP in Chile and by its seasonal variability, we decided to evaluate possible changes in plasma levels of Se in recent years and seasonal fluctuations that could coincide with the epidemiological profile of ICP. To fulfill these objectives, we examined new groups of ICP patients and healthy pregnant and non-pregnant women, and men, applying identical methodology to measure selenium in plasma, so that we could compare our current results with those obtained in the previous study. We also added measurements of zinc and copper concentrations in plasma. Higher serum levels of copper have been reported in patients with ICP than in normal pregnancies (13,14). Other trace elements that participate as cofactors in some enzymatic systems, such as zinc, have not been studied in this clinical situation, and the possibility that they could be related to bile secretory failure has not yet been examined.

Patients and Methods

Population samples

Patients with intrahepatic cholestasis of pregnancy (ICP): During 1994–1995, 21 ICP patients hospitalized in the obstetric wards of our hospital, were selected for this study. All were inhabitants of the Eastern District of Santiago, and their ethnic and socio-economic characteristics were similar to the control groups.

The diagnosis of ICP was based on the finding of pruritus appearing during the second half of pregnancy, without skin lesions before scratching, plus abnormal serum levels of at least one aminotransferase (upper limit in normal pregnancies: 40 IU/l) and/or total bile salts (upper limit: 11 μmol/l) in 2 blood samples taken with a 1-week interval. One patient had a twin pregnancy. Twelve patients were multiparous and 11 of them had had ICP in one or more of their previous pregnancies. None of the ICP patients diagnosed as having ICP had a past history of biliary colicky pain or acute hepatitis during the current pregnancy; no drugs or hormones (i.e. progesterone) had been taken and no other diseases could explain pruritus and abnormal liver tests. Blood samples for trace element determinations were collected during the third trimester of pregnancy, 1 to 8 weeks (median 2.5) after the onset of pruritus. Patients were followed up for at least 2 months after delivery, verifying the disappearance of pruritus and a return to normality in their serum liver tests.

Normal pregnancies: Ninety-eight healthy women in the third trimester of a normal pregnancy were randomly chosen among the population of patients who had periodic check-ups of their pregnancies in a primary care outpatient public facility in the Santiago Eastern District. They lived in a neighborhood sharing a common water supply. In 3 water samples collected during the study period, Se, Zn and Cu were undetected by atomic absorption/mass spectroscopy.

These women had no past history of liver or other acute or chronic diseases and they were non-smokers and non-alcoholics; they had a normal physical examination and normal basic laboratory tests, including blood glucose, urea nitrogen, hematocrit, urine profile, serum total bilirubin, alanine and aspartate aminotransferases, and total bile salts. All had single pregnancies. Fifty-seven multiparous women had a past history of normal pregnancies with deliveries at term. There was no history of medication use for at least 6 weeks before blood sampling, except vitamins and iron, without other trace elements.

These 98 healthy pregnant women were recruited at weekly intervals, trying to balance them in order to have a similar number of

individuals examined in each season during 1 year, from June 1994 to May 1995. Taking advantage of the fact that the climate in Santiago allows clear differences among the four seasons, their limits were established at 3-month intervals, as follows: June–August=winter; September–November=spring; December–February=summer; March–May=autumn.

Non-pregnant women: During the same study period (1994–1995), blood samples were obtained from 29 healthy non-pregnant women selected among individuals applying for family planning with intrauterine devices, in the same public health care facility.

New measurements in individuals studied 9 years before: Blood samples were collected in July 1997 from seven women and six men whose plasma Se levels had been measured in 1988 (12). They were the only members of a group of 43 healthy non-pregnant women and 15 men from our previous study who could be studied again, after 9 years. All of them had remained healthy since their first sampling and the women had not had new pregnancies between the studies.

Blood sampling

After overnight fasting, blood samples were drawn between 8 and 10 AM, using heparinized vacutainers for trace element determinations. Commercially available vacutainers prepared with different anticoagulants, or none, were used for other laboratory tests. The samples were immediately transferred to our laboratory, where plasma was separated after slow-speed centrifugation and stored in plastic vials at −20°C until analysis was performed. All plastic and glass material used in these procedures had been thoroughly washed and rinsed with demineralized and distilled water.

Laboratory determinations

Atomic spectroscopy determinations of Se, Zn and Cu were carried out with a Perkin Elmer 1100B atomic absorption spectrometer. A FIAS 400 System for the hydride vapor generation technique and an electrodeless discharge lamp were used for Se determinations. Plasma samples with 1 ml volume were digested in a thermostatically controlled aluminium block by a nitric acid-perchloric acid mixture (2+1 v/v) based procedure (15,16). Briefly, 3.0 ml of the acid mixture were added and a slow rate of heating was employed to reach 150°C. This temperature was maintained until brown fumes of nitric acid had disappeared and fumes of perchloric acid had become visible. The temperature was raised to 200°C and held for 30 min, to ensure the complete removal of nitric acid and the total conversion of organic forms of Se into selenious acid. The final step included the addition of 0.5 ml of 6 M hydrochloric acid and heating at 150°C for 15 min to reduce Se VI to Se IV. Digests were directly analysed for Zn and Cu concentrations with flame atomization and for Se measurements they were diluted 1:10 in 10% v/v hydrochloric acid. $NaBH_4$ at 0.2% in NaOH 0.05% and HCl 10% v/v were used for the hydride generation.

The method used to measure Se in plasma was identical to the method used in the previous study. Sixteen duplicate plasma samples from healthy non-pregnant women examined in 1988–1990, had been kept frozen at −20°C in tightly closed plastic tubes. These duplicates were defrosted in 1998 and Se concentration was measured in them. In the plasma samples studied in 1988–90, Se concentration was 0.78±0.14 μmol/l and in the duplicates processed now it was 0.79±0.13 μmol/l.

Serum liver tests and other biochemical measurements were performed using routine laboratory techniques. Serum total bile salts were measured with a commercially available kit (Merckotest®), based on its enzymatic measurement using α-hydroxysteroid dehydrogenase.

Ethical considerations

The study protocol was approved by the Ethics Committee, Hospital del Salvador, Santiago, Chile. Individuals included in this study gave their informed consent to participate in it.

Data analysis

The results are reported as group mean values±S.D. Differences were compared by Student's *t*-test for unpaired samples. ANOVA was used in the comparison of trace elements among seasons and in different gestational ages. Statistical significance was established at $p<0.05$.

Results

Clinical and laboratory characteristics of the main population samples (Table 1)

Age and parity were similar in the main groups included in this study: healthy women (non-pregnant or in the 3rd trimester of a normal pregnancy) and in patients with ICP.

Serum liver tests remained normal during the third trimester of normal pregnancies, with the exceptions of an increase in serum total alkaline phosphatases and a decrease in serum total protein and albumin concentrations.

TABLE 1

Baseline clinical and laboratory characteristics of women population samples

	Non-pregnant $n=29$	Normal pregnancies $n=98$	ICP $n=21$
Epidemiological data			
Age, years	29.3±6.8 (19–41)	25.7±6.2 (15–38)	27.7±5.9 (16–40)
Parity	2.1±1.4 (0–7)	1.2±1.5 (0–6)	3.0±0.8 (0–4)
Gestational age, weeks, median (range)	–	35 (28–39)	33 (27–37)
Serum liver tests:			
Bilirubin (mg/dl)	0.56±0.31 (0.15–1.10)	0.42±0.17 (0.16–1.11)	0.74±0.68[a] (0.17–2.82)
ALT (IU/l)	12±6 (4–37)	13±7 (3–40)	78±97[a,b] (6–308)
AST (IU/l)	20±8 (13–40)	20±6 (9–40)	45±43[a,b] (11–194)
Bile salts (μmol/l)	7.2±2.2 (1–9)	8.5±2.1 (1–11)	29.8±35.6[a,b] (3.7–178.4)
AP (IU/l)	80±30 (42–174)	176±66[b] (70–412)	201±105[b] (59–502)
GGT (IU/l)	16±9 (9–29)	12±6 (3–40)	22±19[a] (6–101)
Protein (g/dl)	7.4±0.5 (6.3–8.4)	6.7±0.4[b] (5.7–7.7)	6.5±0.6[b] (5.2–7.3)
Albumin (g/dl)	4.7±0.5 (3.1–5.7)	3.7±0.5[b] (2.6–5.3)	3.1±0.7[a,b] (2.0–4.6)

Values are expressed as mean±SD, (range); n=number of individuals sampled; ICP: intrahepatic cholestasis of pregnancy; ALT: alanine aminotransferase; AST: aspartate aminotransferase; AP: alkaline phosphatase; GGT: γ-glutamyltranspeptidase; [a]$p<0.01$, compared to normal pregnancies, [b]$p<0.001$, compared to non-pregnant women.

TABLE 2

Selenium, zinc and copper concentration in plasma from healthy non-pregnant and pregnant women (3rd trimester of pregnancy), and in patients with intrahepatic cholestasis of pregnancy

	Non-pregnant women	Normal pregnancies			ICP		
		27–32 weeks	33–35 weeks	>36 weeks	27–32 weeks	33–35 weeks	>36 weeks
	n=29	n=34	n=47	n=45	n=14	n=22	n=14
Se (μmol/l)	1.43±0.34	1.27±0.21[b]	1.15±0.20[a]	1.08±0.25[a]	1.01±0.13[c]	1.03±0.16[d]	0.94±0.12[d]
Zn (μmol/l)	19.71±3.21	16.21±5.10[a]	16.84±5.21[b]	17.90±3.61[b]	15.62±1.60	15.57±1.80	15.88±1.92
Cu (μmol/l)	20.62±3.34	31.95±6.28[a]	34.26±5.85[a]	34.35±7.12[a]	46.64±12.02[c]	54.50±11.56[c]	50.80±7.02[c]

Results are expressed as mean±SD; ICP: intrahepatic cholestasis of pregnancy; n=number of plasma samples; [a]$p<0.01$, [b]$p<0.05$, compared to non-pregnant women; [c]$p<0.01$, [d]$p<0.05$, compared to normal pregnancies in the same gestational period.

Patients with ICP had significantly higher mean values of serum total bilirubin, both aminotransferases and total bile salts than normal pregnancies. Only 4/21 ICP patients had hyperbilirubinemia (range 1.11 to 2.82 mg/dl) and in only five ICP patients serum γ-glutamyl transferase activity was over the upper limit detectable in healthy adult women (including normal pregnancies).

Se, Zn and Cu concentrations in plasma from healthy non-pregnant and pregnant women and in patients with ICP (Table 2)

In 29 healthy non-pregnant women, Se concentration in plasma was significantly higher than the values observed 5–7 years before in a similar sample of 43 healthy non-pregnant women: 1.43±0.34 μmol/l *versus* 0.85±0.13 ($p<0.001$).

Because in our previous longitudinal study Se concentration in plasma decreased mildly but significantly during the third trimester of normal pregnancies, in the present cross-sectional study the results were also stratified into three gestational periods: 27–32 weeks, 33–35 weeks and 36 weeks of pregnancy or more.

In normal pregnancies and in the three periods of the third trimester, Se and Zn concentrations in plasma were significantly lower than in non-pregnant women. The magnitude of change was dissimilar for these two trace elements: Se diminished progressively as the age of pregnancy advanced, lowering by the end of pregnancy to 75% of the values observed in non-pregnant controls; linear regression analysis showed $r=-0.36$, $p<0.001$; simultaneously, Zn concentration was mildly low (90% of the values in non-pregnant women) and remained stable during the 3rd trimester of pregnancy. Cu concentration was significantly higher than in non-pregnant women and remained stable during the 3rd trimester of normal pregnancies.

In normal pregnancies studied in 1994–1995, plasma Se concentration was significantly higher ($p<0.001$) than in normal pregnancies studied in 1988–1991, in each one of the 3 time-periods of the third trimester of pregnancy.

Patients with ICP had a lower Se concentration in plasma than normal pregnancies with similar gestational age range, diminishing to 65% of the levels seen in non-pregnant women. In ICP patients, no difference was detected in Zn concentration in plasma when compared to normal pregnancies, but Cu concentration was significantly higher, double the values observed in non-pregnant women.

In patients with ICP, plasma samples were obtained at different times (from 1 to 8 weeks) after the onset of pruritus. No correlation was found between Se concentration in plasma and several indexes of severity of ICP, such as previous time (weeks) with pruritus ($r=0.07$; $p=0.5$), serum levels of bilirubin ($r=0.02$; $p=0.9$), ALT ($r=0.16$; $p=0.5$), AST ($r=0.02$; $p=0.9$) and bile salts ($r=0.2$; $p=0.4$). In contrast, Cu concentration in plasma had a positive correlation with previous time (weeks) with pruritus ($r=0.28$; $p=0.03$), serum levels of bilirubin ($r=0.73$; $p<0.001$), ALT ($r=0.47$; $p=0.04$), AST ($r=0.65$; $p<0.003$) and bile salts ($r=0.61$; $p=0.007$).

Se concentration in plasma from healthy individuals, compared to their own values 9 years before

In 13 individuals (7 women) studied in 1988, new blood samples obtained in 1997 showed a significantly higher plasma Se concentration than 9 years before (Table 3).

TABLE 3

Comparison of Se concentration in plasma from healthy women and men studied 9 years ago

	1988	1997
	μmol/l	
Women, non-pregnant (n=7)	0.86±0.09	1.21±0.05[a]
Men (n=6)	0.87±0.19	1.22±0.09[a]

Values are expressed as group mean±SD. [a]$p<0.001$.

TABLE 4

Selenium, zinc and copper concentration in plasma from normal pregnancies (3rd trimester), in the four seasons

	Winter $n=30$	Spring $n=32$	Summer $n=34$	Autumn $n=30$
Selenium (μmol/l)	1.11±0.21	1.03±0.22	1.34±0.19[a]	1.10±0.18
Zinc (μmol/l)	19.51±2.41	19.97±3.00	16.21±5.37[a]	12.48±2.75[a]
Copper (μmol/l)	33.42±7.29	34.47±6.04	30.57±5.70[b]	36.71±5.51

Results are expressed as mean±SD. [a]$p<0.05$ with every other season; [b]$p<0.05$ in comparison with spring and autumn.

In our previous study, Zn and Cu measurements were not performed in them and therefore these trace elements could not be compared with the present study.

Seasonal differences in Se, Zn and Cu concentrations in plasma from healthy pregnant women (Table 4)
The proportion of individuals at different gestational ages was evenly distributed in every season; therefore, the results obtained during the third trimester were pooled into each season.

Selenium concentrations in plasma from women in the 3rd trimester of a normal pregnancy were significantly higher in samples obtained in summer, while no differences were observed among the other seasons. In contrast, Zn concentrations were lower in summer and in autumn, and Cu concentrations were also lower in summer than in other seasons.

Discussion

The present study shows that in 1994–1995 the concentration of Se in plasma of healthy adults living in Santiago, Chile was significantly higher than 5–7 years before, using identical methodology and measured by the same investigators. This observation includes a comparison between similar groups of non-pregnant women and also an intragroup comparison in a small number of women and men who were studied twice, with a 9-year interval.

The concentration of Se in blood from healthy individuals is accepted as a good reflection of the dietary supply of this trace element and is greatly dependent on agricultural soil composition and the variety of local food, giving rise to an important geographical and seasonal variability (17–20). A measurement of dietary content of Se was not included in our studies. However, Ruz et al. reported that healthy adults from different geographical regions in Chile, sampled also in 1994, had a dietary Se intake (estimated by direct analysis of 24-h duplicate diet composites) of 71.7±54.5 μg/d and the average plasma Se concentration (measured by neutron activation analysis) was similar to our results: 1.44±0.5 μmol/l (21,22). These plasma Se concentrations are comparable to those observed in countries referred to as having an "intermediate" abundance of Se, while the values obtained by us 5–7 years before were then closer to countries with a "marginal" dietary Se availability.

Plasma Se concentration was lower in pregnant women studied in the third trimester of normal pregnancies. Similar to our previous study, plasma Se concentration tended to diminish progressively during the third trimester of pregnancy. In pregnancy, plasma Se concentration is influenced by hemodilution and fetal-placental consumption (23–25); both factors tend to be intensified as pregnancy progresses, lowering progressively the concentration of Se. Our findings coincide with several other studies that reported low Se levels in the blood of pregnant women (23–25,26–37). A low status of Se and/or Zn in pregnancy has been hypothetically related to miscarriage (32), pre-eclampsia and premature delivery (35,37), and fetal anatomical (26) or biochemical abnormalities (33), although with controversial results.

The role of selenium as a cofactor of several enzymes related to oxidative metabolism in the liver and other tissues is well documented, but no relationship has yet been demonstrated with regard to bile secretion (38–42). Experimental studies of bile flow and biliary lipids composition in rats fed low-selenium diets have shown inconsistent or preliminary changes (43–45). Abnormal levels of Se and other essential trace elements have been demonstrated in several acute and chronic liver diseases, in children and in adults (46–55). These changes could theoretically be due to an abnormal hepatic synthesis of plasma binding proteins, to changes in liver uptake, biliary and/or renal excretion, intestinal absorption and enterohepatic circulation, or to a combined effect. With the exceptions of iron and copper, a cause-and-effect relationship between other trace elements and specific liver diseases remains doubtful, as well as the clinical relevance of changes in their blood levels. However, it seems important to explore the possibility that changes in the bioavailability of some trace elements may influence the synthesis, structure or function of circulating or structural proteins important to liver functions and bile formation.

In this study, plasma Se binding proteins and Se-dependent enzymes were not measured. In our previous study, the activity of the Se-dependent glutathione peroxidase (GSH-Px) in plasma was lower in pregnant than in non-pregnant women and it correlated positively with plasma Se concentration. Measurements of selenoprotein P – the main Se-binding protein in plasma, synthetized by the liver – are in progress, in normal pregnant women and in patients with ICP.

The present study confirms that ICP patients have lower concentrations of Se and higher concentrations of Cu in plasma than normal pregnant and non-pregnant women. Some patients with ICP had a low Se concentration in plasma as early as 1 week after the onset of pruritus, suggesting that Se may have already been low before the onset of the disease. In recent years, in Chile, a fall in the prevalence of ICP coincided with an increase in Se concentration in plasma, in ICP patients and in samples of the adult population. While in 1970, ICP could be detected in up to 14% of pregnant women delivering in public hospitals, this prevalence progressively diminished to a third of that value. Se concentration in plasma of healthy adults was relatively low in 1988 and, 5–9 years after that, it was significantly higher, in similar population samples (pregnant and non-pregnant women, and in men) and closer to values reported in individuals living in geographic areas with a "normal" dietary supply of Se. If a low dietary availability of Se and, therefore, low plasma levels, were related to the pathogenesis of ICP, even lower values would have been expected in 1970–1980 but, unfortunately, no data are available from that period.

Seasonal differences in plasma Se levels observed now in normal pregnancies coincide with a seasonal fluctuation in the prevalence of ICP. Most cases of ICP are diagnosed in autumn and in winter, they diminish in spring and there are even fewer in summer. In the present study, Se concentration in plasma from normal pregnant women was significantly higher in summer than in the other seasons, possibly reflecting dietary changes attributable to the availability of different foodstuffs in different seasons. The number of ICP patients collected during the study period was insufficient to stratify them according to seasons.

The present report adds data with regard to two other trace elements:

1. *Zinc.* Zn concentration in plasma was lower in normal pregnancies than in non-pregnant women, but in contrast to Se it remained stable during the third trimester of pregnancy and there was no significant difference in patients with ICP compared to normal pregnancies. Low plasma levels of Zn in pregnancy have been previously detected in some studies (26,29) but reported unchanged in another (28), apparently reflecting differences due to either a marginal or a normal Zn status in the local population examined. In the present report, Zn also had a seasonal variability, showing lower levels in plasma from subjects sampled in summer and in autumn. No specific role has yet been ascribed to Zn in the metabolic routes leading to bile formation and secretion, a fact that should not exclude it from future studies.

2. *Copper.* Our observation of an increased Cu concentration in plasma from healthy pregnant women coincides with previous reports (28,29). This finding contrasts with the dilutional phenomena experienced by several solutes during the final months of pregnancy, including albumin and other proteins. Cu is a constituent of several protein complexes in blood, mainly ceruloplasmin. This, and other metal-binding proteins, were not measured in the present study and therefore their contribution to a raised Cu concentration in plasma was not assessed. Our patients with ICP had a higher concentration of Cu in plasma than in normal pregnancies, coinciding with previous reports (13,14). An impaired biliary excretion of Cu during ICP may explain its retention in blood. Cu is normally excreted in bile and increased blood levels have been observed in other cholestatic disorders (56–58). In the present study, Cu plasma levels correlated with several biochemical indexes of cholestasis and therefore Cu retention in blood may be a good reflection of the severity of the disease.

Conclusion

The present and previous studies illustrate that blood levels of Se, Zn and Cu vary in the third trimester of normal pregnancies. Differences in the direction and magnitude of this phenomenon can be related to changes in water compartmental distribution and other metabolic effects determined by pregnancy and the fetal placental unit. A seasonal variability in plasma levels of these trace elements was also observed and it may reflect changes in their dietary supply. Of the three trace elements studied, selenium appears to be an environmental (dietary) factor that may influence the expressivity of ICP in a genetically predisposed population. However, the lack of correlation between Se plasma levels and the clinical and biochemical expressivity of the disease suggests that the role of selenium in the pathogenesis of ICP is indirect, perhaps as a cofactor in some important step of bile formation or secretion.

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References

1. Reyes H. Intrahepatic cholestasis. A puzzling disorder of pregnancy. J Gastroenterol Hepatol 1997; 12: 211–6.
2. Davidson KM. Intrahepatic cholestasis of pregnancy. Semin Perinatol 1998; 22: 104–11.
3. Back Y. Intrahepatic cholestasis of pregnancy. Clin Liver Dis 1999; 3: 1–13.
4. Reyes H, Simon FR. Intrahepatic cholestasis of pregnancy: An estrogen-related disease. Semin Liver Dis 1993; 13: 289–301.
5. Meng L-J, Reyes H, Palma J, Hernández I, Ribalta J, Sjövall J. Profiles of bile acids and progesterone metabolites in the urine and serum of women with intrahepatic cholestasis of pregnancy. J Hepatol 1997; 27: 346–57.
6. Meng L-J, Reyes H, Axelson M, Palma J, Hernández I, Ribalta J, et al. Progesterone metabolites and bile acids in serum of patients with intrahepatic cholestasis of pregnancy: effect of ursodeoxycholic acid therapy. Hepatology 1997; 26: 1573–9.
7. Meng L-J, Reyes H, Palma J, Hernández I, Ribalta J, Sjövall J. Effects of ursodeoxycholic acid on conjugated bile acids and progesterone metabolites in serum and urine of patients with intrahepatic cholestasis of pregnancy. J Hepatol 1997; 27: 1029–40.
8. Kauppila A, Korpela H, Mäkilä U-M, Yrjänheikki E. Low serum selenium concentration and glutathione peroxidase activity in intrahepatic cholestasis of pregnancy. Br Med J 1987; 294: 150–2.
9. Korpela H, Loueniva R, Yrjanheikki E, Kauppila A. Selenium concentration in maternal and umbilical cord blood, placenta and amniotic membranes. Int J Vitam Nutr Res 1984; 54: 257–61.
10. Mutanen M, Koivistoinen P. The role of imported grain on the selenium intake of Finnish population in 1941–1981. Int J Vit Nutr Res 1983; 53: 102–8.
11. Pyykko K, Tuimala R, Kroneld R, Roos M, Huuska R. Effect of selenium supplementation to fertilizers on the selenium status of the population in different parts of Finland. Eur J Clin Nutr 1988; 42: 571–9.
12. Ribalta J, Reyes H, Hernández I, Fuentes O, Báez M, González M, et al. Can a deficiency of selenium influence the pathogenesis of cholestasis of pregnancy? Gastroenterol Hepatol (Barcelona) 1995; 18: 114–20.
13. Kiilholma P. Serum copper and zinc concentrations in intrahepatic cholestasis of pregnancy: a controlled study. Eur J Obstet Gynecol Reprod Biol 1986; 21: 207–12.
14. Chesta J, Palma R, Antezana C, Aldunate JP, Molina O. Serum copper in intrahepatic cholestasis of pregnancy. Acta Gastroenterol Latinoam 1991; 21: 89–95.
15. Clinton OE. Determinations of selenium in blood and plant material by hydride generation and atomic-absorption spectroscopy. Analyst 1977; 102: 187–92.
16. Mailer RJ, Pratley JE. Evaluation of selenium determination in biological materials by atomic-absorption spectroscopy. Analyst 1983; 108: 1060–6.
17. González C. Contents and distribution of essential elements in Chilean plants and soil. Rev Med Chile 1976; 104: 386–90.
18. Selenium. In: Environmental Health Criteria 58. Geneva: World Health Organization; 1987.
19. Thomson CD, Robinson MF. The changing selenium status of New Zealand residents. Eur J Clin Nutr 1996; 50: 107–14.
20. Levander OA. Selenium requirements as discussed in the 1996 joint FAO/IAEA/WHO expert consultation on trace elements in human nutrition. Biomed Environ Sci 1997; 10: 214–9.
21. Ruz M, Codoceo J, Rebolledo A, Hurtado S, Muñoz L, Gras N. Selenium intake and selenium status of Chilean adults from three distinct geographical locations [abstract]. J Trace Elements Exp Med 1995; 8: 112.
22. Ruz M, Codoceo J, Hurtado S, Muñoz L, Gras N. Characterization of the regional distribution of selenium in Chile using selenium in hens' eggs as a monitor. J Trace Elements Med Biol 1995; 9: 156–9.
23. Rudolph N, Wong SL. Selenium and glutathione peroxidase activity in maternal and cord plasma and red cells. Pediat Res 1978; 12: 789–92.
24. Behne D, Wolters W. Selenium content and glutathione peroxidase activity in the plasma and erythrocytes of non-pregnant and pregnant women. J Clin Chem Clin Biochem 1979; 17: 133–5.
25. Butler JA, Whanger PD, Tripp MJ. Blood selenium and glutathione peroxidase activity in pregnant women: comparative assays in primates and other mammals. Am J Clin Nutr 1982; 36: 15–23.
26. Hinks LJ, Ogilvy-Stuart A, Hambidge KM, Walker V. Maternal zinc and selenium status in pregnancies with a neural tube defect or elevated alpha-fetoprotein. Br J Obstet Gynaecol 1989; 96: 61–6.
27. Wilson DC, Tubman R, Bell N, Halliday HL, McMaster D. Plasma manganese, selenium and glutathione peroxidase levels in the mother and newborn infant. Early Hum Dev 1991; 26: 223–6.
28. Arnaud J, Prual A, Preziosi P, Cherouvrier F, Favier A, Galan P, et al. Effect of iron supplementation during pregnancy on trace element (Cu, Se, Zn) concentrations in serum and breast milk from Nigerian women. Ann Nutr Metab 1993; 37: 262–71.
29. Wasowicz W, Wolkanin P, Bednarski M, Gromadzinska J, Sklodowska M, Grzybowska K. Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in Poland. Relation with birth weight, gestational age, and parity. Biol Trace Elem Res 1993; 38: 205–15.
30. Zachara BA, Wardak C, Didkowski W, Maciag A, Marchaluk E. Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. Gynecol Obstet Invest 1993; 35: 12–7.
31. Lee AM, Huel G, Godin J, Hellier G, Sahuquillo J, Moreau T, et al. Inter-individual variation of selenium in maternal plasma, cord plasma and placenta. Sci Total Environ 1995; 159: 119–27.
32. Barrington JW, Lindsay P, James D, Smith S, Roberts A. Selenium deficiency and miscarriage: a possible link? Br J Obstet Gynaecol 1996; 103: 130–2.
33. Lombeck I, Jochum F, Terwolbeck K. Selenium status in infants and children with phenylketonuria and in maternal phenylketonuria. Eur J Pediatr 1996; 155 Suppl1: S140–4.
34. Navarro M, López H, Pérez V, López MC. Serum selenium levels during normal pregnancy in healthy Spanish women. Sci Total Environ 1996; 186: 237–42.
35. Rayman MP, Abou-Shakra FR, Ward NI, Redman CW. Comparison of selenium levels in pre-eclamptic and normal pregnancies. Biol Trace Elem Res 1996; 55: 9–20.
36. Dobrzynski W, Trafikowska U, Trafikowska A, Pilecki A, Szymanski W, Zachara BA. Decreased selenium concentration in maternal and cord blood in preterm compared with term delivery. Analyst 1998; 123: 93–7.
37. Gromadzinska J, Wasowicz W, Krasomski G, Broniarczyk D, Andrijewski M, Rydzynski K, et al. Selenium levels, thiobarbituric acid-reactive substance concentrations and glutathione peroxidase activity in the blood of women with gestosis and imminent premature labour. Analyst 1998; 123: 35–40.
38. Stadtman TC. Biological functions of selenium. Nutr Rev 1977; 35: 161–6.
39. Sunde RA, Hoekstra WG. Structure, synthesis and function of glutathione peroxidase. Nutr Rev 1980; 38: 265–73.
40. Granger DN, Hernández LA, Grisham MB. Reactive oxygen

metabolites: mediators of cell injury in the digestive system. Viewpoints Dig Dis 1986; 18: 13–6.
41. Tribble DL, Aw TY, Jones DP. The pathophysiological significance of lipid peroxidation in oxidative cell injury. Hepatology 1987; 7: 377–87.
42. Stadtman TC. Biosynthesis and function of selenocysteine-containing enzymes. J Biol Chem 1991; 266: 16257–60.
43. Simon FR, Mackinnon AM. Impaired hepatic heme synthesis in the phenobarbital-stimulated selenium-deficient rat (39442). Proc Soc Exp Biol Med 1976; 152: 568–72.
44. Krieter PA, Ziegler DM, Hill KE, Burk RF. Studies on the biliary efflux of GSH from rat liver due to the metabolism of aminopyrine. Biochem Pharmacol 1985; 34: 955–60.
45. Hernández I, Reyes H, Ruz M, Pineda G. Effect of a selenium-deficient diet on bile formation, in the rat [abstract]. Gastroenterology 1996; 110: 1210A.
46. Aaseth J, Alexander J, Thomassen Y, Blomhof JP, Skrede S. Serum selenium levels in liver diseases. Clin Biochem 1983; 15: 281–3.
47. Dutta SK, Miller PA, Greenberg LB, Levander OA. Selenium and acute alcoholism. Am J Clin Nutr 1983; 38: 713–8.
48. Dworkin B, Rosenthal WS, Jankowski RH, Gordon GG, Haldea D. Low blood selenium levels in alcoholics with and without advanced liver disease. Correlations with clinical and nutritional status. Dig Dis Sci 1985; 30: 838–44.
49. Korpela H, Kumpulainen J, Luoma PV, Arranto AJ, Sotaniemi EA. Decreased serum selenium in alcoholics as related to liver structure and function. Am J Clin Nutr 1985; 42: 147–51.
50. Tanner AR, Bantock I, Hinks L, Lloyd B, Turner NR, Wright R. Depressed selenium and vitamin E levels in an alcoholic population. Possible relationship to hepatic injury through increased lipid peroxidation. Dig Dis Sci 1986; 31: 1307–12.
51. Välimäki M, Alfthan G, Pikkrainen J, Ylikhari R, Salaspuro M. Blood and liver selenium concentrations in patients with liver diseases. Clin Chim Acta 1987; 166: 171–6.
52. Burk RF. Selenium deficiency in search of a disease. Hepatology 1988; 8: 421–3.
53. Välimäki M, Alfthan G, Vuoristo M, Ylikahri R. Effects of selenium supplementation on blood and urine selenium levels and liver function in patients with primary biliary cirrhosis. Clin Chim Acta 1991; 196: 7–15.
54. Thuluvath PJ, Triger DR. Selenium in chronic liver disease. J Hepatol 1992; 14: 176–82.
55. Burk RF, Early DS, Hill KE, Palmer IS, Boeglin ME. Plasma selenium in patients with cirrhosis. Hepatology 1998; 27: 794–8.
56. LaRusso NF, Dickson ER, Pineda AA. Plasma perfusion and studies of copper metabolism in benign recurrent cholestasis. Novel procedures in a rare syndrome. Mayo Clin Proc 1980; 55: 450–4.
57. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985; 88: 108–14.
58. Goksu N, Ozsoylu S. Hepatic and serum levels of zinc, copper, and magnesium in childhood cirrhosis. J Pediatr Gastroenterol Nutr 1986; 5: 459–62.