Abnormal iron parameters in the pregnancy syndrome pre-eclampsia

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Condensation

Iron release, sometimes sufficient to cause iron overload, occurs in pre-eclampsia, decreasing the antioxidant capacity of serum and exacerbating lipid peroxidation and endothelial-cell injury.

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OBJECTIVE: To investigate iron status parameters in pre-eclampsia with a view to exploring their possible contribution to the etiology.

STUDY DESIGN: In prepared serum samples from 40 pre-eclamptic women and matched pregnant controls at the John Radcliffe Hospital, Oxford, a number of iron status parameters were measured. Statistical analysis was by Wilcoxon's Signed Rank Test and linear regression.

RESULTS: Serum iron concentration, ferritin and percent saturation of transferrin were significantly higher in the pre-eclamptic patients than in controls, whereas unsaturated iron-binding capacity and apotransferrin levels were significantly lower. No difference was found in hemopexin concentrations in the two groups. Gestational age at the time of sampling was correlated (positively) with only two parameters: TIBC and UIBC, but only in the pre-eclampsia group. Eighteen percent of pre-eclamptic subjects had percent transferrin saturation levels in the region associated with iron overload.

CONCLUSION: Released iron species in pre-eclampsia may contribute to the etiology and will exacerbate lipid peroxidation and endothelial-cell injury. Given the high prevalence of heterozygosity for haemochromatosis with the associated reduced ability to exclude ingested iron, it may be appropriate to consider the advisability of giving iron supplements to pregnant women at high risk of pre-eclampsia, in the absence of evidence of iron deficiency.

Key words: Pre-eclampsia, iron, transferrin, ferritin, hemopexin, hemochromatosis

Pre-eclampsia is a disorder which is believed to affect one in ten of all pregnancies to some degree. It is the biggest single cause of maternal and fetal mortality and currently there is no cure other than termination of the pregnancy.¹

The causes of pre-eclampsia are complex and are not fully understood but the condition may be associated with poor placentation.² Placentation is complete by about 18 weeks of pregnancy long before symptoms and signs of pre-eclampsia become apparent. It is however not known if poor placentation underlies all cases without exception.² The effect of poor placentation is to leave the spiral arteries smaller than normal for the second half of pregnancy.² It is assumed that this and the associated obstructive lesion of the spiral arteries called acute atherosclerosis lead to placental ischemia for which there is considerable evidence (summarized in Redman and Sargent³). A causal sequence of events in pregnant women cannot be proved. It is possible that pre-eclampsia causes placental ischemia although more likely that placental ischemia causes pre-eclampsia especially as poor placentation is an early preclinical development. Moreover pre-eclampsia-like syndromes can be induced in pregnant animals by surgical restriction of the uteroplacental blood supply.⁴ It is presumed that blood-borne agents arising from the ischemic placenta are the cause of the generalised endothelial-cell damage which gives rise to the symptoms of hypertension, proteinuria, and sudden edema, characteristic of this condition.⁵ The high levels of circulating lipid hydroperoxides believed to be present in pre-eclampsia are among the candidate agents capable of causing such damage to the vascular endothelium.⁶ The malperfused placenta may be responsible for the generation of these reactive chemical species.

When tissues become ischemic, reactive oxygen species such as superoxide and hydrogen peroxide are produced, but neither of these is reactive enough to initiate cellular damage directly.⁷ However, in the presence of catalytic amounts of transition metal ions, particularly iron, which may arise in the ischemic placenta by destruction of red blood cells

from thrombotic, necrotic and hemorrhagic areas,^{8,9} these species can generate the highly reactive hydroxyl radical by Fenton chemistry (Figure 1).^{7,10} This radical can initiate the process of lipid peroxidation which, if uncontrolled, may result in endothelial-cell damage, as hypothesized by Hubel and colleagues.⁶

Extracellular hemoglobin can also support lipid peroxidation either through prior iron release or release of heme from methemoglobin.^{9,10} Free heme can generate lipid hydroperoxides in low density lipoprotein (LDL) particles¹¹ or diffuse into membranes of endothelial cells causing peroxidative damage and cytotoxicity.¹² Even minimal vascular hemolysis is sufficient to yield hemoglobin at a concentration range that can affect LDL peroxidation.

Protection from these deleterious pro-oxidative effects in the plasma is normally afforded by proteins that bind these iron entities in a relatively inert state. Plasma iron is usually almost entirely safely bound to the transport protein transferrin. The greater the extent to which transferrin is saturated with iron, the lower the antioxidant capacity of the plasma.¹³ Iron released intracellularly is normally safely sequestered in ferritin and serum ferritin reflects the amount of the storage protein.¹⁴

The plasma proteins haptoglobin, hemopexin and albumin, bind free hemoglobin and heme, affording protection against iron- and heme-catalysed lipid peroxidation and endothelial-cell injury.¹⁰ Elevated heme catabolism occurs in pre-eclampsia as shown by increased levels of bilirubin and carboxyhemoglobin.⁸ Although hemopexin is clearly an important extracellular antioxidant,¹⁵ its possible importance in the pre-eclampsia syndrome has been previously overlooked.

Disturbances in iron homeostasis have already been observed in pre-eclampsia.^{8,13,16,17} It was decided to investigate further the possible importance of such factors in the condition and to look for the first time at hemopexin levels. This study therefore compares a number of

iron parameters, including hemopexin, serum ferritin and the extent to which transferrin is saturated with iron, in pre-eclamptic women and matched pregnant controls.

Material and methods

Forty obstetric patients at the John Radcliffe Hospital, Oxford, identified as suffering from pre-eclampsia according to specific criteria (new hypertension, from below to consistently at or above 90 mm Hg diastolic pressure; new proteinuria ≥ 75 mg / mmol creatinine, in the absence of urinary-tract infection), were each matched with a healthy pregnant woman of the same age ± 4 years, the same gestation ± 13 days and the same parity group namely 0, 1-3 or ≥ 4 . All controls had conceived spontaneously, had never received a blood transfusion and were not on any medication. . The pre-eclamptic patients received treatment for their blood pressure, methyl dopa and/or nifedipine. Neither pre-eclamptic nor control women received iron supplements. Serum was prepared from venous blood collected from these women and stored at -40°C until analyzed. The mean gestational age at the time of blood sampling was 33 weeks. Samples were obtained in two sets of 20 pairs in consecutive years. Ferritin was determined only in the first set of 20 pairs, while an electrophoretic technique not initially available to us, was applied only to the second set of 20 pairs, to determine percent-transferrin-saturation, apotransferrin and hemopexin. One control sample from the first set was lost giving 19 pairs for statistical analysis.

The clinical characteristics recorded were:- age, gestational age at the time of blood sampling, systolic and diastolic blood pressure, proteinuria, platelet count, aspartate aminotransferase (AST) activity (an indicator of liver damage), gestational age at the time of delivery, infant sex and birth weight.

The study was approved by Central Oxford Research Ethics Committee.

Serum total iron and unsaturated iron binding capacity (UIBC)-were measured on a

Cobas Mira Plus automatic analyzer (Roche Diagnostic Systems, Hertfordshire, UK) at the University of Surrey using a colorimetric method (Unimate 5 iron and Unimate 7 UIBC kits, Roche Products). Accuracy and precision were verified for serum total iron, by analysis of control serum N (Roche Products) and a lyophilized second-generation human serum certified reference material supplied from the lab of Versieck and co-workers (University of Ghent, De Pintelaan 185, B-9000 Ghent, Belgium), while for UIBC, control serum N was used.

Determination of iron concentration in control serum N gave a mean and standard deviation of $14.15 \pm 0.25 \mu\text{mol/l}$ (certified mean $14.00 \mu\text{mol/l}$), coefficient of variation 1.8 and 95% confidence interval 14.02 - 14.29. Iron analysis of the certified reference serum gave a mean and standard deviation of $40.57 \pm 0.94 \mu\text{mol/l}$ (certified mean $42.16 \mu\text{mol/l}$), coefficient of variation 2.3 and 95% confidence limits 39.90 - 41.24 $\mu\text{mol/l}$ (certified serum 39.72 - 44.60 $\mu\text{mol/l}$). These results demonstrate the acceptable accuracy of the iron assay method.

Total iron binding capacity (TIBC) was calculated as the sum of the serum iron and UIBC. The percentage saturation of iron binding capacity was calculated as: serum iron x 100/TIBC.

Percent transferrin saturation was also determined by urea-polyacrylamide gel electrophoresis.¹⁸ This method separates transferrin into the apo-, the two monoferric forms and the diferric form, according to their electrophoretic mobilities. To enable detection of the four transferrin bands, serum samples were treated before being applied to the gel with 6,9-diamino-2-ethoxyacridine lactate (Sigma Chemical Co., UK) to precipitate all serum proteins but β - and γ -globulins. On completion of the electrophoretic run, the gel was stained for 20 min and then repeatedly destained until the background was clear. The relative amounts of the transferrin components were determined by densitometry using the Oast Tech computer

package gel analysis system (Oast Tech, Gillingham, Kent, UK). Percent transferrin saturation was calculated as follows:-

$$\% \text{ transferrin saturation} = \frac{[(\text{C-monoferriic}) + (\text{N-monoferriic}) + (2 \times \text{diferric})]}{2(\text{apo} + \text{C-monoferriic} + \text{N-monoferriic} + \text{diferric})} \times 100$$

Relative apotransferrin concentrations in the two patient groups were also obtained by this method.

There is no hemopexin assay commercially available in the UK. The possibility of determining hemopexin by the same electrophoretic technique as used for the different forms of transferrin was investigated. A purified hemopexin preparation (Bio Products Lab., Borehamwood, Herts., UK) containing 19% heme-hemopexin and 81% apohemopexin was used to ascertain the feasibility of this method. A single band was seen between those of the monoferriic forms of transferrin and the diferric form, suggesting either that heme-hemopexin and apohemopexin have the same electrophoretic mobility in the urea-polyacrylamide gel or, more likely, that under the conditions of the electrophoretic separation, the heme-hemopexin complex dissociates. The band was quantified by densitometry as for the different forms of transferrin.

Serum ferritin was determined by a microparticle enzyme immunoassay method using the Abbott AxSYM system (Abbott Laboratories, Diagnostics Division, Maidenhead, UK). The recommended quality control procedures of the manufacturer were carefully followed.

Data Analysis. Owing to some extremely high values, parametric statistical methods appropriate for normally-distributed data were precluded. Wilcoxon's Signed Rank Test for non-parametric, paired data was used to compare iron parameters in the pre-eclamptic patients and their matched controls. Infant birth weights were allocated to centiles using

“Oxford Standards” charts (Castlemead, Ware, Herts., UK), allowing correction for length of gestation and sex. The χ^2 test was then applied to compare the frequency distribution of centile birth weights in the pre-eclamptic and control groups. Linear regression analysis was used to identify possible correlations between the different iron parameters measured and the clinical data (e.g. gestational age, AST activity, platelet count, systolic or diastolic blood pressure). For all tests significance was accepted for $p < 0.05$.

Results

The pre-eclamptic patients were all suffering from a fairly severe to very severe level of the disease. Ten had AST levels > 42 IU/l suggesting a component of liver damage while thirteen had a low platelet count ($< 175 \times 10^9$ cells/l), also indicative of disease severity, though two control subjects also had a platelet count in this range. Seven of the pregnancies were complicated by the HELLP (hemolysis, elevated liver enzymes, low platelets) or ELLP (elevated liver enzymes, low platelets) syndromes. Gestational age at delivery and centile infant birth weights (corrected for length of gestation and sex) were significantly lower ($p < 0.0001$ and 0.001 respectively) in the pre-eclamptic group, the latter indicating some fetal-growth retardation. Median hematocrit did not differ between the groups.

For all parameters measured, results, including maximum and minimum values and interquartile ranges, are summarized in Table 1.

Median serum iron concentration was around 1.7 fold higher in patients with pre-eclampsia than in pregnant controls (21.7 vs 12.9 $\mu\text{mol/l}$; $p < 0.001$). Results are shown graphically in Figure 2. Of the pre-eclamptic subjects, four had very high values for serum iron ($43.7 - 79.3$ $\mu\text{mol/l}$) and their AST levels indicated liver damage. Omission of these subjects from the analysis reduced the ratio of the median serum iron concentrations from 1.7

to 1.5 ($p < 0.001$).

Both UIBC and TIBC were significantly lower in the pre-eclamptic patients than in the matched controls (47.5 vs 68.7 $\mu\text{mol/l}$; $p < 0.0001$) and (75.5 vs 85.7 $\mu\text{mol/l}$; $p < 0.0001$) respectively.

Median percent saturation of iron-binding capacity (colorimetric method) in women with pre-eclampsia was twice as high as that of matched controls (32.2% vs 15.9 % ; $p < 0.0001$). Four pre-eclamptic subjects with elevated AST levels had values for percent saturation of iron-binding capacity of 50-100%. Even when these subjects were excluded from the analysis, iron-binding capacity was still 1.8 times more saturated in the pre-eclamptic patients than in controls. Seven of 40 pre-eclamptic patients (18%) had percent saturation levels in the range associated with iron overload states (50-100%).¹⁴ Percent saturation of transferrin (electrophoretic method), was more than twice as high in the pre-eclampsia group as in the normal-pregnancy group (median: 27.6 % vs 11.6%; $p < 0.0001$). Two of 20 pre-eclamptic patients (10%) had percent saturation levels in the range associated with iron overload states.

Since transferrin is by far the most important iron binding protein in serum,¹⁹ determination of the percent saturation of iron binding capacity as measured by the colorimetric method is to a good approximation a determination of the percent saturation of transferrin as obtained directly by gel electrophoresis. Indeed there was a highly significant correlation, between the two methods for both patient groups ($r = 0.94$ for combined data; $p < 0.0001$).

Apotransferrin level measured in arbitrary units was significantly lower in pre-eclampsia patients than in matched pregnant controls (289 vs 530; $p < 0.0001$).

Hemopexin level measured in arbitrary units in the pre-eclampsia group did not differ significantly from the hemopexin level in the normal pregnancy group (257 vs 237).

Median serum ferritin was around six-fold higher in the pre-eclamptic patients than in the matched pregnant controls (53.1 vs 9.4 $\mu\text{g/l}$; $p < 0.001$) as shown graphically in Figure 3. Three out of four subjects with very high ferritin levels (257-1259 $\mu\text{g/l}$) had AST values $> 42 \text{ IU/l}$, suggesting a component of liver damage. When those subjects were excluded however, the median ferritin level was still about five-fold higher.

No significant correlations were found between serum iron, TIBC, UIBC, apotransferrin, hemopexin, ferritin or any of the iron saturation parameters and indices of hepatocellular injury (AST), coagulopathy (platelet count), and cardiovascular system abnormalities (diastolic and systolic blood pressure) in the pre-eclamptic patients, with the exception of a weakly significant positive correlation between UIBC and platelet count ($r = 0.55$, $p = 0.03$).

Gestational age at the time of sampling was correlated (positively) with only two parameters: TIBC ($r = 0.71$, $p = 0.0005$; and UIBC ($r = 0.53$, $p = 0.015$) in the pre-eclampsia group. No such correlation was apparent in the control group (TIBC, $r = 0.30$, ns; UIBC, $r = 0.27$, ns).

Comment

The colorimetric and electrophoretic methods used to gauge the extent of transferrin saturation with iron in our subjects gave results which were very significantly correlated and indicated that transferrin saturation in the pre-eclamptic patients was double or more, that in the controls. This reflects the significantly higher serum iron (by 68%) and significantly lower total iron binding capacity, TIBC (by 12%), of the pre-eclamptic subjects compared to the controls. Seven of the 40 pre-eclamptic subjects (18%), had percent saturation levels within the range associated with iron overload.¹⁴

Unsurprisingly, UIBC, being inversely related to percent transferrin saturation, was

significantly decreased (by 31%) in the pre-eclamptic group relative to the control group. This suggests that pre-eclamptic women only have around two-thirds of the capacity of controls to bind additional iron. UIBC reflects the concentrations of both apotransferrin and mono-ferric transferrin species. Apotransferrin alone, measured by the electrophoretic method, was decreased even further than UIBC - by 45% - in the pre-eclamptic group relative to the control group, reflecting the decreased likelihood of finding any iron-free transferrin at the much-higher iron concentrations existing in this group.

Our findings on iron, UIBC, TIBC and % saturation of iron-binding capacity are in agreement with those of previous studies.^{8,13,16,17} The lack of correlation between serum iron and AST levels reflects the poor correlation with parameters of hepatocellular injury found previously^{16,17} and suggests that the raised iron levels cannot be explained by liver damage.

Hemopexin levels, monitored as an indirect measure of whether heme release could be a factor in stimulating lipid peroxidation, were not significantly different in pre-eclamptic patients and their normal pregnant controls. As levels of hemopexin do not always decrease in states of intravascular hemolysis,²⁰ there remains the possibility of some release of heme into the circulation in pre-eclampsia, particularly as increased levels of heme metabolites are certainly observed.⁸ Furthermore, since it was not possible to separate apohemopexin and heme-hemopexin on the urea-polyacrylamide gel, a difference in the heme bound would not have been detected.

Previous studies have come to different conclusions about whether ferritin levels are raised in pre-eclampsia.^{16,17} In our study, even when the three subjects with very high ferritin and AST values were excluded from the analysis, the median ferritin level was still around five-fold higher in the pre-eclamptic group and no correlation was found between serum ferritin and AST levels. This suggests that liver damage is not the principal source of the raised ferritin levels in most subjects, corroborating previous findings.²¹ Ferritin is an acute-

phase protein. The highest levels of ferritin, an acute phase protein, were not found in subjects with the lowest albumin levels, suggesting that an acute-phase response was not responsible for the raised serum ferritin. Serum ferritin was not correlated with serum iron, in line with findings from previous workers.²¹

Gestational age at the time of sampling was significantly and positively correlated with TIBC and weakly with UIBC, but only in the pre-eclampsia group. This correlation was not observed in the control group though it might have been expected as a response to falling iron levels as the pregnancy progressed.²² Hubel and colleagues did not observe a correlation between TIBC and gestational age in pre-eclampsia.¹³ We cannot explain our findings or the difference between the two studies, except to postulate that a mechanism may exist for increasing transferrin expression in response to oxidative stress.

Origin of increased serum iron. The lack of correlation with parameters of liver injury in this and other studies eliminates the liver as the probable source of iron.²⁰ However, data are consistent with a rise in heme catabolism following increased destruction of maternal red cells^{8,13,16,17} and a nine-times greater level of hemoglobin in the serum of pre-eclamptic than control subjects has been reported.¹⁷

The pre-eclamptic placenta presents a histological picture of severe vascular damage in decidual vessels adjacent to infarcted areas,²³ consistent with cell injury and release of iron. Injured red blood cells from necrotic and hemorrhagic areas of infarcted or ischemic placental tissue may be a primary source of potentially toxic iron through the release of hemoglobin or heme into the system.^{8,9}

Is iron involved in the etiology of pre-eclampsia? Oxidative stress in pre-eclampsia correlated inversely with UIBC and positively with % transferrin saturation.¹³ This implies that iron (or an iron species) could be a factor in the generation of the oxidative stress of the condition. Even at very low concentrations, iron entities such as hemoglobin and heme can

increase LDL oxidation,¹¹ suggesting a further mechanism by which iron might be involved in the etiology of the condition. The ability of vitamins C and E to decrease LDL oxidation might explain their effectiveness in reducing the risk of pre-eclampsia.²⁴

The damaged placenta is a likely site for the production of free radicals in pre-eclampsia. Iron species released there from red-cell destruction are clearly capable of initiating and propagating lipid peroxidation²⁵ both in the placenta and in the vasculature, and may be a significant etiological factor in the endothelial-cell damage of pre-eclampsia.

Conclusion. This work has highlighted a significant deficit in the antioxidant capacity of serum by decreased serum-iron buffering in pre-eclampsia. Released iron species may be implicated in the etiology of the condition, but at the very least, are likely to increase oxidative stress, exacerbate lipid peroxidation and promote endothelial-cell injury.

We concur with the previous suggestion²² that raised serum iron and ferritin may have the potential to be used diagnostically to warn of incipient pre-eclampsia.

Eighteen percent of our pre-eclamptic subjects had transferrin saturation levels in the region associated with iron overload. It is now known that as many as 38% of the population are at increased risk of iron overload owing to mutations in the HFE gene associated with haemochromatosis.²⁶

Given the prevalence of heterozygosity for hemochromatosis with the associated reduced ability to exclude ingested iron,^{26,27} and the proportion of our pre-eclamptic subjects with iron overload, it may be appropriate to consider the advisability of giving iron supplements to pregnant women at high risk of pre-eclampsia, in the absence of evidence of iron deficiency. While there is no suggestion that iron supplements are the cause of the excess iron seen in pre-eclampsia, they could be an exacerbating factor in women susceptible to iron overload. Accordingly, the same strictures would apply to the use of parenteral iron. Genetic evaluation of mutations in the HFE gene in a larger study of such women would be

of interest.

Acknowledgements

The authors are grateful to Gerasimoula Sokari for carrying out some of the analyses and to Professor Luc Moens of the University of Ghent for supplying second-generation human serum certified reference material.

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Table I. Serum iron parameters in pre-eclamptic patients and matched pregnant control subjects

		Median	Interquartile	Min / max	p value
Serum iron ($\mu\text{mol/l}$)	Control	12.9	10.0 - 19.7	4.3 / 39.0	
	Pre-ecl	21.7	15.9 - 32.6	6.3 / 79.3	<0.001
UIBC ($\mu\text{mol/l}$)	Control	68.7	61.6 - 87.0	46.7 / 117.1	
	Pre-ecl	47.5	34.3 - 62.6	0.3 / 94.3	<0.0001
TIBC ($\mu\text{mol/l}$)	Control	85.7	80.5 - 97.8	68.7 / 121.4	
	Pre-ecl	75.5	62.6 - 82.7	39.3 / 106.8	<0.0001
% saturation (colorimetric method)	Control	15.9	10.9 - 23.8	3.5 / 42.1	
	Pre-ecl	32.2	21.1 - 47.4	7.3 / 99.6	<0.0001
% saturation (electrophor. method)	Control	11.6	8.7 - 16.0	3.2 / 37.3	
	Pre-ecl	27.6	15.8 - 38.9	10.8 / 73.7	<0.0001
Apotransferrin (arbitrary units)	Control	530	409 - 684	241 / 809	
	Pre-ecl	289	194 - 458	27 / 639	<0.0001
Serum haemopexin (arbitrary units)	Control	237	202 - 317	122 / 505	
	Pre-ecl	257	200 - 316	117 / 471	ns
Serum ferritin ($\mu\text{g/l}$)	Control	9.4	7.7 - 12.2	5.3 / 43.5	
	Pre-ecl	53.1	22.7 - 66.1	8.7 / 1260	<0.001

Figure legends

Fig. 1. Iron-catalysed initiation and propagation of lipid peroxidation

Fig. 2. Scatterplot of serum iron concentrations in pre-eclamptic women and matched pregnant controls. Horizontal bars represent the median values.

Fig. 3. Scatterplot of serum ferritin concentrations in pre-eclamptic women and matched pregnant controls. Horizontal bars represent the median values.

N.B. The four values off-scale in the pre-eclampsia group were all $> 250 \mu\text{g/l}$.

Initiation

Iron-catalysed Haber-Weiss Reaction

The source of hydrogen peroxide for the Fenton reaction is the dismutation of superoxide which is likely to be catalysed by superoxide dismutase (SOD).

Propagation

Iron (II) and certain iron (II) chelates react with lipid hydroperoxides (ROOH), as with hydrogen peroxide, splitting the O-O bond. This gives $\text{RO}^{\bullet}$, an alkoxyl radical, which can also abstract $\text{H}^{\bullet}$ from polyunsaturated fatty acids and from hydroperoxides. The resulting peroxy radicals $\text{ROO}^{\bullet}$ can continue propagation of lipid peroxidation.

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