Transferrin Concentrations in Amniotic Fluid and Blood During Gestation and Early Puerperium

M Terzic´ MD, PhD,
D Plećaš MD, PhD,
A Petronijevic´ MD, PhD.

Department of Obstetrics and Gynaecology,
University Clinical Center,
Belgrade, Yugoslavia

Abstract : *The aims of the present investigation were to determine transferrin concentrations in amniotic fluid and in womens' cubital vein blood during gestation as well as in the early postpartal period and to establish referent curves of this very important nonspecific immunologic protective factor. In the period of 3 years serial amniotic fluid specimens were obtained by amniocentesis under ultrasound control with a free-hand technique in aseptic conditions from 91 patients (239 samples) in varying gestational ages. At the same time specimens of cubital vein blood were taken. Transferrin concentrations were determined by method of immunonephelometry using original Boehring kits. All amniotic fluid samples were cultured for aerobic and anaerobic bacteria within 30 minutes after amniocentesis. Our results indicate that transferrin concentrations in amniotic fluid increased gradually with advancing gestational age from 49.90 mg/l in the 13th week of gestation and reached maximum levels of 260.30 mg/l in the 39th gestational week. In the last week transferrin levels significantly decreased to the 95.05 mg/l at term. Peripheral blood transferrin levels increased from 2.58 g/l in the 13th week of gestation and reached maximal values of 4.67 g/l in the 38th week of pregnancy. In the early puerperal period transferrin concentrations were found to increase, with a peak of 5.80 g/l on the second postpartal day. After that levels decreased. (Thai J Obstet Gynaecol 1993;5: 73-79.)*

Key words : transferrin, amniotic fluid, blood, serial amniocentesis

Recent review has given comprehensive accounts on the functional and other properties of transferrins. Three proteins typify the transferrin family; serum transferrin (the iron transport protein), lactoferrin (found in milk and other secretions, as well as leukocytes), and ovotransferrin or conalbumin (from egg white)[1].

Bacterial colonization of the amniotic fluid has been reported in patients with intact membranes during pregnancy, but microbial invasion of the amniotic cavity during labour, prior

to and especially after rupture of membranes was found to occur more frequently[2]. Microbial invasion of the amniotic cavity may lead to maternal infection and fetal/neonatal sepsis. Antimicrobial components in amniotic fluid becomes the last resort to prevent these complications. A lot of reports have been published regarding the antibacterial activity of amniotic fluid. A number of compounds such as lysozyme, transferrin, peroxidase, 7s immunoglobulin, spermine, betalysin, beta 1a/beta 1c globulin and zinc have been found in amniotic fluid and are assumed to exhibit bacterial growth-inhibitory activities[3]. However, how these compounds inhibit the bacterial growth in amniotic fluid is not clearly understood, because investigators have shown contradictory evidence with respect to growth of certain bacterial species, especially in amniotic fluid. The reasons for these differences need to be clarified. An electronic literature search (Medline) has not retrieved any reference about transferrin blood and intraamniotic reference curves (up to December 1992).

Materials and Methods

Serial amniotic fluid samples were obtained under ultrasound control by a free-hand technique in aseptic conditions from patients treated in the Department of Obstetrics and Gynaecology, University Clinical Center in Belgrade, during a period of 3 years, in varying gestational ages. In 91 women, 239 amniocenteses were performed for different purposes. Indications for serial amniocenteses were: Rh-alloimmunisation, diabetes mellitus, polyhydramnios, pregnancy induced hypertension, genetic and amniocentesis at term. Amniotic fluid specimens contaminated with blood, those from patients with ruptured membranes or who were receiving antibiotics were discarded. The primary condition for inclusion in this study was the sterile first amniotic fluid specimen. Each sample was centrifuged at 10° C for 10 minutes at 1000 X g to remove particulate materials and was frozen at -70° C until studied. Transferrin concentrations were determined by method of immunonephelometry using original Boehring kits. All amniotic fluid samples were cultured for aerobic and anaerobic bacteria immediately after amniocentesis. Microorganisms were identified with standard methods. During the amniocentesis and also in the first 5 postpartal days womens' cubital vein blood specimens (3 ml) were taken for analysis. Peripheral cubital vein blood samples of 30 healthy nongravid women were obtained and served as controls. Obtained data were tested by one way variance analysis.

Results

Transferrin intraamniotic concentrations increased gradually with advancing gestational age from 49.90 mg/l in 13th week of gestation and reached maximum levels of 260.30

mg/l in the 39th gestational week (Fig. 1 and Table 1). In the last week of pregnancy, transferrin concentrations significantly decreased.

Transferrin blood concentrations were low in the 13th week of gestation (2.58 g/l) and increased according to the pregnancy progress, with some variations. Maximal concentrations were in the 38th week

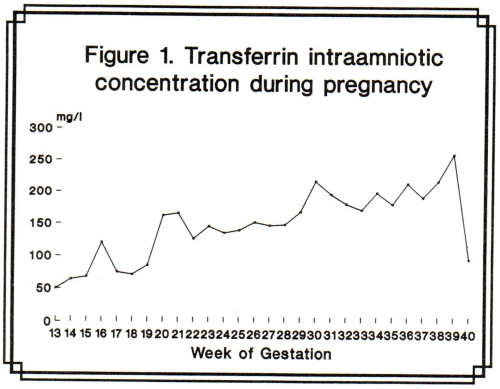

Table 1 Transferrin concentrations in amniotic fluid during pregnancy (mg/l)

Weeks of gestation	$\bar{x}$	SD	Min	Max
13	49.90	-	49.9	49.9
14	63.96	17.91	33.8	82.0
15	68.21	0.22	61.0	75.0
16	120.12	63.23	36.1	267.0
17	75.08	7.97	52.0	80.0
18	77.36	21.59	42.5	125.0
19	85.78	43.16	40.1	156.0
20	162.50	34.64	138.0	187.0
21	166.30	-	166.3	166.3
22	126.50	61.56	75.0	223.0
23	146.02	42.11	62.1	185.1
24	136.00	-	136.0	136.0
25	140.21	32.11	102.1	163.5
26	152.34	28.25	132.2	179.5
27	147.52	28.41	126.1	166.5
28	149.00	-	149.0	149.0
29	168.80	-	168.0	168.0
30	217.00	3.28	214.0	220.0
31	196.52	20.22	172.1	225.4
32	181.00	-	181.0	181.0
33	172.00	-	172.0	172.0
34	199.00	44.91	158.0	240.0
35	180.50	12.59	169.0	192.0
36	213.84	39.20	116.0	259.0
37	191.76	59.58	55.0	290.0
38	217.46	40.28	137.0	272.0
39	260.30	46.59	184.0	308.0
40	95.05	11.30	89.4	112.0

(4.67 g/l). In the puerperium transferrin levels increased and reached a peak on the 2nd postpartal day (5.80 g/l). After that, concentrations decreased and on the 5th day were found to be 3.91 g/1 (Fig. 2 and Table 2). One way variance analysis confirmed statistically significant differences between transferrin concentrations after the 32nd week of gestation

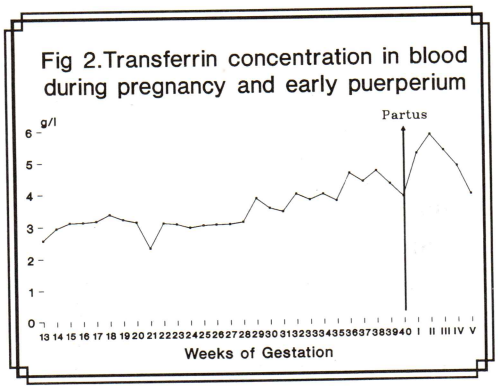

Fig 2.Transferrin concentration in blood during pregnancy and early puerperium

Table 2 Transferrin concentration in blood during pregnancy and early gestation (g/l)

Gestational weeks	x	SD	Min	Max
13	2.58	-	2.58	2.58
14	2.94	0.08	2.9	3.1
15	3.11	0.22	3.0	3.3
16	3.12	0.52	2.2	4.5
17	3.16	0.29	2.8	3.7
18	3.37	0.71	2.5	5.1
19	3.21	0.65	2.2	3.9
20	3.12	0.17	3.0	3.3
21	2.31	-	2.31	2.31
22	3.09	0.41	2.7	3.8
23	3.06	0.24	3.0	3.3
24	2.95	-	2.95	2.95
25	3.02	0.22	2.9	3.3
26	3.04	0.21	3.0	3.2
27	3.05	0.17	3.0	3.3
28	3.11	-	3.11	3.11
29	3.84	-	3.84	3.84
30	3.54	0.36	3.2	3.9
31	3.42	0.42	3.1	4.1
32	3.97	-	3.97	3.97
33	3.78	-	3.78	3.78
34	3.96	0.01	3.8	4.1
35	3.74	0.35	3.4	4.1
36	4.06	0.56	3.9	5.4
37	4.34	0.64	3.5	5.5
38	4.67	0.58	3.6	5.5
39	4.25	0.61	3.4	4.9
40	3.86	0.11	3.7	3.9
I	5.22	0.45	5.1	5.3
II	5.80	0.48	5.6	6.2
III	5.30	0.62	5.2	6.2
IV	4.82	0.51	4.6	5.3
V	3.91	0.31	3.7	4.1

compared to the levels before($p<0.05$). Maximal transferrin blood levels on the 2nd postpartal day confirmed the significance of this nonspecific immunologic defense factor during the period when the antimicrobial protection is the most necessary. In healthy nongravid persons transferrin levels in blood were 2.92 ± 0.57 g/l.

Bacterial growth studies showed proliferation of microorganisms in only 1 amniotic fluid specimen (0.42%), while other samples were sterile. In this patient after the 2nd, repeated genetic amniocentesis in the 18th week of gestation Staphylococcus epidermidis was isolated. Clinical signs of intraamniotic infection were present. She was treated according to the antibiogram and delivered at term without fetal and/or maternal complications. Intraamniotic transferrin concentrations in the 16th and 18th week were 36.1 mg/l and 42.5 mg/l, and at term 55.0 mg/l, while in blood levels were 3.28 g/l, 3.35 g/l in the same gestational ages respectively. This patient had intraamniotic transferrin level at the end of pregnancy significantly lower compared to the other patients without microbiological substrate in amniotic fluid. Also, blood transferrin levels in this patient were significantly lower than in the others ($p<0.05$).

Discussion

Transferrins (transferrin, lactoferrin and ovotransferrin) have been found to exert a bacteriostatic effects on a number of bacteria, and these effects could be eliminated by saturating both transferrin and iron-binding sites with iron[4,5]. The presence of lactoferrin in the specific granules of neutrophils suggest an extracellular site of action for lactoferrin[2]. Polymorphonuclear leukocytes exposed to highly purified human lactoferrin exhibit an increased random motility and are primed to produce more superoxide. This action seemed to be specific, because it could be abolished by simultaneous addition of antilactoferrin antibodies. So, polymorphonuclears became more effective after exposure to lactoferrin by having a greater motility and producing superoxide at a faster rate. The bacteriostatic effect of lactoferrin appears related to its ability to deprive bacteria of iron required for growth. Evidence has also been presented suggesting that lactoferrin participates in the alteration of the physicochemical properties of the neutrophil membrane during degranulation, the generation of granulopoiesis, and the modulation of complement function[6]. Besides antimicrobial properties exhibition, transferrins are involved in other processes during pregnancy. It has been confirmed that the liver and the yolk sac stimulate kidney differentiation by producing the soluble factor-transferrin[7]. Tubulogenesis in vitro is influenced and regulated by transferrin[8]. Transferrins specifically stimulate dermatan- and chondroitin-sulphate proteoglycan accumulation around lung cells, and in the extracellular

matrix of lung tissue in vitro[9]. Transferrin mRNA levels increased in liver throughout gestation with maximum expression at term[10].

Results obtained in the current study indicate that transferrin concentrations in the blood of pregnant women are modesty elevated during pregnancy. These findings are in agreement with the results obtained in other investigations[2,3,10].

It is well known that intraamniotic infection plays a role in increased fetal and/or neonatal morbidity and mortality, as well as maternal morbidity. Terzic´ [11] stressed on the fact that antibacterial activity of amniotic fluid may protect patients from chorioamnionitis and resultant preterm delivery. Transferrin is known to be a very important bacterial growth inhibitor[5,12]. A number of authors have previously described transferrin concentrations in amniotic fluid[2-4]. But until now there is no appropriate study that systematically presents transferrin concentrations in amniotic fluid and blood of pregnant women. In our prospective investigation we demonstrated that amniotic fluid transferrin exhibits a pattern of activity related to the gestational age, it increases according to the progression of gestation reaching maximum near term. Our results indicate a strong relationship between high transferrin level and sterile amniotic microenvironment. Up to now, only Nazir et al[13] have published results of antibacterial activity in amniotic fluid specimens obtained by serial amniocentesis. In the cited study in only 4 patients amniotic fluid was taken more than once, and therefore no conclusions could be drawn. The present study showed that incidence of intraamniotic infection after repeated amniocentesis was 0.42%. In the patient with positive microbial finding transferrin blood and intraamniotic levels were lower than in the all other investigated patients.

In conclusion,we can say that transferrin intraamniotic levels increased according to the progression of gestation, reaching maximal values at the 39th week of gestation. Transferrin blood concentrations were found to be increasing in a similar pattern, but it is important to point out that in the puerperium, when the antimicrobial protection is the most important, transferrin had maximal blood levels on the 2nd postpartal day. In addition, we confirmed that this nonspecific factor plays an important role in the antimicrobial activity of amniotic fluid.

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