Med. J. Cairo Univ., Vol. 62, No. 2, June (Suppl.) : 63-71. 1994.

Amniotic Fluid Transferrin in the Detection of Chorioamnionitis

MOHAMED ABOUL GHAR, M.D.; AHMED ABOUL NASR, M.D.; SHERIF EL SHARKAWY, M.D.; NASHWA ABDEL KADER, M.D. and EKRAM FATEEN, M.D.

The Obstetrics and Gynecology Department, Faculty of Medicine, Cairo University and the Human Genetics Department, National Research Center.

Abstract

The purpose of this study was to determine the transferrin concentration in amniotic fluid in cases of premature rupture of membrane (PROM) before and after 12 hours from rupture of membranes. In order to assess the amniotic fluid transferrin as a parameter for early detection of intrauterine infection. The present study includes a total number of 45 pregnant females, 30 with PROM (15 cases within 12 hours and 15 cases after 12 hours from rupture of membranes) and 15 normal cases with intact membranes as a control group. Amniocentesis under direct ultrasonic guidance was performed to all patients. The obtained amniotic fluid samples were subjected to aerobic bacterial culture and transferrin assay. Moreover, C-reactive protein and WBCs count were estimated in those patients. The present study reveals : Amniotic fluid cultures obtained from the patients with PROM were positive in 16 cases, 10 of the them showed clinical chorioamnionitis, while the remainder 6 did not show any sign of infection. There was a significant increase in the level of amniotic fluid transferrin in cases of PROM $>$ 12 hours as compared with control group ($P < 0.01$). Moreover, there was marked increase in amniotic fluid transferrin level in +ve cases of clinical chorioamnionitis as compared with —ve cases and the difference was statistically highly significant ($P < 0.001$). Also, amniotic fluid transferrin level showed marked increase in cases of PROM with +ve culture «whether, there was clinical chorioamnionitis or not» as compared with —ve culture. The difference was statistically highly significant. ($P < 0.001$). In conclusion, the determination of transferrin in amniotic fluid could be done in cases with PROM as a reliable predictor and diagnostic tool for infection.

Introduction

PREMATURE rupture of membranes (PROM) is one of the most common complications of pregnancy, it is occurring in approximately 10% of all pregnancies[1]. The major complication of PROM is chorioamnionitis which occurs if the expectant management is applied in order to gain more time for in utero fetal development. However, this policy has the

disadvantage of the increased risk of maternal and/or fetal infections. For this reason, early detection of intra-uterine infection is required in order to ensure the most favorable outcome of pregnancy, minimizing the fetal risks and the later sequelae of this hazard to mother and her neonate[2].

In order to diagnose chorioamnionitis certain clinical and laboratory criteria should be established, e.g. rise of body temperature of 37.8C or more, maternal and/or fetal tachycardia, uterine tenderness and foul smelling vaginal discharge. Moreover, conseming laboratory data the following could increase; WBCs count, ESR and C-reactive protein level in the sera of those patients[3]. In addition, amniotic fluid sample should be withdrawn to allow bacterial culture[4].

The transferrin is a protein bound to iron and has a bacteriostatic property[5].

The aim of this work is to determine the transferrin concentration in amniotic fluid in cases of PROM before and after 12 hours from rupture of membranes compared to control group and to study the relation of transferrin level in amniotic fluid to bacterial colonization, maternal serum C-reactive proteins and WBCs count in cases of silent and manifested chorioamnionitis compared to the control group.

Material and Methods

This study includes 45 patients classified into two main groups :

1. **Control group :** comprised 15 patients in normal labor with intact membrane.
2. **Studied group :** comprised 30 patients with confirmed PROM. The studied group is divided into 2 subgroups :
   a) 15 patients seen within 12 hours after rupture of membrane.
   b) 15 patients seen after 12 hours after rupture of membrane.

These cases were of gestational ages ranging from 30-40 weeks, and their ages ranged from 18-40 years. In the 15 cases of PROM within 12 hours, 8 of them were delivered by normal vaginal deliveries and the remainder 7 by C.S.

Meanwhile, in the other subgroup of PROM after 12 hours, 12 patients delivered vaginally and 3 by C.S. However, the control group delivered vaginally, PROM were documented by sterile speculum examination and nitrazine test. All patients were subjected to amniocentesis under direct ultrasonic guidance to obtain A-F samples for acrobic bacterial cultures and transferrin assay. Moreover, blood samples were taken for both C-reactive proteins of WBCs count determination. The rocket electroimmunodiffusion technique of Laurel was used for quantitative estimation of C-reactive proteins.

The amniotic fluid sample is about 10 c.c. in volume, it was divided as 5 c.c. for immediate aerobic bacerial culture. by using the bacturcult culture medium (sample Laboratories, Cranburg, New-Jersy). It is a disposable plastic tube, the inner surface of which is coated with a special nutrient indicator culture medium.

The remaining half of the sample was collected in a closed sterile plastic tube and stored frozen in deep freeze at-20°C Till being analysed by transferrin assay. The method which was applied for this assay, the quantitative estimation of transferrin by the use of Rocket electroimmunodiffusion technique of Laurel[6].

The white blood cell count was done by the method described by Dacie and Lewis using the Neubauer counting chamber after diluting the sample with 2% acetic acid.

All patients in this study did not receive any antibiotics until all samples were taken.

Results

Table (1) shows the results of WBC counts, serum C-reactive proteins levels and amniotic fluid transferrin assay in the 3 studied groups. We noticed that the difference in WBCs counts between PROM before (group A) and after 12 hrs (group B) is statistically highly significant ($P < 0.005$) compared to the controlled group. Mean while, the serum C-reactive proteins in group A only show a significant difference compared to control ($P < 0.05$). Moreover, the differences in amniotic fluid transferrin levels between group B and the controlled group are statistically highly significant ($P < 0.01$), whereas, it show insignificant differences in other groups.

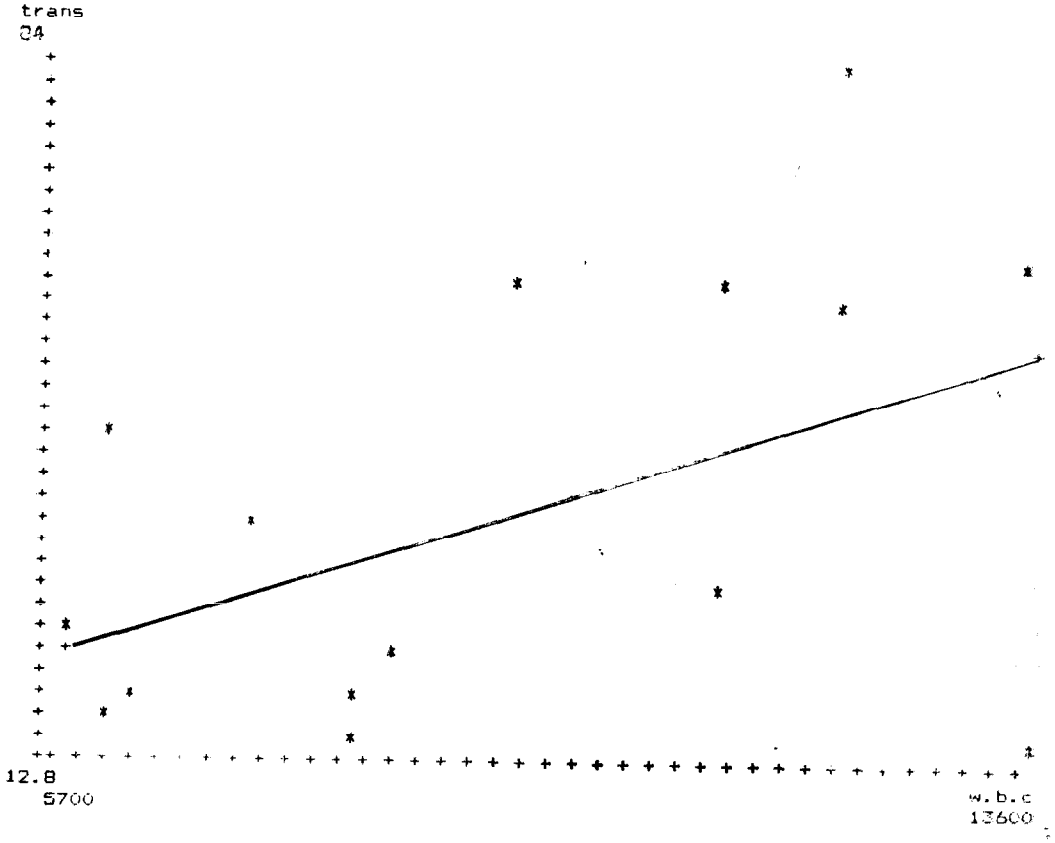

Fig. 1. Linear corelation between WBCs and transferrin in cases of PROM > 12 hours

Table (1) : The WBCs Count, Serum CRP Level and A.F. Transferrin in the Three Studied Groups.

	WBCs/cc Mean ± S.D.	«t» value - «p» valuc
PROM < 12 hr.	10586 ± 3667	*3.2 - < 0.001
PROM > 12 hr.	9193 ± 2793	**2.28 - < 0.01
control	7440 ± 1025	***1.17 - > 0.05
F	5.01	
P	< 0.01	Highly significant
	CRP g/ml	«t» value - «p» value
PROM < 12 hr.	3696 ± 3387	*1.98 - < 0.05
PROM > 12 hr.	2826 ± 2497	**1.37 - > 0.05
control	1802 ± 1461	***0.8 - > 0.05
F	2.03	
P	> 0.05	Insignificant
	Transferrin mgZ	«t» value - «p» value
PROM < 12 hr.	27.69 ± 21.46	*1.6 - > 0.05
PROM > 12 hr.	37.28 ± 22.98	**3.05 - < 0.01
control	17.64 ± 9.68	***1.18 - > 0.05
F	4.01	
P	< 0.05	Significant

Table (2) shows 3 major parameters in this study (WBCs count, serum C-reactive protein and A.F. transferrin in +ve and —ve cases of clinical chorioamnionitis.

The WBCs count is statistically highly significant between +ve and —ve cases ($P < 0.001$). Meanwhile, the difference in C-reactive protein in both group is statistically insignificant ($P < 0.005$). Wehreas, the difference in amniotic fluid transferrin between +ve and —ve cases is statistically highly significant ($P < 0.001$).

Table (3) shows a comparison betweer —ve and +ve cultures as regard WBC count, C-reactive proteins and amniotic fluid transferrin levels.

We noticed a highly significant increase ($P < 0.001$) between +ve versus —ve culture as regard WBCs counts. Moreover there is a statistically highly significant difference ($P < 0.001$) in serum C-reactive protein and amniotic fluid transferrin in +ve and —ve culture cases.

Table (2) : WBCs Count, Serum CRP Levels and A.F. Transferrin in +ve and —ve Cases of Clinical Chorioamnionitis.

	WBCs/cc
—ve	7857 ± 1309
+ve	13330 ± 3260
t	8.06
p	< 0.001
	CRP g/ml
—ve	2456 ± 2686
+ve	3890 ± 2198
t	1.54
p	> 0.05
	Transferrin mg%
—ve	19.44 ± 10.02
+ve	55.88 ± 21.89
t	7.57
p	< 0.001

Fig. (1) shows linear correlations between WBCs and transferrin in cases of PROM > 12 hours.

The results of bacterial cultures are as follows : 7 cases among the 15 patients with PROM within 12 hours showed no growth. Meanwhile, 5 cases had streptococci and 3 cases had E.coli infections.

In the other group of PROM after 12 hours, 7 cases showed no growth. Meanwhile, 5 cases had +ve cultures for streptococci and 2 cases had +ve culture for E coli and one case had staph infection.

Table (3) : A Comparison between —ve and +ve Culture as Regard WBCs Count CRP and A.F. Transferrin Levels.

	WBCs/cc	«t» value - «p» value
—ve culture	7531 ± 1139	*6.2 - < 0.001
Strept.	12370 ± 3779	**4.9 - < 0.001
E-coli	10520 ± 1826	
F	21.5	
P	< 0.001	Highly significant
	CRP g/ml	«t» value - «p» value
—ve culture	1544 ± 1430	*4.89 - < 0.001
Strept.	4580 ± 2317	**6.2 - < 0.001
E-coli	6760 ± 3083	
F	22.2	
P	< 0.001	Highly significant
	Transferrin mg%	«t» value - «p» value
—ve culture	18.8 ± 10.69	*6.6 - < 0.001
Strept.	53.4 ± 21.6	**1.7 - < 0.05
E-coli	29.0 ± 19.6	
F	20.27	
P	< 0.001	Highly significant

Discussion

The tests which are involved in this study to detect early infection in cases of PROM are amniotic fluid transferrin, culture of amniotic fluid, maternal serum C-reactive protein and leukocytic count. The importance of these parameters were stressed by Berardi et al.[7] as more sensitive tests compared to the conventional clinical signs of chorioamnionitis e.g. hyperthermia, fetal tachycardia, tender uteru and discolored amniotic fluid.

Ten patients from the thirty witl PROM, developed clinical chorioamnioniti based on elevation of maternal tempera ture to 38°C or more in absence of othe caused of fever.

A.F. culture was done to identif specific organisms and to select prope antibiotics prescription. By means of thi

nethod clinically silent chorioamnionitis ·ould be easily diagnosed. In our study he results of amniotic fluid culture obtaned from the patients with PROM were ›ositive in 16 patients, 10 of them showed :linical chorioamnionitis while the remainler 6 did not show any sign of infection silent infection).

This agrees with the study of Hollander et al[8], who found that culure results from amniotic fluid obtained n cases with PROM demonstrates that :linically silent infection may constitute ›ne third of these patients.

Transferrin has bacteriostatic properties, he antibacterial activity is exerted by :helating iron which is necessary factor for ›acterial growth[5]. In our study, we ound an apparent relationship between mniotic fluid transferrin level, PROM and nfection.

There was marked increase in AF ransferrin level in positive cases of clini:al chorioamnionitis as compared with ıegative cases, the mean value in negative ·ases was 19 44 ± 10.02 mg%, meanvhile in positive cases it was 55.88 ± :1.89 mg, the differences between the mean vere statistically highly significant (P <).001).

Moreover, there is significant increase vith level of A.F. transferrin in positive :ulture 81.4 ± 41 mg% compared to :ases of negative culture 18.8 ± 10 mg% P < 0.001).

Our study demonstrate the importance ›f using A.F. transferrin level as early predictor of silent chorioamnionitis and as a diagnostic tool for the already established cases of clinical chorioamnionitis. To the best of our knowledge, no studies investigated amniotic fluid transferrin in cases of PROM and its relation to infection.

Our work demonstrates no statistical significant difference in the mean C-reactive proteins positive and negative cases of clinical choroamnionitis. Meanwhile, there is significant elevation of C-reactive proteins level in cases of +ve cultures if compared to —ve cultures. This means that C-reactive protein is an early predictor of silent chorioamnionitis, this coincides with results of Hammed et al[9].

These findings apply with the view of Evaldson et al.[10] and Salzer et al. [11] who found that C-reactive protein is a reliable early predictor of clinical chorioamnionitis. In addition, Ismail and Coworker[12], found that C-reactive protein was more sensitive than other standard laboratory or clinical tests in predicting clinical chorioamnionitis.

Normal pregnancy was associated with leukocytic changes causing significant rise in number of these cells. Therefore, the ability of this serum marker in diagnosis of clinical chorioamnionitis may be limited [13].

In our study, there was significant elevation in WBCs count in both +ve clinical chorioamnionitis, and in +ve culture compared to —ve cases of clinical

chorioamnionitis and —ve culture ($P < 0.001$), these findings coincide with many authors [**14,15**].

In conclusion :

Chorioamnionitis is one of the major complications of PROM. Transferrin concentration was increased in cases of PROM with +ve bacterial culture possibly because transferrin has a bacteriostatic effect. Meanwhile, WBCs count should be used as early marker for chorioamnionitis.

As amniotic fluid transferrin was found to be significantly increased in PROM cases with +ve bacterial culture «either with clinically manifested or silent chorioamnionitis», so we recommend that, determination of transferrin in amniotic fluid could be done in cases with PROM as a reliable early predictor and diagnostic tool for infection.

Amniocentesis will provide amniotic fluid suitable for both bacterial culture and transferrin determination. However, if there is any technical difficulty in doing the amniocentesis, transferrin could be determined in amniotic fluid collected from the vagina.

References

1. NINTZILEOS, A.M.; CAMPELL, W.A.; NOCHIMSON, D.J. and WEINBAUM, P.J. : Degree of oligohydraminus and pregnancy outcome in patients with preterm premature rupture of membranes. Obstet. Gynecol., 67 : 162, 1985.
2. SPERLING, R.S.; RAMAMURTHY R.S. and GIBBS, R.S. : A compariso of intrapartum, immediate post-partun treatment of intraamniotic infectior Obstete Gynecol., 70 : 861, 1987.
3. SATIN, A.J.; MABERRY, M.C. LEVENO, K.J. and SHERMAN, M. Chorioamnionitis : A harbinger of dys tocia. Obstet Gynecol., 79 : 913, 1992.
4. OHLSSON, A. and WANG, E. : A analysis of antenatal tests to detect in fection in preterm premature rupture o membranes. Am. J. Obstet. Gynecol., 162 809, 1990.
5. SCHADE, A. and CAROLINE, L. : A iron binding component in human plasm Science, 104 : 340, 1976.
6. LAURELL, C.B. : Quantitative estima tion of proteins by electrophoresis i agarose gel containing antibodies. Ana Biochem. 15 : 45, 1966.
7. BERARDI, J.C.; HUTIN, S.; GODORI J. and MADINIA, V. : The value o C-reactive protein in the detection o *chorioamnioitis in cases of prematur* rupture of membranes (Abstract). Rey Fr-Obstet Gyneacol, 86 : 229, 1991.
8. HOLLANDER, R.; SAVITZ, D.A. an THROP, J.M. : Infection and prema ture. Clin. Obstet. Gynecol., 31 : 55 1986.
9. HAMED, C.; TEJANI, N.; VERNA an ARCHIBALD, F. : Silent chorioamnic nitis as a cause of preterm labor refrac tory to tocolytic therapy. Am. J. Obstet Gynecol., 149 : 726, 1984.
10. EVALDSON, G.; LAGNELUS, A. an WINIARSKI, J. : Premature ruptur of membranes. Acta. Obstet. Gynecc Scand, 59 : 385, 1980.
11. SALZER, H.R.; GENEGER, H.; MUHAI U.; LISCHTA, A.; SCHATTEN, C. an POLLAK, A. : C-reactive protein : a early masker for neonatal bacterial in fection due to prolonged rupture of am niotic membranes and/or amnionitis Acta. Obstet. Gynecol. Scand, 66 : 36 1987.

12. ISMAIL, M.; ZINAMAN, M.; LOWENSOHN, R. and MOAWARD, A. : The segnificance of C-reactive protein leve's in women with premature rupture of membranes. Am. J. Obstet. Gynecol., 151 : 541, 1985.

13. GIBBS, R.S.; CASTILLO, M.S. and RODGERS, P.J. : Management of acute chorioamnionitis. Am. J. Obstet. Gynecol., 136 : 709, 1980.

14. HARGER, H.J.; HASING, W.A.; TUOMALA, E.R. and GIBBS, S.R. : Risk factors for preterm premature rupture of membranes. A malticenter case control study. Am. J. Obstet Gynecol., 163 : 130, 1990.

15. GOYERT, G.; WOLFE, H.; BHATAI, R. and NORMAN, G. : Evaluation of potential early markes of chorioamnionitis associated with preterm rupture of membranes. Am. J. Perinatol., 7 : 33, 1990.