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Relationship between some obstetric landmarks and the concentration of alpha-fetoprotein in maternal blood

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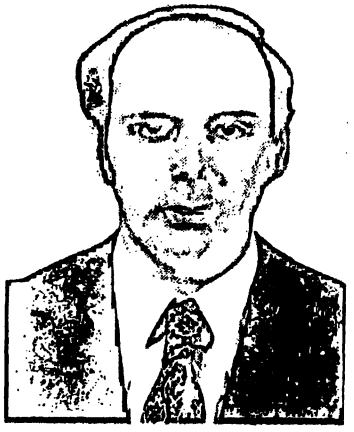
1 Introduction

Alpha-fetoprotein (AFP), a protein of fetal origin encountered in pregnancy serum, cord serum and amniotic fluid, has been largely used in prenatal diagnosis of open neural tube defects and other fetal abnormalities by its quantitation in maternal blood and amniotic fluid [2, 3]. High levels of AFP are also associated with fetal distress and missed abortion [11, 12]. This makes AFP measurement another potentially useful tool to the study and management of complicated pregnancies. There is a great variability of AFP values in maternal circulation at the different periods of gestation taken separately. This variability may be due to a number of factors. Some of these factors were described by HABIB [6], but in the whole they are not well investigated. The present report attempts to study the variation of the levels of AFP in pregnancy serum with some obstetric landmarks, in the hopes that part of this variability could be clarified.

2 Material and methods

Peripheral venous blood samples were collected from 89 women at 32 to 34 weeks of pregnancy and from 115 women at term. The sera were separated and kept frozen at − 20 °C until further processing. Gestation period was calculated from the date of the last menstrual period and confirmed by clinical examination. Patients were excluded from the study for the following reasons: uncertain gestation age, symptoms or signs of threatened abortion, fetal death, suspected twins, previous birth of a child with neural-tube defect, maternal diabetes or hypertension. All females attended the Antenatal Clinic of the Newcastle General Hospital. Data on smoking habit, parity and pregnancy number, newborn sex and 1 − minute Apgar score

Curriculum vitae



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were obtained from the patients. Maternal serum AFP was measured using radioimmunoassay kits (Hoechst-Behringwerke) and computed from double determinations. Statistical examination was carried out using the electronic system SPSS [9]. Linear correlation coefficient of Pearson and analysis of variance (F, Duncan new multiple range test) were employed. The level of significance was set at 5%.

3 Results

3.1 Relationship between AFP levels and either parity or pregnancy number

No significant difference was observed between maternal serum AFP mean levels of nulliparous, women with one child or with two or more children in the last two months of pregnancy, although there was a slight suggestion that AFP decreases as parity increases at 32–34 weeks gestation (table I). Similar results were found when pregnancy number was considered (table II). When parity and pregnancy number were correlated with AFP concentration in maternal blood the coefficients of correlation were respectively 0.002 ($n = 89$, $P = 0.492$) and -0.0184 ($n = 89$, $P = 0.432$) at 32–34 weeks and -0.0093 ($n = 114$, $P = 0.461$) and 0.0006 ($n = 115$, $P = 0.498$) at term.

3.2 Relation between AFP concentration and 1 – minute Apgar score

Table III shows that higher levels of AFP are present in blood of mothers whose babies presented Apgar scores below 8, and this difference became significant at 32–34 weeks gestation or in the last two months of pregnancy (32–41 weeks).

3.3 Effect of smoking on maternal serum AFP levels

Table IV indicates that smokers have higher AFP values than nonsmokers, particularly

Table I. Relation between parity and AFP concentrations (mean ± S.D.) at 32–34 weeks of pregnancy and at term.

Parity	Concentration of AFP (ng/ml)	
	32–34 wks gestation	At term
Nulliparous	235 ± 96 (n = 36)	172 ± 97 (n = 57)
Parous (1 child)	236 ± 115 (n = 29)	178 ± 93 (n = 39)
Parous (2 or more children)	219 ± 94 (n = 24)	171 ± 71 (n = 18)
F Probability	0.7790	0.9478

Table II. Relationship between pregnancy number and AFP levels (mean ± S.D.) at 32–34 weeks of pregnancy and at term.

Pregnancy number	Concentration of AFP (ng/ml)	
	32–34 wks gestation	At term
One	241 ± 100 (n = 30)	176 ± 102 (n = 48)
Two	224 ± 107 (n = 28)	170 ± 95 (n = 37)
Three or more	228 ± 95 (n = 31)	172 ± 71 (n = 30)
F Probability	0.8129	0.9654

when they smoke more than 20 cigarettes per day. It should be noted that the group of nonsmokers corresponds to women who not only did not smoke during pregnancy but also had never smoked at all. The overall statistical differences between all groups were however not significant; but, when the difference between the means of the various groups were computed separately, a significance appeared between nonsmokers and smokers of more than a pack per day in samples from 32 weeks to term ($P = 0.032$).

3.4 Influence of newborn sex on the levels of AFP in maternal circulation

Table V shows a clear tendency of higher AFP values in mothers of boys, and the difference

Table III. Comparison between AFP serum values (mean ± S.D.) of mothers whose babies had Apgar scores equal or superior to 8 and below 8.

1-minute Apgar scores	Maternal serum AFP concentration (ng/ml)		
	32–34 wks gestation	At term	32–41 wks gestation
Equal or above 8	217 ± 90 n = 67	168 ± 93 n = 82	190 ± 95 n = 149
Below 8	276 ± 115 n = 22	187 ± 87 n = 33	223 ± 107 n = 55
F Probability	0.014	0.294	0.032

Table IV. Maternal AFP levels (mean ± S.D.) of smokers and non-smokers in late pregnancy.

Maternal smoking habits	Maternal serum concentration of AFP (ng/ml)		
	32–34 wks of gestation	At term	32 wks of gestation to term
Non-smokers	222 ± 96 (n = 32)	170 ± 101 (n = 51)	190 ± 101 (n = 83)
Smokers (up to 20 cigarettes per day)	247 ± 118 (n = 18)	165 ± 81 (n = 29)	196 ± 104 (n = 47)
Smokers (more than 20 cigarettes per day)	262 ± 114 (n = 10)	228 ± 114 (n = 12)	244 ± 113 (n = 22)
F Probability	0.507	0.135	0.096

Table V. Maternal serum AFP concentration in relation to the sex of the fetus in late pregnancy.

Sex of fetus	Maternal serum AFP concentration (ng/ml)	
	32–34 wks of gestation	At term
Males		
Mean ± S.D.	251 ± 108	186 ± 95
n	45	60
Females		
Mean ± S.D.	209 ± 87	161 ± 88
n	43	51
F Probability	0.044	0.153

computed by analysis of variance became significant when AFP reaches its peak in maternal blood (32–34 weeks gestation).

4 Discussion

AFP concentration in maternal blood is regulated by various and complex not yet fully understood mechanisms. Basically, it depends on the rate of biosynthesis of the protein by the fetal liver, its transference to maternal circulation and also on the rate of catabolism. Any factor which acts on these mechanisms would certainly modify AFP values in maternal blood. Hence, the present study investigated the relationship between these values in the last two months of pregnancy and some obstetric landmarks such as parity, pregnancy number, 1 – minute Apgar score, smoking habit and newborn sex, in the hopes that some significant association would elucidate the possible role of some of these factors in controlling the levels of AFP. It was seen that parity and number of pregnancies do not correlate with the concentration of AFP in maternal serum. Therefore, these results do not indicate a general immunosuppressive role of AFP in late pregnancy in the maternal system, as postulated by some investigators [7, 8], since the larger the antigenic stimulation provoked by a great number of pregnancies the higher the levels of AFP would be expected in order to explain fetal nonrejection.

The relationship between Apgar score, smoking habit and AFP concentration in maternal blood gave rise to interesting findings. Our results suggest that serum AFP levels in late gestation are elevated in heavy smokers compared to nonsmokers, observations not yet reported according to our knowledge. THOMSEN et al. [13] have recently described an augmentation of AFP levels in blood of pregnant smokers at 16 weeks gestation. Therefore, it is possible that this elevation occurs throughout gestation. Since the habit of cigarette smoking provokes important alterations in the ultrastructure of the umbilical artery, umbilical vein and placenta [1], similar to the ones encountered in diabetic angiopathy with increased microvascular permeability to plasma proteins [10], it is feasible that the greater maternal blood AFP values found by ourselves and by THOMSEN et al. [13] reflects a leakage of the protein across the placenta and/or through the membranes. Therefore, since albumin is most like AFP in molecular size and configuration, it is perfectly understandable that albumin leakes in diabetics as AFP does in pregnant smokers. In fact, since maternal serum AFP is nearly totally derived from the fetus via amniotic fluid and amniotic fluid albumin is totally derived from maternal circulation in a proportional bidirectional protein movement, it is expected that in pregnant smokers there is also a leakage of albumin from mother's blood towards amniotic fluid. This may represent an interesting field to study.

Results of the present study also confirm previous findings of a correlation between high maternal serum AFP levels and fetal distress [11, 12, 15]. We found a negative association between maternal serum AFP levels and Apgar scores of the newborns, seeming that higher levels of the protein are present in the blood of mothers who gave birth to babies of lower Apgar scores. This can also be interpreted as an altered fetal-maternal barrier, prejudicing well-being of the fetus. Although this relationship must not be considered as proof of a causal association, it may represent a potentially useful tool to the obstetrician and pediatrician in the management of a complicated pregnancy and of the future newborn respectively.

Finally the degree of relationship between sex of the newborn and the AFP levels was examined. Mothers of boys had significantly higher levels of the protein in their blood. These findings agree to some extent with those of CABALLERO et al. [4] who, while using maternal blood at delivery, found that mothers of boys contained higher concentrations of AFP in their circulation than did mothers of girls, although they did not find significance in their analyses; this difference became significant when these investigators studied serum AFP levels in newborn

males and females (males contained greater levels). In an effort to analyze the results of the present study and those of CABALLERO et al. [4], it may be postulated that male fetuses (heavier than female fetuses) produce more AFP in late pregnancy and this is reflected not only in fetal circulation but in the mother's blood too. It is also possible that some factors present in males or females stimulate or inhibit respectively AFP production and/or secretion. When AFP level in maternal circulation is maximum (32—34 weeks gestation), the sex variation is more evident. Various hormones have been described to suppress or encourage the protein fabrication by the liver [5, 14, 16]. More investigation in this area will probably uncover the mechanisms responsible for the sex variation in the AFP maternal serum values.

To the authors' knowledge some of the approaches examined in this study are original. The results must be taken with caution. It is advisable to extend the surveys performed to all periods of gestation. The importance of the screening test of AFP quantitation in pregnancy serum for the prenatal diagnosis of malformations, particularly the open neural tube defects, is well established at the present time and largely used in developed countries. A correct interpretation of this test may need special tables of AFP normal ranges, which should regard the factors briefly discussed above. Not appreciating these elements could result in erroneous interpretation of the test result and in turn lead to an aggressive plan for clinical management which might include premature and unjustified delivery.

Summary

Alpha-fetoprotein (AFP) quantitation in maternal blood has been used for prenatal diagnosis of anencephaly, spina bifida and some other congenital abnormalities. The levels of AFP in pregnancy serum are greatly dispersed within each gestational period, what makes the test of difficult interpretation mainly in border line cases. The factors which contribute to this variability are still poorly understood. They must act either on the synthesis or catabolism of the protein, or on the permeability of the fetal-maternal barrier. In an attempt to elucidate some of these factors, the levels of AFP in serum of 89 women at 32 to 34 weeks gestation and of 115 women at term were measured by radioimmunoassay and related to several obstetric variables such as parity, pregnancy number, 1 — minute Apgar score, smoking habit and sex of newborn. It was seen that parity and pregnancy number do not correlate with the levels of AFP in pregnancy serum (tables I and II). Smokers, particularly those who smoke more than a pack per day, however, had higher levels of the protein in their blood (table IV). The interpretation of these data should take into consideration the fact that smoking causes vasoconstriction and important vascular alterations similar to the ones encountered in diabetic angiopathy. It is possible that an increased microvascular permeability of placenta and/or membranes to proteins of the size of AFP from the fetus to maternal circulation may explain the greater levels of the protein in serum of smokers. Higher AFP levels were also found in blood of gravidas who gave birth to babies with lower 1 — minute Apgar scores (table III) what may be of interest to obstetric and pediatric care as indicative of a high risk pregnancy with a possibly feeble newborn. Finally we observed greater levels of AFP in blood of mothers who gave birth to male newborns (table V), what cannot be presently explained. From the above mentioned results, it is concluded that some of the variables described should be taken into account when one analyzes AFP values in maternal serum for prenatal diagnosis purposes.

Keywords: Alpha-fetoprotein, Apgar score, newborn, pregnancy serum, smoking.

Zusammenfassung

Beziehungen zwischen einigen geburtshilflichen Parametern und der Alpha-Fetoproteinkonzentration im mütterlichen Blut

Die quantitative Bestimmung des Alpha-Fetoproteins (AFP) im mütterlichen Serum dient der pränatalen Diagnostik eines Anencephalus, einer Spina bifida und einiger anderer kongenitaler Mißbildungen. Während der einzelnen Gestationsabschnitte zeigt der AFP-Spiegel eine große Streubreite, was die Interpretation der Werte, besonders in Grenzfällen, erschwert. Über die Ursachen dieser Variabilität ist bisher wenig bekannt. Möglicherweise sind die Synthese und der Katabolismus des Pro-

teins oder die Permeabilität der feto-maternalen Schranke daran beteiligt. Zur Abklärung einiger Faktoren wurde der AFP-Spiegel im Serum von 89 Frauen in der 32. bis 34. Schwangerschaftswoche sowie von 115 Frauen am Termin über einen Radioimmunassay bestimmt und dann zu verschiedenen geburtshilflichen Parametern wie Parität, Anzahl der Schwangerschaften, Apgar-Score nach 1 Minute, Rauchgewohnheiten und Geschlecht des Neugeborenen in Beziehung gesetzt. Wir konnten zeigen, daß zwischen der Parität sowie der Anzahl der Graviditäten und dem mütterlichen AFP-Spiegel keine Korrelation besteht (Tabellen I und II). Raucherinnen, besonders solche mit mehr als 20 Zigaretten pro Tag, hatten höhere AFP-Spiegel im Serum (Tabelle IV). Hier gilt es zu berücksichtigen, daß das Rauchen eine Vasokonstriktion sowie Veränderungen an den Blutgefäßen verursacht, vergleichbar den Veränderungen bei einer diabetischen Angiopathie. Denkbar ist eine erhöhte Permeabilität von Mikrogefäßen der Plazenta für Proteine von der Größe des AFP. Höhere AFP-Spiegel wurden auch im Blut von Schwangeren gefunden, deren Neugeborene einen niedrigen Apgar-Score 1 Minute p. p. aufwiesen (Tabelle III). So könnte ein höherer AFP-Spiegel als Hinweis für eine Risikoschwangerschaft sowie ein möglicherweise deprimiertes Kind gewertet werden. Schließlich konnten wir beobachten, daß die AFP-Spiegel bei Müttern von männlichen Neugeborenen höher waren, ohne daß wir eine Erklärung dafür finden konnten (Tabelle V). Wir meinen, daß einige der oben beschriebenen Parameter berücksichtigt werden sollten, wenn AFP-Bestimmungen zum Zweck der pränatalen Diagnostik durchgeführt werden.

Schlüsselwörter: Alpha-Fetoprotein, Apgar-Score, Neugeborenes, Rauchen, Serum bei Schwangeren.

Résumé

Relations entre quelques paramètres obstétricaux et la concentration maternelle sanguine d'alphafœtoprotéine

Le dosage de l'alpha-fœtoprotéine (AFP) dans le sérum des femmes enceintes a été employé pour le diagnostic prénatal d'anencephalie de spina bifida et d'autres anomalies congénitales. Les taux d'alpha-fœtoprotéine dans le sérum des femmes enceintes sont très dispersés à chaque période de la grossesse ce qui rend difficile l'interprétation du test, surtout pour les dosages limites. Les facteurs qui contribuent à cette variabilité sont encore mal compris. Il peuvent agir aussi bien sur la synthèse que sur le catabolisme des protéines, ou sur la perméabilité de la barrière materno-fœtale. Pour en élucider quelques-uns, les taux d'AFP sérique de 89 femmes de 32 à 34 semaines de gestation et de 115 femmes à terme, ont été mesurés par radioimunoessai et corrélationnés avec quelques variables obstétricales (parité, numéro de gestations, tabagisme, score d'apgar à la lère minute, poids du nouveau-né et du placenta et sexe du nouveau-né). Les corrélations ont été calculées parmi quelques paramètres obstétricaux.

Parité et numéro de gestations ne sont pas corrélés avec le taux d'AFP dans le sérum des femmes enceintes (tableaux I et II). Les fumeuses, particulièrement celles qui fumaient plus d'un paquet par jour, cependant, ont eu les taux d'AFP les plus élevés (tableau IV). L'interprétation de ces données doit faire considérer le fait que fumer cause une vasoconstriction et d'autres importantes altérations vasculaires semblables à celles trouvées dans l'angiopathie diabétique. Il est possible, donc, qu'une perméabilité microvasculaire augmentée, du placenta et/ou des membranes pour les protéines de la taille de l'AFP, du fœtus vers la circulation maternelle puisse expliquer les niveaux assez élevés d'AFP dans le sérum des fumeuses. Les niveaux les plus élevés d'AFP ont été observés dans le sérum des mères dont les nouveaux-nés avaient les scores d'apgar (tableau III) les plus bas ce qui peut être intéressant pour les soints obstétricaux et pédiatriques comme indicatifs de grossesse à haut risque et de la possibilité d'un nouveau-né faible.

Finalement, nous avons observé les taux les plus élevés d'AFP dans le sérum des mères dont les nouveaux-nés étaient de sexe masculin, ce qui ne peut pas encore être expliqué (tableau V). Les résultats présentés ici permettent de conclure que quelques-unes de ces variables décrites doivent être considérées quand on analyse les sérum des femmes enceintes pour le diagnostic prénatal.

Mots-clés: Alpha-fœtoprotéine, fumeuses, nouveau-né, scores d'Apgar, sérum des femmes enceintes.

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