ORIGINAL RESEARCH

SERUM ALPHA FETOPROTEIN LEVELS IN HEALTHY FULL-TERM NEONATES AND INFANTS

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ABSTRACT

Objective: Alpha fetoprotein (AFP) is an important tumor marker in childhood. However, AFP levels remain high during the first few months of life, making clinical interpretation difficult in this period. The aim of the present study is to determine normal AFP levels in healthy full-term neonates and infants followed-up at Kocaeli University Hospital, Department of Pediatrics.

Materials and Methods: Healthy full-term neonates born in Kocaeli University Hospital and <12 month-old infants attending the well baby outpatient clinic were enrolled in the study. Blood samples were drawn from the umbilical cord vein at delivery or peripheral vein after delivery. Serum AFP concentration was determined by solid phase, two-site chemiluminescent immunometric commercial diagnostic assay.

Results: Ninety-four neonates with a gestational age of 37-41 weeks and <12 month-old infants (48 male, 46 female) were enrolled. Mean AFP level of cord blood samples was 67.246±52.137 (105-226.000) ng/ml. Serum AFP levels of all 94 babies had a significant negative correlation with the postnatal age (r=-0.877, p<0.001). Results of regression analyses did not support a significant sex dependency (r= -.096, p=0.35).

Conclusion: Normal range of AFP in healthy full-term neonates and infants is very wide. Pediatric oncologists must consider moderately high values carefully in the follow-up period and if the decrease in the follow-up measurement is slower than the expected half-life for that age, the probability of an AFP-producing tumor could be high. This approach would prevent unnecessary interventions depending on false positive AFP results.

Keywords: *Alpha fetoprotein, Normal range, Newborns, Infants*

SAĞLIKLI TERM YENİDOĞAN VE İNFANTLARDA SERUM ALFA FETOPROTEİN DÜZEYLERİ

ÖZET

Amaç: Alfa fetoprotein (AFP) çocukluk çağında önemli bir tümör belirleyicisidir. Bununla birlikte yaşamın ilk birkaç ayında AFP düzeyinin yüksek kalması bu periyodda klinik yorumu zorlaştırmaktadır. Bu çalışmanın amacı Kocaeli Üniversitesi Hastane, Pediatri Bölümü'nde izlenen sağlıklı term yenidoğan ve infantlarda serum AFP düzeylerinin belirlenmesidir.

Materyal ve Metod: Kocaeli Üniversitesinde doğan sağlıklı term yenidoğanlar ve sağlam çocuk polikliniğine başvuran sağlıklı <12 aylık infantlar çalışmaya alındı**.** Kord veninden (doğumda) veya periferik venlerden kan örnekleri alındı. Serum AFP konsantrasyonu solid faz, ikili-bölümlü kemilüminesen immünometrik tanısal yöntemle değerlendirildi.

Bulgular: Doksan dört örnek (48 erkek, 46 kız) değerlendirildi. Kord kanı ortalama AFP düzeyi 67.246±52.137 (105-226.000)ng/ml bulundu.Doksan dört bebekte serum AFP düzeyi ile postnatal yaş arasında anlamlı negatif korelasyon saptandı (r= -0.877, p<0.001).Regresyon analizinde, cinsiyete bağlı farklılık gösterilemedi (r= -.096, p=0.35).

Sonuç: Sağlıklı yenidoğan ve infantlarda AFP normal sınırları çok geniştir. Pediartik onkolog orta derecede yüksek AFP değerlerini dikkat ile değerlendirmeli ve izlemde AFP düzeyindeki düşme, yaş için uygun yarılamna süresinden yavaş ise AFP üreten tümör olasılığının yüksek olabileceği düşünülmelidir. Bu yaklaşım, yanlış pozitif AFP sonuçlarına bağlı gereksiz girişimleri önleyecektir.

Anahtar Kelimeler: Alfa fetoprotein, Normal sınır,Yenidoğan, İnfant

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INTRODUCTION

Alpha fetoprotein (AFP) is one of the important tumor markers in pediatric oncology [1]. AFP secretion is characteristic for malignant germ cell tumor with yolk sac component and hepatoblastoma in childhood [2]. As a tumor marker AFP has an important role in initial diagnostic evaluation, and follow-up during therapy, but the physiologically elevated AFP levels in infancy or in benign conditions, such as hepatic diseases or hereditary disorders, must also be considered in the differential diagnosis [2]. AFP levels remain high during the first few months of life, thus making clinical interpretation difficult in this early period and normal variations in AFP levels were found to be extremely large in early postnatal life[2-6]. Very few and incomplete data regarding the determination of reference values for AFP in healthy newborn are available [4]. Postnatal serum AFP levels are age dependent and gradually decline as the infant matures [6]. Sex and race-related differences in serum AFP levels, especially in early postnatal life, have been conflicting and the cause for these controversies remain obscure [3,7,8]. The aim of the present study is to determine normal AFP levels in healthy full-term neonates and infants who attended Kocaeli University Hospital, Department of Pediatrics.

METHODS

Healthy full-term neonates born in Kocaeli University Hospital and <12 month-old infants attending the well baby outpatient clinic were enrolled in the study after parental consent. The study respected the guidelines of the Helsinki declaration concerning medical research in humans and received local Ethics Committee approval. Gestational age was calculated by the mother's last menstrual period, by fetal ultrasonic evaluation and by new Ballard scoring system[9]. Babies who had an appropriate birth weight for their gestational age were included in the study. Exclusion criteria were: prematurity (<37 gestational weeks), small or large for gestational age, hyperbilirubinemia, hepatobiliary disease or abnormal liver function profile results, positive hepatitis-B surface antigen, respiratory distress, respiratory or cardiac disease, severe infection and congenital malformations, and history of high maternal serum AFP during pregnancy follow-up.

Blood samples were drawn from the umbilical cord vein at delivery or peripheral vein after delivery. Residual serum from the venous umbilical cord or peripheral blood samples collected for routine biochemical analyses essential to patient care was used for estimation of AFP. Repeated venopunctures were avoided. Serum AFP concentration was determined by solid phase, two-site chemiluminescent immunometric commercial diagnostic assay developed for the Immulite automated immunoassay system (Diagnostic Products Corporation, Los Angeles, CA, USA) [10,11]. Whitin-run coefficient variation was 6.3% at a mean AFP concentration of 0.80U/ml and 2.4% at a mean concentration of 182U/ml. The assay requires 10μL of serum. Proper dilution was required due to much higher serum AFP concentrations compared to adult samples. Results were reported in ng/ml. One international unit (1IU) of AFP of the standard preparation of the World Health Organisation (72/225) was equivalent to 1.21ng in our method.

Patient characteristics were summarized using descriptive statistics; mean±SD was used for the expression of continuous variables. Chi-square test or Fisher's exact test was used to evaluate the differences among the cathegorical variables. Kruskal Wallis test was used for continous variables. Spearman correlation, linear regression and exponential regression analyses were used to assess the relation between postnatal age and AFP level. Statistical significance was determined at $p<0.05$.

RESULTS

Six samples were excluded from our study due to congenital anomalies and respiratory disease. Ninety-four neonates (48 male, 46 female) with an appropriate birth weight for their gestational age and infants (<12 month-old) were enrolled. All subjects were delivered vaginally or with cesearian section without any significant perinatal complication. No babies had a diabetic mother or abnormal maternal plasma AFP level that was evaluated during rutin pregnancy follow-up. Eighty-nine babies were singletons, two babies were from twin pregnancies and another 3 were triplets.

In the first step of statistical evaluation, we evaluated AFP levels of cord blood samples taken at delivery from 35 babies. Demographic data of 35 newborns with gestational ages between 38-40 weeks is shown in Table I. Mean AFP level of 35 cord blood samples was 67.246±52.137 (105-226.000) ng/ml. Mean cord blood AFP levels of full-term newborns with regard to gestational age and sex are shown in Table II.

Table I: Demographic characteristics of full-term newborns

Gestational age	**All newborns (38-40 weeks)**	**38 weeks**	**39 weeks**	**40 weeks**	**p**
Number	35	12	17	6	
Male/Female	14/21	6/6	7/10	1/5	0.392
Birth weight (g) (range)	3193±327 (2500-3980)	3318±388 (2720-3980)	3081±293 (2500-3500)	3258±188 (2900-3450)	0.216

Table II: The concentration of AFP* in the cord blood of full-term newborns partitioned by gestational age and sex

Gestation (week)	AFP(ng/ml) mean±SD (range)	AFP(ng/ml) mean±SD(range) Male	AFP(ng/ml) mean±SD(range) Female	p
38	67.108±61.314 (105-226.000)	44.700 (105-226.000)	69.566±38.544 (25.700-121.000)	0.485
39	70.566±54.495 (17.600-198.000)	89.553±61.181 (17.600-198.000)	57.275±48.035 (19.400-175.000)	0.364
40	58.116±23.934 (33.100-100.800)	52.500	59.240±26.581 (33.100-100.800)	0.770

*single values demonstrate median level or patient's AFP level when there is only one patient in the subgroup.

Peripheral venous blood was collected from 59 newborns or infants with postnatal age of 4-335 days (median 7 days). In the second step of statistical evaluation, we evaluated AFP levels of all newborns and infants (35 cord blood samples and 59 peripheral blood samples; total 94 samples). Serum AFP levels of study group partitioned by age groups and sex, is shown in Table III. No statistically significant difference was found between sex and postnatal age groups (p=0.817, 0.924, respectively).

Table III: Serum AFP* levels of infants partitioned by age groups and sex

Postnatal age	No. (M/F)	AFP (ng/ml) mean±SD (range)	AFP(ng/ml) mean±SD (range) Male	AFP(ng/ml) mean±SD (range) Female	p
0-1 month	56 (27/29)	50.838±48.759 (28-226.000)	26.272 (105-226.000)	48.501±40.065 (28-175.000)	0.694
2 months	7 (4/3)	1.621 (296-12.251)	1.328±623 (296-3.772)	4279 (916-12.251)	0.114
3-6 months	6 (3/3)	84 (27-788)	105 (27-397)	64 (27-788)	0.827
7 months	11 (6/5)	39 (13-3.175)	32±17 (13-64)	96 (29-3.175)	0.03
8-9 months	8 (4/4)	22 (7-2.300)	23±21 (7-53)	47 (14-2.300)	0.343
10-12 months	6 (3/3)	18 (6-590)	18 (17-590)	14 (6-560)	0.4

*single values demonstrate median level or patient's AFP level when there is only one patient in the subgroup.

Serum AFP levels of all 94 babies (newborns and infants) had a significant negative correlation with postnatal age (r=-0.877, p<0.001). Results of linear regression analyses demonstrated a significant negative correlation between plasma AFP concentrations and postnatal age (Table IV and Fig. 1). Linear regression analyses did not support a significant sex dependency (r= -0.096, p=0.35).

Table IV: Least squares regression of plasma AFP (ng/ml) concentration by infant age (day)

		Linear regression (y=a+bx) y=46359.09-225.422 x			
	n	Correlation coefficient	r^2	95% CI	P value
All infants	94	-0.484	0.234	36346.2-56371.9	0.000

(y=AFP; x=Age)

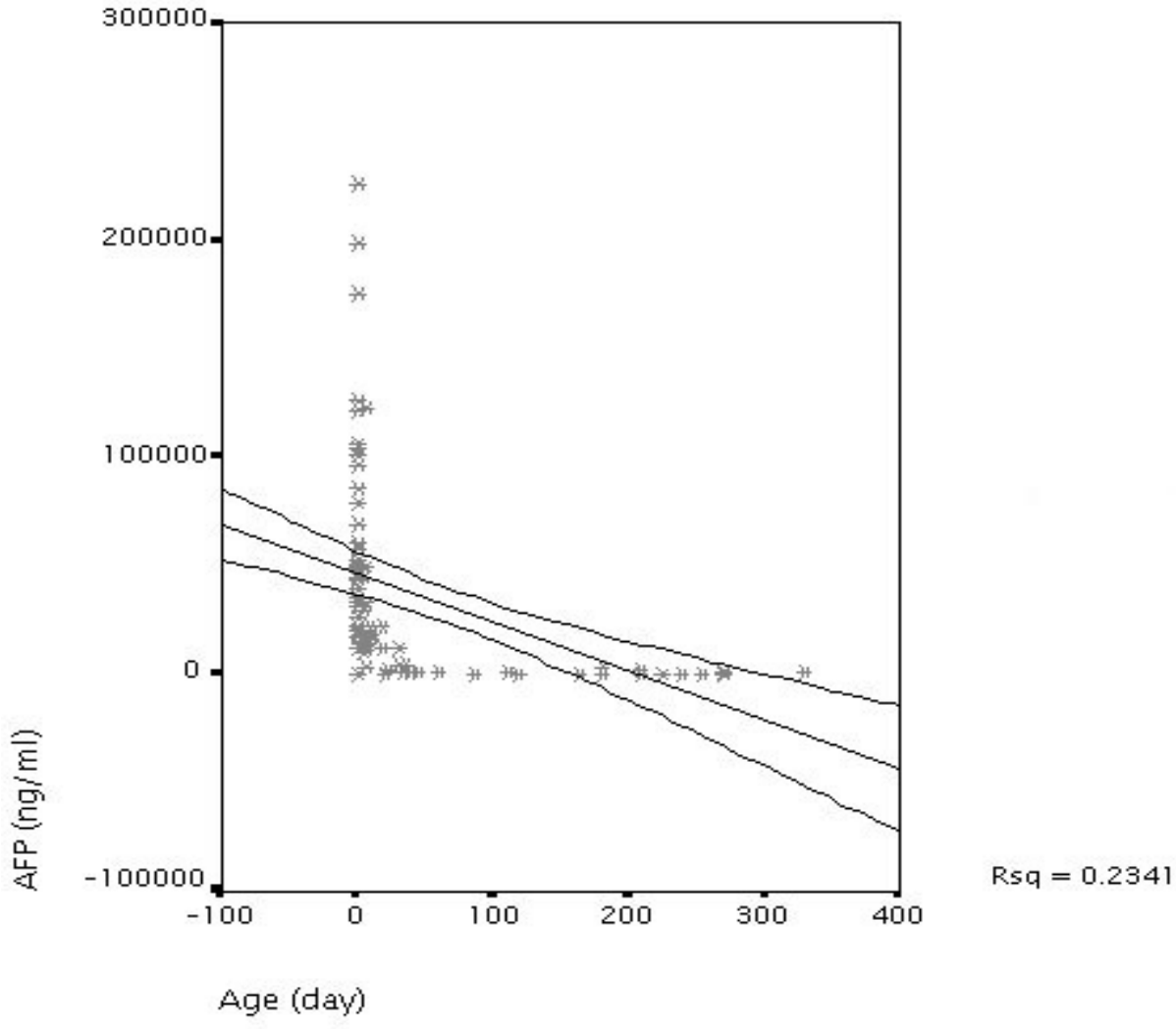

Fig. 1: Normal ranges of serum AFP in early infancy

DISCUSSION

AFP is an oncofetal protein with molecular weight of 68,000 Da [2]. AFP is produced in the developing fetus in equal amounts by the yolk sac and the fetal liver [7]. Because the human yolk sac involutes at the 9th week, the fetal liver and to lesser extent, the gastrointestinal tract is responsible for most of the AFP production during fetal development [12]. During early pregnancy AFP is the predominant serum protein, showing maximum fetal serum levels of 3-4g/L in the 12th week of pregnancy. As AFP crosses the placenta it can be measured in the serum of the pregnant women [2]. The synthesis of AFP does not cease entirely at term, but a small amount of AFP is continuously synthesized until 8 months of age. The infant plasma concentration falls exponentially from a mean of 50.000U/ml to a typical adult concentration of less than 10 U/ml at 6 to 8 months of age [1,7]. The explanation for the observation of AFP synthesis not ceaseing entirely at term may be the presence of fetal hepatocytes, that sustain transient production of AFP during the early postnatal period, although they decrease in number [7].

The value of serum AFP for detection and/or differentiation of a great number of infantile diseases is well established, especially for germ cell tumor with yolk sac component and hepatoblastoma, and benign liver disorders [2,12]. But serum AFP levels are high in normal fetuses and children in early infancy, making the interpretation of AFP levels very difficult at this age group [1]. Abnormal concentrations of AFP in biologic fluids during embryonic and fetal development have been found to be associated with congenital malformations and hereditary disorders [12-18].

Reference values for AFP in healthy full-term newborns at birth are not well-defined [4]. The postnatal serum AFP levels are age-dependent and gradually decline as the infant matures [1,3,5,6]. Low birth weight infants have a clearly greater mean concentration of AFP than other newborns [5]. When the low birth weight group was partitioned into two subgroups to distinguish small for gestastional age from premature infants, a distinctively high mean was found for both groups [5]. On the other hand, birth weight itself has been correlated with gestational age [6]. Since the estimation of gestational age without sonography can be highly subjective, Mizejewski GJ, et al. [5] suggested that birth weight is the parameter of choice upon which to measure postnatal AFP blood concentrations. In the present study, we excluded infants with birth weight inappropriate for gestationel age to eliminate any factor that may cause a change in serum AFP concentration. In our study fetal sonography is a routine examination for the determination of gestational age and it is also confirmed by the mother's estimated date of her last menstrual period and physical examination of the neonatologyst by the new Ballard scoring system.

Bellini C, et al. [4] examined AFP levels in 150 healthy newborns and preterms within the postnatal 24 hours. All babies included in their study were of appropriate birth weight. They found a significant correlation between AFP values and both birth weight and gestational age. In their study mean AFP values in male and female newborns were 151±61mg/L and 150±59mg/L, respectively. These were higher than our AFP values as a result of presence of premature babies in their study group. Blohm ME, et al. [19,] found that, at birth mean serum AFP levels were 41,687 ng/ml in 256 term babies. We found higher mean AFP values (67.246 + 52.137 ng/ml) in a similar group with a wide range (105-226.000). Our mean values in newborns and infants are similar to Wu JT, et al. [20] but show wider AFP ranges in all postnatal age groups.

In most infants, serum AFP levels decrease to normal adult levels within the first 8-10 months, but in a significant proportion of children AFP levels do not normalize until the end of the 2nd year of life [19,21]. Our study group was limited to 12 month-old infants. Some babies had normal adult values even in the 7th month but this study is inefficient to make a conclusion about the age for AFP decrease to normal adult values.

Findings of sex-related differences in serum AFP levels, especially in early postnatal life, have been conflicting, and the cause of these controversies remain obscure [3,7]. The levels of fetal AFP at parturition are found to be significantly higher in the male infants [8,22]. In a study fetal AFP serum concentration was found higher in boys than girls and this sex related difference remained during the first week of life [22]. Lee PI, et al. [3] investigated the normal developmental pattern of serum AFP level. Their results support the presence of a sex-related difference in AFP levels during early postnatal life. Higher AFP levels are noted for newborn boys during the first 2 postnatal weeks. In newborn girls, the mean serum AFP level decreased rapidly from postnatal day 1 to 2 and remained relatively constant thereafter until 7 days of age. It is hard to explain the different

developmental patterns of AFP in male and female neonates. It may also be that the biodynamics of AFP is influenced by such sex-determined factors as hormones. Sex related difference diminish with increasing age[3]. However, Bellini C, et al. [4] found that there was no significant difference between AFP values in males vs. females in 150 healthy newborns and preterms [4]. In the present study AFP values were not statisticaly different in males and females except values of the 7th month which were higher in females. We showed with exponential and linear regression analyses that a significant sex dependency does not exist in AFP values of 0-12 month-old infants. But we cannot make a final conclusion with this small group of infants and such a wide variation of AFP levels.

Observation on the relationship between race and AFP levels has been inconsistent. In fact, maternal serum AFP values have been shown to be higher in blacks and Orientals than in Caucasians [3]. Maternal serum AFP concentrations are affected by a number of variables including ethnicity [23,24]. However, there is no evidence about ethnical differences in infant serum AFP levels [3]. More studies are necessary to explain wide variations in ethnicity.

In conclusion, we showed that the normal range of AFP in healthy infants who were followed-up in our center is very wide and pediatric oncologists must consider moderately high values carefully and if the decrease in the follow-up measurement is slower than the expected half-life for that age, the probability of an AFP-producing tumor could be high. This approach would prevent unnecessary interventions depending on false positive AFP results.

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