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Study of concentration of amniotic fluid alpha-fetal protein in thalassemia fetus

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ABSTRACT

Objectives: To test the hypothesis that concentration of amniotic fluid alpha-fetal protein (AFAFP) is increased in thalassemia fetus.

Methods: A total of 135 cases of amniocentesis admitted from July 2013 to December 2014 were included in this study. Among them 98 cases of normal fetuses were assigned into control group and 37 cases of thalassemia fetus were included as thalassemia fetus group. Alpha-fetoprotein levels detected by enzyme linked immunosorbent assay and the alpha-fetoprotein concentration were compared between the two groups. There is no significant difference in gestational age between the two groups.

Results: 1. AFP concentration in thalassemia fetus group was significantly higher than that of normal control group [(1 541.65 ± 734.78) μg/mL *vs.* (2 728.84 ± 1 539.97) μg/mL], and amniotic fluid AFP concentration was related to fetal thalassemia. 2. AFAFP concentration in pure α-thalassemia fetus was higher than that of β-thalassemia fetus or mixed α- and β-thalassemia fetus, but the difference was not significant.

Conclusions: Concentration of amniotic fluid alpha-fetal protein is increased in thalassemia fetus. AFP concentration in α-thalassemia fetus was higher than that of β-thalassemia or mixed α- and β-thalassemia fetus but difference was not significance. Further studies are needed to explore the possible correlation between Down syndrome and biochemical markers of thalassemia.

1. Introduction

AFP (Alpha-Fetal Protein, AFP) is a glycoprotein, mainly from embryonic liver cells which reaches the peak at 30 weeks of pregnancy, and then decrease gradually [1,2]. AFP exists in amniotic fluid or plasma, can be used for prenatal diagnosis of fetal. AFP is associated with many diseases, such as liver cancer, Down syndrome, fetal central neural defect, congenital nephrosis and ventral wall defect. Its concentration increased significantly and has diagnostic value [3,4]. Thalassemia is an autosomal recessive genetic incomplete chronic hemolytic diseases. Globin gene deletion due to hemoglobin peptide chains with one or several synthetic reduce or unable to synthesize. Constituent of hemoglobin changes may cause hemolytic disease. Diagnosis and currently accepted treatment of thalassemia could prevent the birth of children with severe by prenatal diagnosis. In this study, by researching the amniotic fluid alpha-fetoprotein concentrations of thalassemia fetus, relationship between AFP concentration and fetal thalassemia were studied in order to provide guidance for prenatal diagnosis.

2. Materials and methods

From July 2013 to December 2014, 135 pregnant women of singleton pregnancy between 18 and 29 weeks who have taken amniocentesis in the Local Tertiary Prenatal Diagnosis Center were included in this study. (1) A total of 37 cases of them were included as thalassemia group. Gestational age of this group were between 18 and 27 weeks with an average of (20.80 ± 2.90) weeks. These cases were diagnosed as fetal α-thalassemia or β-thalassemia by genetic testing. The other 98 healthy cases were included as control group and their gestational age were between 18 and 29 weeks with an average of (21.51 ± 2.49) weeks. There was no significant difference

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between the two groups. (3) No Down syndrome, fetal central neural defect, congenital nephrosis or ventral wall defect was found in all cases.

2.1. Reagents and instruments

Value of AFP of Amniotic fluid was detected by Human Alpha-Fetoprotein ELISA Kit Reagents purchased from Beijing Bomingsai Technology Co. Ltd. (Product number: H21798). Microplate reader used in this study was from Laboratory in Grade III Class A Teaching Hospital.

2.2. Methods

Extracting amniotic fluid 5 mL by Amniocentesis after B-positioning under sterile conditions which was stored at −80 °C. ELISA method was applied to detect the AFP value of amniotic fluid using Human Alpha-Fetoprotein ELISA Kit after 1:100 dilution. Grouped statistical AFP value was detected and follow-up was conducted on all cases to track and record pregnancy outcomes.

2.3. Statistical analysis

Using SPSS 20.0 statistical software for data analysis and processing, all data were expressed as (Mean ± SD). *T* test was used for comparison between groups.

3. Results

3.1. Comparison of gestational age

The gestational age of pregnant women were analyzed statistically. There was no significant difference in gestational age between the thalassemia group and the control group [(21.51 ± 2.49) weeks *vs.* (20.80 ± 2.90) weeks] (Table 1).

Table 1
Comparison of gestational age and AFP concentration between the two groups.

Groups	*n*	Gestational age mean (weeks)	AFP concentration (μg/mL)
Thalassemia group	37	20.80 ± 2.90	2728.84 ± 1539.97
Control group	98	21.51 ± 2.49	1541.65 ± 734.78

3.2. Comparison of AFP concentrations

Mean amniotic fluid AFP concentration was (1541.65 ± 734.78) μg/mL in control group, while (2728.84 ± 1539.97) μg/mL in the halassemia group showing significant difference between the two groups ($P < 0.05$) which indicated that AFP concentration of fetal amniotic fluid are related to thalassemia (Table 1).

3.3. Comparison of AFP concentrations between pure α-thalassemia fetus and β-thalassemia fetus

Comparison of AFP concentrations between pure α-thalassemia cases and β-thalassemia cases (including mixed α- and β-thalassemia) were shown in Table 2.

Table 2
Comparison of AFP concentrations between pure α-thalassemia fetus and β-thalassemia fetus (μg/mL).

Groups	*n*	AFP concentration
α-Thalassemia only	28	3050.137 ± 1726.624
β-Thalassemia and mixed α- and β-thalassemia	10	2070.225 ± 787.883
Normal	100	1550.930 ± 732.136

4. Discussion

AFP is a widely used prenatal screening biomarker for Down's syndrome and fetal neural tube defect. There are a mass cases of thalassemia patients in south China. Compared with other advanced countries of the thalassemia prevention, there is still much room for improvement in prevention and diagnostic techniques for thalassemia.

In this study of 37 thalassemia cases and 98 normal cases, we found that the concentration of fetal amniotic fluid AFP is increased in thalassemia group, this provide new ideas for clinical screening for thalassemia. However, cause of elevated amniotic fluid AFP remains unclear. Previous study demonstrated the inverse relationship between fetal anemia and AFAFP. It is supposed that elevated AFAFP relates to fetal hematopoiesis [6,7].

There are two types of thalassemia, α-thalassemia and β-thalassemia, in some cases are both α- and β-thalassemia type (mixed α- and β-thalassemia).α-thalassemia cause fetal period anemia whileβ-thalassemia shows anemia after birth.

Mixed α- and β-thalassemia has similar characters with β-thalassemia. In this study, the initially analysis between α-thalassemia and β-thalassemia group showed that AFP concentration in α-thalassemia fetus group was significantly higher than β-thalassemia type (including mixed α- and β-thalassemia type) but the difference was not significant. This result was coordinate to that hypothesis that α-thalassemia fetus are anemic while β-thalassemia fetus are not. Studies on larger number of cases are needed to provide further evidence.

AFAFP relates to fetal disease, yet maternal blood AFP (MBAFP) is influenced by both AFAFP and placental transfusion [1,6]. For fetal diseases, MBAFP is significantly correlated with fetal hemoglobin and fetal middle cerebral artery blood flow velocity [8] in fetal anemic disease such as red blood cell alloimmunisation and parvovirus infection [9,10]. It was also reported that MSAFP increases earlier than the increase in MCA velocity [11,12]. Here we initial the study on AFAFP in fetal thalassemia and also subgenotype group of thalassemia as a very common local congenital disease in Hainan province, south China which will be with great potential in improving prenatal screen and diagnose [13].

In conclusion, this research explored the relationship between the amniotic fluid AFP concentration and thalassemia, and compared AFAFP concentration in α-thalassemia fetus and β-thalassemia fetus (mixed α- and β-thalassemia). AFAFP is increased in thalassemia fetus and that in α-thalassemia fetus group was significantly higher than β-thalassemia (including mixed α- and β-thalassemia) but not meet statistic significance. Further larger studies are needed to establish whether adjustments for thalassemia are needed when giving Down syndrome risks based on biochemical markers. There is also great potential for combined MBAFP with MCA velocity and other tools for

screen or non-invasive prenatal diagnose of thalassemia, and provide new ideas for early prevention and treatment of the disease.

Conflict of interest statement

The author(s) declare(s) that there are no conflicts of interests regarding the publication of this article.

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