Original Article

Comparison of leptin level in amniotic fluid and human serum between fetuses with and without Down syndrome

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ABSTRACT

Introduction: Down syndrome is a genetic disorder and the leading cause of mental retardation, which leads to a delay in motor skills, power, and motor-vision control. The leptin level plays a role in the growth of the embryo and the normal evolution of the brain. Therefore, the present study aimed at comparing and finding the relationship between the leptin level in amniotic fluid in fetuses with and without Down syndrome. **Methods and Materials:** This is a case-control study. The sample size included 15 cases with Down syndrome and 30 cases without Down syndrome. The convenience sampling was performed among the patients who were referred to Ali Ebn Abitaleb Hospital in 2017-2018. The leptin level was measured using ELISA. Finally, the leptin level was compared in two groups of fetuses with and without Down syndrome. Data were analyzed using SPSS V.21. **Results:** The results showed that there was no significant difference between the mean leptin level in human serum in two groups ($p>0.957$). However, the mean leptin level in the amniotic fluid in the healthy group and Down syndrome group was 3.703 ± 1.721 and 2.847 ± 1.32, respectively, which showed a no significant difference ($p>0.682$). **Conclusion:** The results showed that the leptin level in amniotic fluid in mothers with fetuses with Down syndrome was less than mothers with healthy fetuses. Also, the leptin level of serum in the Down syndrome group was insignificantly higher than the control group.

Keywords: Leptin, Amniotic Fluid, Down Syndrome

Introduction

Down syndrome (Trisomy 21) is a type of genetic disorder that arises due to the full presence or part of an additional 21 chromosome (Paterson). Down syndrome is the most common genetic cause of mental retardation and includes 9.5-11.8% of every 10,000 living births [1]. About 20 % of the total fetuses and 0.5-1% of infants suffer from chromosomal abnormalities, which one -third of them suffer from trisomy of autosomal chromosomes. One of the most common is Down syndrome with a frequency of about one per 600-700 infants. In 95% of those suffering from this syndrome, three copies (trisomy) of chromosome number 21 are found, the translocation of chromosome 21 with one Acrocentric chromosome occurs in 4% of the patients, and the Mosaic condition is observed in 1% of patients [2]. Patients with Down syndrome suffer from severe Mental retardation, Hypotonia, Brachycephaly, delay in voluntary and involuntary patterns, the impairment of learning, and congenital heart disorders (40 % of cases). The average age of those with this syndrome is about 60 years [3]. Since congenital anomalies, especially Down syndrome, have a massive economic, social, and cultural burden for the family and society, the prenatal screening and early diagnosis of this disorder is now the only possible way to reduce the burden of the disease in society

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and prevent the birth of infants with this disorder [4]. Prenatal diagnosis is performed too precisely by applying Cytogenetic methods and using samples such as amniotic fluid, Chorionic villus, or fetal blood. However, due to the high cost and invasion of these procedures, they cannot be used in all pregnancies. The solution to this problem is to avoid employing invasive methods and imposing heavy costs on patients and using non-invasive and less expensive screening methods. By employing these screenings, the pregnancies which are exposed to risk are diagnosed, then using more precise techniques the fetus condition in terms of suffering or lack of suffering from this disorder is investigated [4, 5]. Leptin is the 4 -stranded helix protein with a long chain of Cytokine cells of group 1 of obese (ob) genes, which is located in chromosome 6 and 7 [6, 7]. The leptin receptors have long LEPRL isoforms in the hypothalamus, the short LEPRS isoforms in the organs and tissues [8], as well as soluble isoforms SolLEPR only in humans. SolLEPR may be responsible for the leptin resistance in mothers and leptin paired transport for fetus growth [9-11]. Leptin contains 167 amino acids and is caused by obesity [12]. This protein is mainly produced by the white adipose tissue and released into the bloodstream in two forms, free and attached to the transmitter protein into the bloodstream, in which the biological and biochemical effects are applied to the target tissues via its free form [13, 14]. Hypothalamus is one of the target tissues of leptin. One of the neurons of the target tissue of leptin is the neuropeptide y in the Arcuate nucleus. These neurons play a role in the increasing appetite signal and increase tending to eat food; it also reduces energy consumption and causes fat accumulation, weight gain, and obesity [15]. In the past, leptin was known as a regulator of food intake and energy consumption. Still, with more recognition of this molecule, it was found that it has extensive physiological and pathological functions in the tissues [16]. Anomaly in the leptin gene and its receptor can cause problems such as disorder in the reproductive process, infertility and decrease of gonadotropin secretion, impaired hormonal balance, insulin resistance, diabetes, overeating, obesity, and anomaly in the blood circulation system [17]. Placenta produces 14% of leptin in the pregnant female. Leptin is produced by adipose cells and fetal syncytiotrophoblast. It is also produced by human amniotic epithelial cells and secreted in amniotic fluid [18-23]. During pregnancy, the amount of serum leptin increases progressively and reaches its peak in the second three months; then reaches a Plateau, which is 2-3 times of non-pregnant women [24]. Some hypotheses argue that the chronic increase of leptin and increasing the leptin resistance during pregnancy is associated with the increase in progesterone, prolactin, lactogen, and stopping the progressive secretion of cyclic estradiol serum [24]. Leptin enters from the placenta to the mother's and fetus's body and plays a significant role in the growth of the fetus, and by reducing insulin sensitivity, the embryo uses the mother's glucose [25]. Various studies have shown significant correlation between the umbilical cord leptin and the weight index of the infant[26].

There are some studies that investigated the relationship between leptin and Down syndrome [27, 28]. In a study conducted by Demir et al. (2012) in Turkey on 15 fetuses suffered from Down syndrome and 48 fetuses with normal karyotype, the level of leptin in amniotic fluid had no significant difference between the two groups in the second three months of pregnancy [29]. In a study conducted by Choi et al. (2009) in South Korea, compared to the leptin level in amniotic fluid in the second three months of pregnancy of Down syndrome and normal fetuses. They reported that amniotic fluid in the Down syndrome group was significantly lower than that of the control group [30]. Radunovic et al. (2003) conducted a study US to compare the level of Leptin in human serum of healthy and with Down syndrome fetuses. They stated that the leptin level of fetus serum (not the mother's serum) in the fetuses with Down syndrome were significantly lower than healthy fetuses [31]. Therefore, the present study aimed to compare the level of leptin in amniotic fluid and fetus serum of fetuses with and without Down syndrome.

Method

This is a case-control study. The sample size included all pregnant mothers who were referred to Ali Ebn Abitaleb Hospital of Zahedan in 2017-2018. The inclusion criteria were mothers with 16-20 weeks of gestational age, who were under the Amniocentesis test. Mothers' dissatisfaction to participate in the study was one of the exclusion criteria. The leptin level in the amniotic fluid of 30 fetuses with and without Down syndrome (15 cases suffered from Down syndrome and 30 healthy fetuses) were evaluated. The convenience sampling method was performed. The present study aimed to compare the leptin level in amniotic fluid and serum leptin levels in maternal blood between the fetuses with and without Down syndrome. I n addition to the required amount of checks required of amniotic fluid for the test, additional 2 cc of maternal blood was centrifuged for 5 min with 3000 rpm and the supernatants were kept at 80 ° C for further investigation. Finally, the results of the supernatants were obtained using the performed tests after the Down syndrome diagnosis based on the national protocols and conventional procedures. The sample size was estimated regarding restrictions on access to the cases (with Down syndrome) and according to the previous studies [29]. In this study, 15 fetuses with Down syndrome positive test and 30 fetuses whose tests were negative were selected, and the level of Leptin in amniotic fluid is measured from them. Also, the serum leptin level, which was obtained from 2cc of maternal blood and kept at 80°C with amniotic fluid was studied in both groups. Finally, the leptin level of amniotic fluid infants in Down syndrome and healthy fetuses group and their association with serum leptin levels of the mother were compared. Serum leptin levels were measured by ELISA kit (Invitrogen, Carlsbad, Calif., USA) according to the manufacturer's instruction.

The diagnosis of down syndrome was carried out using free cell DNA or karyotype analysis of amniotic fluid cells separately in the laboratory and out of this plan . Meanwhile, the

amniocentesis was performed under the guidance of sonography using Trans abdominal and in the sterile condition. At the end of the study, the information obtained from each patient was analyzed using descriptive statistics including mean, standard deviation, T-test, ANOVA using SPSS V.22. The significance level was $p<0.05$.

Results

According to the results in this study, the mean age of mothers who had a healthy fetus and fetus with Down syndrome was 33.23 ± 8.44 and 32.33 ± 8.79, respectively. The independent t-test showed that the mean age of individuals in the two groups had no significant differences with each other ($p =0.745$). Also, the mean weight of mothers who had a healthy fetus and fetus with Down syndrome was 65.20 ± 14.49 and 63.60 ± 13.30, respectively. The independent t-test showed that the mean weight of individuals in the two groups had no significant differences with each other ($p = 0.694$).

According to Table. 1, the average serum leptin level in the mothers who had a healthy fetus and fetus with Down syndrome was 3.42 ± 2.172 and 3.46 ± 3.060, respectively. The independent t-test showed that the average serum leptin level of individuals in the two groups had no significant differences with each other ($p > 0.957$). The average of leptin level of amniotic fluid in the mothers who had a healthy fetus and fetus with Down syndrome was 3.703 ± 1.721 and 2.847 ± 1.32, respectively. The independent t-test showed that the average leptin level in the amniotic fluid of individuals in the two groups had no significant differences with each other ($p > 0.682$). Also, the average gestational age of the mothers who had a healthy fetus and fetus with Down syndrome was 107.300 ± 12.412 days (15 weeks and 28 days) and 99.667 ± 12.009 days (14 weeks), respectively. The independent t -test showed that the average gestational age in the two groups is not different. In other words, two groups are not significantly different in terms of pregnancy age ($p > 0.056$).

Table 1: Comparison of leptin level in serum and amniotic fluid for fetuses with and without Down syndrome

	Down Syndrome group Mean ± SD	Healthy group Mean ± SD	P-value
Serum leptin level	3.46 ± 3.060	3.42 ± 2.172	0.957
Leptin level in amniotic fluid	2.847 ± 1.32	3.703 ± 1.721	0.682
Gestational age	99.667 ± 12.009	107.300 ± 12.412	0.056

Discussion

The results of this study showed that the confounding variables such as age and weight are homogeneous in the two groups. In a consistent study conducted by Basbug et al. to investigate the relationship of the leptin level of amniotic fluid and the mother serum in the neural tube disorders (NTD), it was found that the two groups of intervention and control were homogeneous in terms of mothers' age and BMI [32]. Also in the study of Radunovic et al. (2003) that were coordinated and consistent with our study, it was found that the two groups of pregnant mothers with a healthy fetus and a fetus with Down syndrome were homogenous in terms of mother age [31]. Molvarec et al. (2011) concluded that the two groups of pregnant mothers with healthy fetuses and Preeclampsia were identical in terms of confounding variables such as age, BMI, and weight of infant birth [33].

Also, the results of the t-test showed that the leptin level in amniotic fluid and serum had no significant difference in healthy and Down syndrome groups. The average leptin level in amniotic fluid in Down syndrome groups was lower than the healthy group. On the other hand, the two groups had no significant differences in terms of gestational age. In a study conducted by Choi et al. (2009), which was consistent with the present study, they aimed to investigate the reduction of leptin level in amniotic fluid in the second three months of pregnancy of mothers with Down syndrome fetuses and concluded that the leptin level in amniotic fluid was significantly lower than the same period in mothers with healthy fetuses [30]. In a study in 2017, which its results were consistent with the results of our study, researchers concluded that the levels of inflammatory factors such as angiopoietin 1 (ANG-1), angiotensin, Leptin, Epidermal growth factor (EGF), interleukin 1-beta (IL-1β), and interleukin 4 (IL-4); in amniotic fluid with Down syndrome were significantly lower [34]. In another study conducted by Demir et al. (2012), they found that demographic variables such as the age and weight of mothers and gestational age between the control group and mothers with fetuses which suffered from trisomy 21 had no significant differences. Also, in this study, it was shown that the leptin level in amniotic fluid in mothers with fetuses that suffered from this disorder was significantly lower than mothers with healthy fetuses [29]. In a study (2003) with the results which were inconsistent with the results of the present study, researchers concluded that the serum leptin level in mothers with fetuses that suffered from Down syndrome was significantly lower than that in mothers with healthy fetuses [31]. Finally, in a study conducted in 2003, researchers concluded that the serum leptin level and the leptin level in amniotic fluid was significantly more in the group consisted of mothers with fetuses which had nervous tube defect than mothers with healthy fetuses. The results of this study [32] were in consistent with the results of our study.

Conclusion

The results of our study showed that variables such as mother's age and weight had no significant effect on mothers with fetuses who suffered from Down syndrome. On the other hand, it was found that the leptin level in amniotic fluid in mothers with fetuses who suffered from Down syndrome was lower than the control group, but the serum leptin level of fetuses with Down syndrome was insignificantly more than that of healthy fetuses.

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