Supplementary Material

Table S1: Participating patient clinical profiles and the classification of primary Sjögren's syndrome according to both the American-European consensus group criteria and the 2016 American College of Rheumatology-European league against rheumatism criteria

Number	Age	Gender	Ocular dryness‡	Oral dryness‡	Schirmer's test¶	Salivary scintigraphy	Autoantibodies			Salivary gland histopathology§	AECG criteria	2016 ACR/EULAR criteria
							ANA	SSA	SSB			
1	62	Female	Yes	Yes	+	Abnormal	+	+	+	N/A	Yes	Yes
2	39	Male	Yes	Yes	+	Abnormal	−	−	−	+	Yes	Yes
3	65	Female	Yes	Yes	+	Abnormal	+	−	−	+	Yes	Yes
4	41	Female	Yes	Yes	N/A	Abnormal	+	+	−	N/A	Yes	N/A
5	50	Female	Yes	Yes	N/A	Abnormal	+	+	+	N/A	Yes	N/A
6	41	Female	Yes	Yes	+	Abnormal	−	−	−	+	Yes	Yes
7	36	Female	Yes	Yes	N/A	Abnormal	+	+	+	N/A	Yes	N/A
8	46	Female	Yes	Yes	+	Abnormal	+	+	+	N/A	Yes	Yes
9	53	Female	Yes	Yes	+	Abnormal	−	−	−	+	Yes	Yes
10	53	Female	Yes	Yes	+	Abnormal	+	+	+	N/A	Yes	Yes
11	25	Female	Yes	Yes	N/A	Abnormal	−	+	−	N/A	Yes	N/A

‡Symptoms for at least 3 months, ¶A Schirmer's test ≤5 mm/5 min in at least one eye was considered positive, §The presence of focal lymphocytic sialadenitis was considered positive. Patients 2 and 9 received labial salivary gland biopsy in another hospital. ANA: Antinuclear antibodies, SS: Sjögren's syndrome, AECG: American-European consensus group, 2016 ACR-EULAR: 2016 American College of Rheumatology-European League Against Rheumatism, N/A: Not available, +: Positive, -: Negative

Does the Presence of Extended Jugular Lymphatic Sacs Add More Risk to Nuchal Thickness for Genetic and Structural Abnormality?

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Abstract

Background: The risks added by extended jugular lymphatic sacs (EJLS) to increased nuchal translucency (NT) including genetic and structural abnormalities and pregnancy outcomes have not been previously investigated, which this study aims to investigate. **Methods:** The data of 155 singleton pregnancies with increased fetal NT (≥95th percentile) of these 20 with fetal EJLS were evaluated retrospectively. Patients were stratified according to NT thickness such that ≥95th percentile - 3.5 mm, 3.6–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, ≥6.5 mm, and grouped according to the presence of EJLS. Pregnancy outcomes, genetic and structural abnormalities were assessed by comparing EJLS with non-EJSL cases (n-EJLS). **Results:** Associated with NT, the incidence of the presence of EJLS increased with NT, from 4.5% at the ≥95th percentile - 3.5 mm to 30.8% when NT ≥5.5 mm. In the n-EJLS group, the proportion of fetuses with structural and genetic abnormalities increased as the measurement of NT increased. This correlation was not observed in the EJLS group. Compared to n-EJLS, cases with EJLS had a higher rate of fetal structural (38.5% vs. 75%, $P = 0.003$) and genetic (18.5% vs. 45%, $P = 0.005$) anomalies and a lower term live birth rate (59.3% vs. 15%, $P < 0.001$). **Conclusion:** The increasing rate of EJLS was seen as NT increased. Compared to n-EJLS, the EJLS cases had a higher rate poor pregnancy outcomes and fetal genetic and structural abnormalities.

Keywords: Abnormal karyotype, cystic hygroma, jugular lymphatic sac, nuchal thickness, structural abnormality

Introduction

Nuchal thickness (NT) is the ultrasonographic expression of subcutaneous fluid accumulation posterior to the neck of the fetus, whether the accumulation is septated and confined to the neck or encompasses the entire fetus.[1] Enlarged NT is defined as the measurement of vertical thickness in the mid-sagittal region of the fetus during the first trimester that is equal to or greater than the 95th percentile of the reference range.[1,2] The incidence of enlarged NT is 8.6%[3] and has been associated with various fetal genetic or structural abnormalities. However, enlarged NT can also occur in normal fetuses.[3-5] The pathophysiology of enlarged NT is not fully understood, but theories such as heart failure, alteration of extracellular matrix composition, and disorders of lymphangiogenesis have been proposed.[6,7] Studies have shown that pregnancies with increased fetal NT have a higher rate of fetal structural abnormalities, including congenital heart defects and musculoskeletal abnormalities.[3] In addition, pregnancies with elevated fetal NT have been found to have an increased rate of adverse pregnancy outcomes, such as amniotic fluid abnormalities, preterm births, miscarriages, pregnancy terminations of pregnancy (TOP), intrauterine fetal death (IUFD), and a low rate of live births, even in those without genetic and chromosomal abnormalities.[5-8] The incidence of extended jugular lymphatic sacs (EJLS) in the normal pregnant population ranges from 1.6% to

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2.4%.[8-10] However, the incidence of EJLS in cases with increased NT is much higher, ranging from 23.5% (415 / 98) to 84.6% (22 / 26).[3-10] The most widely accepted theories to explain the NT increase are cardiac dysfunction/failure or venous congestion in the fetal head and neck,[9] alterations in extracellular matrix components,[10] conditions such as fetal anemia, infection, and hypoproteinemia,[11] and disorders of lymphangiogenesis.[12] However, these theories could not explain the reason for the localization of such thickening to the neck of the fetus or the transient nature of this finding. It has also remained unexplained why concomitant EJLS appeared simultaneously in some cases and not in others.[13] Furthermore, the significance of fetal EJLS remains to be elucidated.[3-5,14-18]

In this study, we aimed to investigate whether the presence of EJLS in the first trimester increases the risk of genetic and structural abnormalities and pregnancy outcome in cases with increased NT.

Materials and Methods

After approval by the Health Sciences University, Etlik Zübeyde Hanım Woman's Health Training and Research Hospital's Institutional Review Board (no: 11/30-2021), and obtained patient consent, we retrospectively searched medical records between January 2017 and January 2021 for cases with increased NT detected by routine first-trimester ultrasound (USG). We identified 230 such pregnancies, 22 of which were associated with EJLS. The cases with multiple pregnancies (n = 12; 11 had twins and another had a triplet pregnancy) or incomplete data or follow-up (n = 73) were excluded. Finally, 155 patients, 20 of whom had EJLS, were enrolled in the study [Figure 1]. We collected data on patients' demographic characteristics, first- and second-trimester combined test results, ultrasonographic findings, type of invasive tests, and genetic results. We retrieved the data on pregnancy outcomes, including abortion, IUFD, TOP, postnatal death, preterm birth, term birth, and live birth (T and L) from the hospital database. According to our clinical protocol, if a patient was found to have an elevated fetal NT or other abnormal findings on first-trimester USG screening, were examined in detail by a perinatologist who had at least 8 years of experience in obstetric USG evaluation using the Voluson E6 device (GE Healthcare GmbH and Co OG, Zipf, Austria). These were evaluated with both transvaginal (via a 7-MHz probe) and abdominal (via a 5-MHz probe) USG screening. USG findings included crown-rump length, NT and absent or reversed a-wave of the ductus venosus (DV), tricuspid regurgitation, jugular lymphatic sacs (JLS), fetal structural abnormalities, and minor markers such as fetal echogenic intracardiac focus and hypoechogenic intestines.

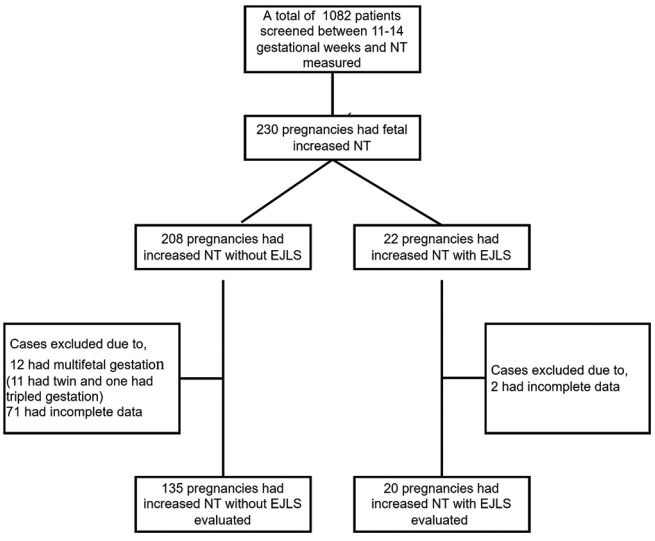

Figure 1: Diagram of study follow-up

The presence of JLS was studied in the transverse plane of the fetus through the mandible and spinal cord and confirmed in the coronal and sagittal planes.[8] Invasive tests were offered in all pregnancies with elevated NT, the type of which was selected according to the gestational week in which the patients were admitted: chorionic villus sampling if between 11 and 14 weeks of gestation, amniocentesis if between 16 and 22 weeks of gestation, or postnatal karyotyping if beyond 22 weeks of gestation.

The presence of an echogenic intracardiac focus, hyperechogenic bowel, or absent nasal bone was considered a mild marker, whereas other fetal anomalies were considered severe. For cases with genetic or structural anomalies, the decision of TOP was made by the perinatology council.

We stratified cases with or without EJLS and according to their NT categories: ≥95th percentile - 3.5 mm, 3.5–4.4 mm; 4.5–5.4 mm, 5.5–6.4 mm, and ≥6.5 mm. Study variables, including patient characteristics, chromosomal or structural abnormalities, and pregnancy outcomes, were determined based on these subgroups.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 26 (SPSS Inc., Chicago, IL, USA). The distribution of parameters was assessed by Kolmogorov–Smirnov's normality tests. Descriptive analyses were given (using tables of frequencies for the categorical variables and using means and minimum–maximum values for the normally distributed variables). One-way analysis of variance was used for the normally distributed and Kruskal–Wallis test was used for the nonnormally distributed continuous data. Comparison of categorical variables was performed by the Chi-square test or the Fisher's exact test where appropriate. The correlation between fetal abnormalities, adverse pregnancy outcomes, and EJLS and NT was assessed using Spearman-Rho correlation. P <0.05 was considered statistically significant.

Results

The mean maternal age of the study population (n = 155) was 29.18 ± 6.11 and the majority of patients had a fetal NT <5.5 mm. The distribution of cases according to stratified NT was 43.2%

(*n* = 67), 27.7% (*n* = 43), 15.5% (*n* = 24), 8.4% (*n* = 13), and 5.2% (*n* = 8) in the ≥95th percentile - 3.5 mm, 3.6–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, and ≥6.5 mm, respectively. There were 20 (12.9%) pregnancies with fetal ELJS. The lower rate of ELJS cases was seen in pregnancies with fetal NT <4.5 mm compared to those had ≥4.5 mm. The distribution of EJLS in NT groups was such that 4.5% (*n* = 3), 9.3% (*n* = 4), 29.2% (*n* = 7), 30.8% (=4), 25% (*n* = 2) in ≥95th percentile - 3.5 mm, 3.6–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, and ≥6.5 mm, respectively. An increasing rate of fetal structural and genetic abnormalities was observed as the NT increased. Furthermore, the higher rate of abortion, TOP and a lower rate of live birth were observed as the fetal NT increased [Table 1].

Table 2 demonstrates fetomaternal characteristics and pregnancy outcomes of the non-EJLS (n-EJLS) cases. The majority of cases had a fetal NT <5.5 mm. The higher rate of poor pregnancy outcomes and the lower rate of T and L births were observed as NT increased in these cases. The overall T and L ratio was 59.3% (T and L birth ratio ranging from 68% in ≥95th percentile - 3.5 mm group to 0% in ≥6.5). In addition, the higher rate of fetal structural and karyotypic abnormality was seen as NT increased. The overall rate of karyotypic anomaly was 18.5% (ranging from 10.9% in ≥95th percentile - 3.5 mm group to 50% in ≥6.5). Followed cystic hygroma (*n* = 27, 20%), the most common structural abnormalities detected were cardiovascular system (CVS) (*n* = 15, 11.1%) and central nervous system (CNS) (*n* = 9, 6.7%). Although the increasing rate of cystic hygromas and CVS anomalies were seen as the NT increased, this correlation was not seen with CNS or other systems anomalies. We found structural abnormalities in 75% of cases with EJLS, with cystic hygromas being the predominant condition (40%). The EJLS cases with NT of 4.5–6.4 mm had the highest rate (100%) of structural abnormalities. Karyotypic abnormalities and TOP were found in 45% and 55% of EJLS cases, respectively. The highest rate of karyotypic abnormalities (100%) and TOP (100%) was found in those with NT of 3.6–4.4 mm [Table 3].

Compared to cases without EJLS, those with EJLS had significantly higher rates of abnormal fetal anatomy (38.5% vs. 75%, $P = 0.003$), karyotype (18.5% vs. 45%, $P = 0.005$), reversed a-wave in the DV (10.4% vs. 30%, $P = 0.026$), TOP (14.1% vs. 55%, $P < 0.001$), and a lower rate of T and L births (59.3% vs. 15.0%, $P < 0.001$). The predominant karyotypic abnormality in both groups, trisomy 21, was significantly more common in cases with EJLS than in those without EJLS (35.0% vs. 8.9%, $P = 0.00$) [Table 4].

Table 1: Fetal and maternal features and pregnancy outcomes of all cases included in study

Variable	NT					Total (*n*=155), *n* (%)
	≥95th percentile-3.5 mm (*n*=67), *n* (%)	3.6-4.4 mm (*n*=43), *n* (%)	4.5-5.4 mm (*n*=24), *n* (%)	5.5-6.4 mm (*n*=13), *n* (%)	≥6.5 mm (*n*=8), *n* (%)	
Maternal age (mean±SD)	27.8±5.77	28.6±6.84	31.67±5.68	31.8±8.24	30±6.35	29.18±6.11
Gravida (mean±SD)	2.09±1.28	2.56±1.55	2.54±1.47	3±1.41	2.38±1.69	2.38±1.42
Parity (mean±SD)	0.82±0.87	1.21±1.1	1.13±1.19	1.15±1.28	0.88±0.83	1±1.028
Abortus (mean±SD)	0.28±0.71	0.42±0.96	0.3±−0.7	0.92±1.04	0.50±1.07	0.38±0.84
EJLS	3 (4.5)	4 (9.3)	7 (29.2)	4 (30.8)	2 (25)	20 (12.9)
Reverse a wave in DV	5 (7.5)	6 (14)	5 (20.8)	2 (14.4)	2 (20.4)	20 (12.9)
Fetal anatomy						
Normal	39 (58.2)	22 (51.2)	6 (25)	1 (7.7)	0	68 (43.9)
Abnormal	18 (26.9)	14 (32.6)	17 (70.8)	11 (84.6)	7 (87.5)	67 (43.2)
M.M	10 (14.9)	7 (16.3)	1 (4.2)	1 (7.7)	1 (12.5)	20 (12.9)
Fetal karyotype						
Refused	8 (11.9)	5 (11.6)	3 (12.5)	2 (15.4)	1 (12.5)	19 (12.3)
Normal	51 (76.1)	27 (62.8)	13 (54.2)	7 (53.8)	4 (50)	102 (65.8)
Abnormal	8 (11.9)	11 (25.6)	8 (33.3)	4 (30.8)	3 (37.5)	34 (21.9)
Invasive testing						
CVS	44 (65.7)	29 (67.4)	20 (83.3)	11 (84.6)	5 (62.5)	99 (63.8)
Amniocentesis	12 (17.9)	7 (16.3)	8 (33.3)	0	2 (25)	29 (18.7)
Postnatal karyotyping	1 (1.4)	1 (2.3)	2 (8.3)	0	0	4 (2.5)
Pregnancy outcome						
Abortion	7 (10.4)	4 (9.3)	3 (12.5)	3 (23.1)	2 (25)	19 (12.3)
TOP	6 (9)	8 (18.6)	6 (825)	5 (38.5)	5 (62.5)	30 (19.4)
IUFD	1 (1.5)	1 (2.3)	0	0	0	2 (1.3)
T and L	45 (67.2)	23 (53.5)	10 (41.7)	5 (38.5)	0	83 (53.5)
PD	2 (3)	3 (7)	3 (12.5)	0	0	8 (5.2)
PB	6 (9)	4 (9.3)	2 (5.3)	0	1 (12.5)	13 (8.4)

NT: Nuchal translucency, EJLS: Extended jugular lymphatic sac, SD: Standard deviation, DV: Ductus venosus, CVS: Chorionic villus sampling, M.M: Minor marker, TOP: Termination of pregnancy, IUFD: Intrauterine fetal death, T and L: Term and live birth, PD: Postnatal death, PB: Preterm delivery

Table 2: Feto-maternal characteristics and pregnancy outcomes of nonextended jugular lymphatic sac cases

Variables	NT					Total (*n*=135), *n* (%)
	≥95th percentile-3.5 mm (*n*=64), *n* (%)	3.6-4.4 mm (*n*=39), *n* (%)	4.5-5.4 mm (*n*=17), *n* (%)	5.5-6.4 mm (*n*=9), *n* (%)	≥6.5 mm (*n*=6), *n* (%)	
Maternal age (mean±SD)	28.08±5.23	28.57±5.58	31±6.46	33.6±7.16	29.3±4.68	29.07±5.96
Number of gravida (mean±SD)	2.13±1.19	2.56±1.56	2.47±1.5	3.11±1.36	2±0.89	2.38±1.44
Reverse a wave in DV	4 (6.3)	4 (10.3)	4 (23.5)	1 (11.1)	1 (16.7)	14 (10.4)
Fetal anatomy						
Normal	38 (59)	21 (53.8)	6 (35.3)	1 (11.1)	0	66 (48.9)
Abnormal	16 (25)	13 (33.3)	10 (58.8)	7 (77.8)	6 (100)	52 (38.5)
MM	10 (15)	5 (12.8)	1 (5.9)	1 (11.1)	0	17 (12.6)
Affected organs or systems						
CH	9 (14)	8 (20.5)	5 (29.4)	3 (33.3)	2 (33)	27 (20)
CNS	3 (4.7)	1 (2.6)	0	0	1 (16)	5 (3.7)
CVS	4 (6.3)	3 (7.7)	4 (23.5)	2 (22.2)	2 (33)	15 (11.1)
GIS	7 (10)	1 (2.6)	0	1 (11.1)	0	9 (6.7)
Hydropic placenta	0	0	1 (5.9)	0	0	1 (0.7)
MSS	0	1 (2.6)	0	0	1 (16)	2 (1.5)
Hypoplastic NB	1 (1.6)	0	0	0	0	1 (0.7)
SUA	0	2 (5.1)	0	0	0	2 (1.5)
Isolated HF	1 (1.6)	1 (2.6)	0	2 (22.2)	0	4 (3)
GIS + MSS	1 (1.6)	0	1 (5.9)	0	0	2 (1.5)
Face + CVS	0	1 (2.6)	0	0	0	1 (0.7)
Karyotype						
Refused	7 (10.9)	5 (12.8)	2 (11.8)	1 (11.1)	0	15 (11.1)
Normal	50 (70.8)	27 (69.2)	10 (58.8)	5 (55.5)	3 (50)	95 (70.4)
Abnormal	7 (10.9)	7 (17.9)	5 (29.4)	3 (33.3)	3 (50)	25 (18.5)
Trisomy 21	2 (3.1)	4 (10.3)	4 (23.5)	1 (11.1)	1 (16.7)	12 (8.9)
Trisomy 18	1 (1.6)	1 (2.6)	0	2 (22.2)	1 (16.7)	5 (3.7)
Other	4 (6.3)	2 (5.1)	1 (5.9)	0	1 (16.7)	8 (5.9)
Pregnancy outcome						
Abortion	6 (9.4)	4 (10.3)	2 (11.8)	3 (33.3)	1 (16.7)	16 (11.9)
TOP	5 (7.8)	4 (10.3)	4 (23.5)	2 (22.2)	4 (66.7)	19 (14.1)
IUFD	1 (1.6)	1 (2.6)	0	0	0	2 (1.5)
T and L	44 (68.8)	23 (59)	9 (52.9)	4 (44.4)	0	80 (59.3)
PD	2 (3.1)	3 (7.7)	1 (5.9)	0	0	6 (4.4)
PB	6 (9.4)	4 (10.3)	1 (5.9)	0	1 (16.7)	12 (8.9)

NT: Nuchal translucency, SD: Standard deviation, DV: Ductus venosus, CH: Cystic hygromas, CNS: Central nervous system, CVS: Cardiovascular system, GIS: Gastrointestinal system, MSS: Musculoskeletal system, NB: Nasal bone, SUA: Single umbilical artery, HF: Hydrops fetalis, M.M: Minor marker, TOP: Termination of pregnancy, IUFD: Intrauterine fetal death, T and L: Term and live birth, PD: Postnatal death, PB: Preterm delivery

There was a significant but a mild correlation between EJLS and NT (correlation coefficient [CC] =0.283), fetal abnormalities (CC =0.273), and adverse pregnancy outcomes (CC =0.308), ($P < 0.05$). In addition, there was a significant correlation between NT and fetal anomalies (CC =0.404) and unfavorable pregnancy outcomes (CC =0.320), ($P < 0.05$) [Table 5].

Discussion

The presence of EJLS was in 12.9% of all cases with increased NT, and 75% of fetuses in this group had abnormal anatomy, 45% had an abnormal karyotype, and only 15% achieved T and L birth. Compared to cases with EJLS, n-EJLS cases had better pregnancy outcomes, including lower rates of fetal structural and karyotypic abnormalities and higher rates of T and L. However, the rate presence EJLS increased as NT increased, and this increase was more pronounced when NT ≥4.5 mm. In addition, there was an increased rate of fetal structural and karyotypic abnormalities and poor pregnancy outcome as NT increased which is making hard to detect the exact effect of EJLS on these outcomes. Nonetheless, in the presence of EJLS, this study provides practical information, particularly for those using elevated NT to predict fetal karyotypic and structural abnormalities and obstetric outcomes without further stratification.

We believe that if EJLS is associated with an increase in NT, this should be a warning sign that fetal anatomy and karyotype should be carefully examined, considering that 75% of them have structural abnormalities and almost half have karyotypic abnormalities.

Table 3: Characteristics of cases with extended jugular lymphatic sac

Variables	NT					Total cases (*n*=20), *n* (%)
	≥95th percentile-3.5 mm (*n*=3), *n* (%)	3.6-4.4 mm (*n*=4), *n* (%)	4.5-5.4 mm (*n*=7), *n* (%)	5.5-6.4 mm (*n*=4), *n* (%)	≥6.5 mm (*n*=2), *n* (%)	
Maternal age (mean±SD)	22±3.0	31.5±5.59	33.29±3.82	27.75±10.14	32±12.73	30±7.7
Gravida (mean±SD)	1.33±0.58	2±0.82	2.71±1.5	2.75±1.71	3.5±3.54	2.45±1.56
Reversed a wave in DV	1 (33.3)	2 (50)	1 (14.3)	1 (25)	1 (50)	6 (30)
Fetal anatomy						
Normal	1 (33.3)	1 (25)	0	0	0	2 (10)
Abnormal	2 (66.7)	1 (25)	7 (100)	4 (100)	1 (50)	15 (75)
M.M	0	2 (50)	0	0	1 (50)	3 (15)
Affected organ or systems						
CH	1 (33.3)	1 (25)	3 (42.9)	2 (50)	1 (50)	8 (40)
CNS	0	0	1 (14.3)	0	0	2 (10)
CVS	1 (33.3)	2 (50)	0	1 (25)	0	3 (15)
GIS	0	0	0	0	1 (50)	1 (5)
MSS	0	0	0	1 (25)	0	1 (5)
Isolated HF	0	0	3 (42.9)	0	0	3 (15)
Fetal karyotype						
Refused	1 (33.3)	0	1 (14.3)	1 (25)	1 (50)	4 (20)
Normal	1 (33.3)	0	3 (42.9)	2 (50)	1 (50)	7 (35)
Abnormal	1 (33.3)	4 (100)	3 (42.9)	1 (25)	0	9 (45)
Trisomy 21	0	3 (75)	3 (42.9)	1 (25)	0	7 (35)
Trisomy 18	0	1 (25)	0	0	0	1 (5)
Others	1 (33.3)	0	0	0	0	1 (5)
Pregnancy outcome						
Abortion	1 (33.3)	0	1 (14.3)	0	1 (50)	3 (15)
TOP	1 (33.3)	4 (100)	2 (28.6)	3 (75)	1 (50)	11 (55)
T and L	1 (33.3)	0	1 (14.3)	1 (25)	0	3 (15)
PD	0	0	2 (28.6)	0	0	2 (10)
PB	0	0	1 (14.3)	0	0	1 (5)

NT: Nuchal translucency, SD: Standard deviation, DV: Ductus venosus, CH: Cystic hygromas, CNS: Central nervous system, CVS: Cardiovascular system, GIS: Gastrointestinal system, MSS: Musculoskeletal system, HF: Hydrops fetalis, M.M: Minor marker, TOP: Termination of pregnancy, T and L: Term and live birth, PD: Postnatal death, PB: Preterm delivery

Indeed, this raises the question of whether EJLS is a cause or a result in cases with increased NT. The pathophysiology of enlarged NT has been discussed for four decades. It has been associated with many fetal structural or genetic abnormalities. Therefore, it is not thought to be caused by a single pathophysiological mechanism. Abnormalities in extracellular matrix composition, hemodynamic alterations in first trimester, and abnormal endothelial development due to several reasons have been the most accepted origins for enlarged NT.[6,7,13,19,20] Furthermore, it has been suggested that the pathophysiology of increased NT may be caused by a delayed reorganization of the lymph nodes. Lymphatic endothelial cell buds originate from the internal jugular veins and lymphatic development begins with the formation of JLS in the neck and forms the main drainage area for lymphatic fluid to enter the systemic circulation as the right thoracic duct grows toward the left JLS at 14 weeks of gestation.[20,21]

Similar to our study, existing studies reported a correlation between NT distance and presence of EJLS.[13,22,23] In agreement with a study by Bronshtein *et al*., in which the authors attributed the pathophysiological basis of cystic hygroma to the failure of the lymphatic system to communicate with the nuchal venous system, we observed that the most common accompanying abnormality to be cystic hygroma in increased NT cases both with and without EJLS.[24] Complete but delayed remodeling of the lymphatic system could explain both the regional and transient features of the increased NT and the high T and L birth rates found in this study, 59.3% in increased NT without EJLS. However, compared with this group, the fetuses with EJLS had a lower T and L birth rate, a higher rate of reversed a-wave in DV, and a higher rate of karyotypic and structural abnormalities. This may suggest another pathophysiological mechanism that endothelial signaling is crucial in embryogenesis and is involved in the processes of heart formation and blood and lymphatic vessel development. Indeed, processes in genes involved in endothelial differentiation may affect both cardiac and lymphatic vasculature, and it is likely that environmental, epigenetic, or genetic influences are responsible.[25,26]

In this study, there was a higher rate of reversed a-wave in DV when increased NT was accompanied by EJLS. In addition to the theory suggesting the aforementioned developmental pathophysiological mechanism of the DV, another theory

Table 4: Comparison of outcomes between cases with and without extended jugular lymphatic sac

Variables	Cases without EJLS (*n*=135), *n* (%)	Cases with EJLS (*n*=20), *n* (%)	*P*
Maternal age (mean±SD)	29.07±5.96	30±7.7	0.576***
Gravida (mean±SD)	2.38±1.44	2.45±1.56	0.962****
NT			
≥95th percentile-3.5 mm	64 (47.4)	3 (15)	0.003*
3.6-4.4 mm	39 (28.9)	4 (20)	
4.5-5.4 mm	17 (12.6)	7 (35)	
5.5-6.4 mm	9 (6.7)	4 (20)	
≥6.5 mm	6 (4.4)	2 (10)	
Reversed a wave in DV	14 (10.4)	6 (30)	0.026*
Fetal anatomy			
Normal	66 (48.9)	2 (10)	0.003**
Abnormal	52 (38.5)	15 (75)	
M.M	17 (12.6)	3 (15)	
Fetal karyotype			
Refused	15 (11.1)	4 (20)	0.005**
Normal	95 (70.4)	7 (35)	
Abnormal	25 (18.5)	9 (45)	
Trisomy 21	12 (8.9)	7 (35)	
Trisomy 18	5 (3.7)	1 (5)	
Others	8 (5.9)	1 (5)	
Pregnancy outcome			
Abortion	16 (11.9)	3 (15)	<0.001**
TOP	19 (14.1)	11 (55)	
IUFD	2 (1.5)	0	
T and L	80 (59.3)	3 (15)	
PD	6 (4.4)	2 (10)	
PB	12 (8.9)	1 (5)	

*Fisher's exact test, **Chi-square test, ****t*-test, ****Mann-Whitney test. *P*<0.05 considered as significance. NT: Nuchal translucency, EJLS: Extended jugular lymphatic sac, SD: Standard deviation, DV: Ductus venosus, M.M: Minor marker, TOP: Termination of pregnancy, IUFD: Intrauterine fetal death, T and L: Term and live birth, PD: Postnatal death, PB: Preterm delivery

Table 5: Spearman-Rho correlations between extended jugular lymphatic sac, nuchal translucency, fetal abnormalities, and adverse pregnancy outcomes

Spearman Rho	Variables	CC	*P*
EJLS	NT	0.283	<0.001
	Fetal abnormalities	0.271	0.001
	Adverse pregnancy outcomes	0.308	<0.001
NT	EJLS	0.283	<0.001
	Fetal abnormalities	0.404	<0.001
	Adverse pregnancy outcomes	0.320	<0.001

P<0.05 considered as significance. EJLS: Extended jugular lymphatic sac, NT: Nuchal translucency, CC: Correlation coefficient

indicated that high venous pressure due to EJLS, abnormal innervation of the jugular vein, and DV may contribute to abnormal flow rates in fetuses with elevated NT.[22]

In this study, cystic hygromas (40%), isolated hydrops fetalis (15%), and cardiovascular abnormalities (15%) were the most common abnormalities noted in EJLS cases. Among these, being specific organ anomalies, the cardiovascular anomalies had the highest rate of poor obstetric outcome and abnormal karyotype. In the n-EJLS group, the most common abnormalities were cystic hygromas (27%), cardiovascular (15%), and gastrointestinal system (9%) anomalies. Our results are consistent with previous studies that found cystic hygromas and hydrops fetalis to be the most frequently observed abnormalities in EJLS cases. Considering the increased NT, our results seem to differ from studies that described cardiovascular and musculoskeletal abnormalities as the first two most common abnormalities both with and without EJLS.[4,27]

We found a higher rate of abnormal fetal karyotypes in cases with EJLS (45%) than in those without EJLS (18.5%). The most frequently detected abnormal karyotype in both groups, trisomy 21, was more common in cases with EJLS than in cases without EJLS (77% and 48%, respectively). The study by Haak *et al*. reported that the development of EJLS preceded the increase of NT in mouse embryos with trisomy 16, which is the animal model for trisomy 21 in humans.[23] Therefore, EJLS may be a more specific finding than increased NT, as there are more pathophysiological pathways leading to increased NT, as mentioned previously.[4,25-27]

The rate of T and L births was much higher in cases without EJLS (59.3%) compared with those with EJLS (15%). This

may be due, in part, to the higher frequency of normal fetal anatomy and karyotype in the former. For example, we observed that when fetal anatomy was normal, the rate of T and L birth was 73% in cases without EJLS and 66% in cases with EJLS. Similar to our study, positive pregnancy outcomes were reported in 50%–71% of cases with increased NT between 4.5 and 5.4 mm with normal fetal anatomy.[16]

Although studies showed the occurrence of early childhood developmental delays in up to 9% of such cases.[15] Senat *et al*. reported that no fetal structural malformations occur when the karyotype is normal and that when nuchal translucency (NT) improves, prognosis at 2 years of age is not affected.[28] However, similar to our study, increased NT is known to adversely affect pregnancy outcomes even in fetuses with normal karyotype and anatomy, particularly by reducing viable pregnancy rates above 3.5 mm.[15]

There are strengths in this study, although both examination and clinical management were performed by the same team. However, whether it can be classified as a research feature is still a question. Because the same team is also more prone to specific recommendations. The authors are advised to include the features of this study.

Weaknesses of this study: First, that patient selection was variable in some situations, including fetal structural and karyotypic abnormalities, that may affect rates of miscarriage, TOP or preterm birth, and T and L. Second, because of the relatively small number of patients in each NT stratum, we could not demonstrate the exact risk added by EJLS on NT. This was another limitation due to the wide range of data and the difficulty in presenting them, as each fetus undergoes its process in a unique way.

Conclusion

The rate of EJLS became higher with increasing NT and the majority of EJLS cases NT had ≥4.5 mm. Cases with EJLS were more likely to have poor pregnancy outcome, abnormal karyotype, and structural abnormalities. The most common structural and karyotypic abnormalities detected were cystic hygromas and trisomy 21 in both groups, with and without EJLS, with both being more pronounced in EJLS.

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Conflicts of interest

There are no conflicts of interest.

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