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ANTİMİCROBİAL ACTİVİTY OF HUMAN AMNİOTİC FLUİD AND KARYOTYPE

Microbiology

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ABSTRACT

In this study, the antimicrobial activity of 40 amniotic fluid samples had been examined. Relationships were also examined between the antimicrobial activity of the amniotic fluids and maternal age, gestational week, number of birth, number of gestation and karyotype.
Thirteen (32,5 %) of 40 amniotic fluid samples showed inhibitory effect on *Escherichia coli,* and 3 (7.5%) on *Candida albicans.* Both *E. coli* and *C. albicans* was not effective in the sample. According to the results, there were no significant statistical relationship between the antimicrobial activity and maternal age, gestational week, number of birth, number of gestation, karyotype. In this study, a total of 40 amniotic fluid samples from mothers between 16-18 weeks gestation.

KEYWORDS

amniotic fluid, antimicrobial activity, karyotype.

INTRODUCTION

Approximately 2-4 out of 100 babies have birth defects defined as abnormalities of structure, function or body metabolism, which is a serious and costly public health problem as well as a family problem when combining medical and social factors (1,2). A routine prenatal diagnostic test to detect birth defects in the fetus or embryo before it is born has been introduced for either performing a timely appropriate treatment or giving the parents a chance to decide the fate of the unborn babies with health problems (3,4). The most common indication for prenatal diagnosis is advanced maternal age at risk of having babies with chromosome abnormalities (5,6). The monogenic disorders are also a common reason why couples opt for prenatal genetic testing (7). Conventional methods for prenatal genetic testing are based on invasive procedures, such as chorionic villus sampling, amniocentesis and cord blood sampling, to obtain fetal genetic materials for prenatal genetic analysis (8,9). Amniocentesis is typically carried out under ultrasonographic control, between 15 and 17 weeks of pregnancy. The procedure specific risk is 0.5% to 1% (10) .

The traditional view is that the amniotic cavity is sterile and does not contain viable microorganisms (11). Bacteria can gain access to the amniotic cavity by passing through intact membranes (12,13) or by transplacental passage in cases of hematogenous dissemination bacteremia in the context of periodontal disease or other distant infections (11,14-17) .

Amniotic fluid is known to have antimicrobial properties (18-20). Yet, a comprehensive description of all the natural antimicrobial agents present in amniotic fluid has not been achieved. This antibacterial activity is due to the combined effect of several antibacterial factors such as lysozyme, peroxidase, transferrin, lactoferrin, immunoglobu lins, beta -lysin, zinc and so on (21-23). Clear Amniotic fluid was found to have antibacterial activity, reaching its peak at 36 to 40 weeks of gestation (23). In this study , the inhibitory effect on *Escherichia coli* and *Candida albicans* of 40 amniotic fluid samples had been examined .All of amniotic fluid samples were obtained from women between 16-18 weeks gestation. Relationships were also examined between the antimicrobial activity of the amniotic fluids and maternal age gestational week, number of birth, number of gestation and karyotype.

MATERIALS AND METHODS

Amniotic fluid

For genetic analysis, 40 amniotic fluid samples were obtained from women undergoing mid-trimester amniocentesis. Their antimicrobial activity was tested against Escherichia coli (ATCC 25922) and *Candida albicans* (ATCC 10231). The amniotic fluid contains numerous free-floating fetal cells that can be grown in a laboratory. When these cells multiply and reach a certain number, their chromosomes are extracted and analyzed. Amniocenteses were carried out at the Department of Gynecology and Obstetrics, Dicle University Hospital in the city of Diyarbakir, Southeast Turkey. All samples were analyzed in the Medical Biology and Genetic Department Laboratory at Dicle University for cytogenetic analyses. Around 15 mL of amniotic fluid would normally be aspirated. Chromosomal analysis requires prior cell culture, which takes on average two weeks. After this metaphase chromosomes are analyzed numerically and structurally. The same time amniotic fluid was immediately sent to the microbiology laboratory for cultures. Blood agar, MacConkey's agar, chocolate agar, and Thayer-Martin agar were used for aerobic cultures. Blood agar, bacteroides, and anaerobic agar were used for anaerobic culture. The amniotic fluid samples culture were negative. Antimicrobial activity determination for the amniotic fluid samples were centrifuged at 4°C for 15 min at 3,000 X g, the clear supernatant passed through 0.20 mikronm pore size filter (Biotech) and studied on the same day.

Microbial growth inhibition study

Escherichia coli (ATCC 25922), *Candida albicans* (ATCC 10231) were used throughout the study to determine the antimicrobial properties of amniotic fluid. The inoculum was an overnight culture of *E. coli in* Mueller-Hinton (MH) broth (Difco), of in C.albicans in Sabouraud-Dextrose Broth (SDB –Merck) diluted to McFarland standard No. 1 with normal saline; 0.1 ml of which was further diluted with saline to contain 1 x 10^4 colonyforming units/ml; 0.1 ml of this was added petri dishes containing 40 ml of Mueller Hinton Agar (MHA - Difco) or Sabouraud dextrose agar (SDA –Merck). The black discs were placed on the Mueller-Hinton agar and Sabouraud-Dextrose agar surfaces, after and 50 ml of the test amniotic fluid were added into each black discs (OXOID)(24). The plates were incubated at 37°C for 24 h and observed. Fluids were regarded as inactive if the growth of the test organisms at the sides of the test discs .

Statistical analysis

Spearman Correlatin Coefficient method, for descrete variable was used to determine the correlation among. Antimicrobial activity, karyotype and other variables respectiveler (age, gestational week, number of birth, number of gestation). The statistical analysis were

carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS Inc. Chicago, IL, USA). Two-sided p values were considered statistically significant at $p \leq 0.05$

RESULTS

In this study, thirteen (32,5%) of 40 amniotic fluid samples showed inhibitory effect on *E.coli* and 3 (7,5%) on *C.albicans*. Both E. coli and *C. albicans* was not effective in the sample. Table I shows distribution the age, weeks of gestation, number of birth, number of gestation of the mothers in relation to antimicrobial activity of the amniotic fluids. There was no correlation between antimicrobial activity and the age, weeks of gestation, number of birth ,number of gestation of the women (Table II).

Table III shows the karyotype of the mothers in relation to antimicrobial activity of the amniotic fluids. There was no correlation between antimicrobial activity and the karyotype of the fetus.

DISCUSSION

The fact that *E. coli* represent the major pathogens for early - onset neonatal infection. Although *C. albicans* is a frequent inhabitant of the female genital tract, chorioamnionitis is rarely caused by this fungal organism (25,26).

We demonstrated that clear amniotic fluid has inhibition of *E. coli* and *C.albicans* growth. The bacteriostatic properties of amniotic fluid have been attributed to various organic and nonorganic components, including transferrin, lysozyme, a zinc peptide, and the zinc / phosphate ratio. In particular, the role of zinc and phosphate has been emphasized by many with reports of the inhibitory effect on the growth of *E. coli, Staphylococcus aureus,* and *Streptococcus faecalis* by zinc or a zinc -dependent peptide and the growth enhancing effect of zinc on GBS (27-30). Oka et al. (22) reported that the growth of *E. coli* was inhibited in AF with apotransferrin, but stimulated in amniotic fluid with holotransferrin. According to the results of Thadepalli and his colleagues (31) amniotic fluid could be largely divided into the two categories, inhibitory amniotic fluid and non-inhibitory amniotic fluid. In the inhibitory amniotic fluid, iron concentration was low, apotransferrin that do not bind to iron was elevated, and finally iron-saturation of transferrin was around 30%. On the other hand, in the non-inhibitory AF, iron concentration was elevated, apotransferrin was decreased, and finally iron-saturation of transferin was more than 50%. This categorization was based on the fact that the higher iron-saturation of transferrin is, the easier bacteria can capture iron from transferrin (32). Similarly, Ahn et al. (33) reported that antibacterial activity of amniotic fluid is closely related with low iron-availability. According to the results of Vaısbuch et al. (34) the AF concentration of immunoreactive total hemoglobin increases with advancing gestational age, and is elevated in pregnancies that are complicated with intra-amniotic infection/inflammation. Spontaneous labor at term is associated with higher AF concentrations of total hemoglobin. Hemoglobin can be detected in the amniotic fluid of all pregnant women.

Tzu-Hao Wang et al. (35) investigated the functional roles of differentially expressed proteins in the amniotic fluid supernatant (AFS) with abnormal karyotypes. Samples of AFS from 34 fetuses with normal-karyotype, 17 with trisomy 18, and 19 with trisomy 21 .noted that the levels of apolipoprotein A1, AP-3µ, and antitrypsin were significantly decreased in trisomy-18 AFS, whereas placental protein-14 was increased. On the other hand, apolipoprotein A1 was decreased in trisomy-21 AFS, but antitrypsin, prealbumin, and transferrin were increased in trisomy 21. Biologic network analyses revealed that the proteins of the trisomy-18 AFS network were involved in immune processes, dysfunction of skin pigmentation, and platelet disorders, whereas those of trisomy 21 were associated with dysfunctional lipid and cholesterol metabolism, processes of metal ion transport, adenosine triphosphate metabolism, and energy-coupled protein transport. In our study, there was no correlation between antimicrobial activity and the karyotype of the fetus, but covering different periods of pregnancy and a greater number of different karyotypes more should be done in future studies.

Florman and Teubner (36) studying an in vitro model, noted that the addition of small amounts of meconium to amniotic fluid reversed the inhibitory effect of amniotic fluid and led to enhancement of growth of both *E. coli* and *Listeria* organisms. Human b-defensin-2 (HBD-2) is a potent antimicrobial peptide that is part of the innate immune response. The presence of several antimicrobial peptides has been documented in amniotic fluid, including human neutrophil peptides (HNP) 1, 2 and 3,bactericidal/permeability-increasing protein, and calprotectin (MRP8/14) (37). Harder et al. (38) reported that HBD-2 preparations have antimicrobial activity against *E. coli, Pseudomonas aeruginosa* (LD90:10 mg/mL) and *C. albicans* (LD90: 25 mg/mL). Recent investigations with recombinant HBD-2 revealed that this peptide is even more potent, giving an LD50 near 100 ng/mL (39) suggest that HBD-2 has a preferential effect on Gram-negative bacteria and yeast (38). Soto et al. (11) suggest that HBD-2 may contribute to the antimicrobial activity of amniotic fluid, and that this antimicrobial peptide participates in the innate immune response against intra-amniotic infection or other insults eliciting an inflammatory response. Despite extensive studies the antimicrobial properties of amniotic fluid fully elucidated. There are many factors that affect chemistry of the amniotic fluid known and unknown In our study; a total of 40 AF samples from patients between 16-18 weeks gestation. Thirteen of these fluids (33.5%) were inhibitory to *E. coli*, and three of these fluids (7.5%) were inhibitory to C.albicans. Antimicrobial property of AF increases with the increasing period of gestation, reaching a peak level during the 36th-40th week of pregnancy (31). Nazir at al.(40) in the study was to determine the bacterial growth inhibitory property of amniotic fluid (AF) from patients in the early third trimester and to examine its relationship to preterm labor and delivery. A total of 38 AF samples from patients between 27 and 35 weeks gestation. Five of these fluids (33%) were inhibitory to *E. coli.* Vincent et al. (41) in the study fifty-one amniotic fluids were aspirated via the vaginal route from 51 pregnant (between 37 and 42 weeks gestation) Nigerian mothers. Their antimicrobial activity was tested against *E. coli* and *C. albicans.* Inhibition rates were 19.6% for *E. coli* and 41.2% for ***C. albicans.*** Akın and Seyrekbasan (24) reported that twenty two (42,3%) of 52 amniotic fluid samples between 32 and 41 weeks gestation showed inhibitory effect on E.coli, 23 (44,2%) and 29 (55,8%) on *C.albicans.*

In conclusion, our results suggest that a greater number of different karyotypes more should be done in future studies for antimicrobial activity of amniotic fluid.

Table I. The samples of amniotic fluid distribution and age ,weeks of gestation, number of birth, number of gestation and antibacte rial activity

Samples No	Age	Weeks of gestation	Number of birth	Number of gestation	Antimikrobiyal activity (mm)
1	37	18	2	4	0
2	35	18	2	3	0.4
3	33	18	1	2	0
4	37	16	0	1	0
5	35	17	1	2	0
6	34	17	5	7	0.3
7	22	16	1	3	0.5
8	43	16	2	5	0.5
9	33	17	3	4	0
10	21	18	0	1	0.7
11	29	16	2	4	0
12	33	17	1	3	0
13	28	17	0	1	0.8
14	39	16	4	6	0
15	23	17	2	3	0
16	41	18	4	5	0
17	34	17	3	5	0
18	36	16	0	2	0.5
19	38	17	4	7	0
20	34	18	4	6	0.5
21	33	17	1	3	0
22	23	17	2	3	0.7
23	40	18	3	5	0
24	37	17	2	3	0
25	22	17	3	4	0.5
26	27	18	1	2	0.3
27	38	17	4	5	0
28	34	17	2	4	0.3
29	22	16	1	2	0

30	21	16	1	2	0
31	43	18	3	5	0.3
32	33	16	3	4	0.5
33	21	18	0	2	0
34	29	16	2	4	0
35	36	18	0	3	0
36	38	17	4	5	0.6
37	37	17	2	3	0
38	40	18	3	5	0.5
39	37	17	2	3	0
40	22	17	0	2	0

Table II. Correlation between antimicrobial activity of the amniotic fluids and the age, weeks of gestation, number of birth , number of gestation of the women

	rs	P
Antimicrobial activity-age	-0,10	0,541
Antimicrobial activity- weeks of gestation	0,047	0,775
Antimicrobial activity- number of birth	0,047	0,772
Antimicrobial activity- number of gestation	0,027	0,867

rs:Spearman Rank-order Coefficient
The p value ≤ 0.05

Table III. Karyotype distribution and antimicrobial activity (zone diameters, mm) of amniotic fluids

Samples No	*E. coli*	*C.albicans*	Karyotype
1	0	0	46,XY
2	0.4	0	46,XX
3	0	0	46,XX
4	0	0	46,XX,9qh+
5	0	0	46,XX
6	0.3	0	46,XY
7	0.5	0	46,XX
8	0.5	0	46,XX
9	0	0	46,XY
10	0	0.7	46,XX
11	0	0	46,XY,9qh+
12	0	0	46,XY
13	0.8	0	46,XX
14	0	0	46,XX
15	0	0	46,XX,9qh+
16	0	0	46,XY
17	0	0	46,XY
18	0.5	0	46,XX
19	0	0	46,XX
20	0.5	0	46,XX
21	0	0	47,XX,+21
22	0	0.7	46,XY
23	0	0	46,XX
24	0	0	46,XX,inv(9)
25	0.5	0	46,XX
26	0	0.3	46,XX
27	0	0	46,XY
28	0.3	0	46,XX
29	0	0	46,XX
30	0	0	46,XX
31	0.3	0	46,XY
32	0.5	0	46,XX
33	0	0	46,XX
34	0	0	46,XX
35	0	0	46,XX,inv(9)
36	0.6	0	46,XY
37	0	0	46,XX
38	0.5	0	46,XY,9qh+
39	0	0	46,XY
40	0	0	46,XY,9qh+

Table IV. Correlation between antimicrobial activity of the amniotic fluids and the karyotype of the fetus

	rs	P
Karyotype-E.coli antimikrobial activity	-0,054	0,742
Karyotype-C.albicans antimikrobial activity	-0,107	0,511

rs:Spearman Rank-order Coefficient
The p value $p \leq 0.05$

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