Original Article

The Comparison of Second Trimester Screening Tests With the White Blood Cell Counts

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Abstract

Objective: Our aim was to determine the possible differences in the maternal immune system by comparing white blood cells (WBCs) count with the sub-parameters in pregnant women with high-risk and no-risk for seconder trimester screening.

Materials and Methods: The results of complete blood count (CBC) and second trimester screening (STS) tests made between January 2011 and September 2015 of women meeting the inclusion criteria were analyzed retrospectively. All test results of pregnant women with high-risk for trisomy 21 (n = 55), neural tube defect (NTD) (n = 45) and with no-risk (n = 55) were compared. Trisomy 18 could not be evaluated due to limited number of cases.

Results: The monocyte count ($P < 0.001$) of T21 group were significantly increased compared to control group, but neutrophil to lymphocyte ratio (N/L) ($P = 0.027$), lymphocyte to monocyte ratio (L/M) ($P < 0.001$) and neutrophil to monocyte ratio (N/M) ($P < 0.001$) were significantly decreased. The WBC ($P = 0.02$), monocyte ($P < 0.001$) and lymphocyte ($P < 0.001$) count of NTD group were significantly increased compared to control group, but N/L ($P = 0.02$), L/M ($P = 0.034$) and N/M ratio ($P < 0.001$) were decreased.

Conclusion: The interaction of maternal immune system with the abnormal fetus may change the compositions of peripheral blood WBC and sub-parameters. Some of these changes may increase the predictive sensitivity of STS test. Further prospective studies are needed to confirm these findings.

Keywords: Blood cell count, Neural tube defects, Prenatal diagnosis, Pregnancy Trimester, Second, Trisomy 21, White blood cell

Introduction

Several studies reported the close relationship between maternal age and fetal chromosomal abnormalities such as trisomy 21, trisomy 18 and also neural tube defects (NTDs) (1,2). Determining such abnormalities within a reasonable time is important to maternal health and termination of fetus. The second trimester screening (STS) is helpful for early determination of fetal abnormalities. This is carried out along with the maternal age, gestational age, ultrasonography and several blood tests like β-hCG (human chorionic gonadotropin), AFP (alpha fetoprotein) and uE3 (unconjugated estriol) tests (1).

Early prenatal screening gives important information about the anomaly of the fetus during the second trimester. Prenatal screening may detect 60%-70% of aneuploidies (trisomy 21 and 18) with 5% false positive result (3,4). Also, approximately 70%-95% of NTDs and anencephaly maybe detected by evaluating the maternal serum AFP (5,6). In multiple pregnancies, STS detection rates may have decreased to 50% (7). STS cannot be diagnosed, but abnormal test results should be confirmed with the advanced diagnostic tests such as amniocentesis or chorionic villus sampling.

During pregnancies, physiological changes in the peripheral blood cells distribution may occur. An example reported in a research article is leukocytosis (8). During pregnancy, neutrophils are the dominant peripheral blood leukocytes and their activity is decreased by the fetal inhibitory factors (9,10). The lymphocyte part of peripheral blood decreases in the first and second trimester (11). The monocytes and monocyte-lymphocyte ratio increases in the first trimester of pregnancy (11,12). Some of the complete blood count (CBC) changes may also be detected in pathological pregnancies (13-15). White blood cell (WBC) levels decrease in the molar pregnancy compared to healthy pregnancy (14). In ectopic pregnancy, monocyte counts are higher compared to normal pregnancy (13). In pre-eclampsia, the neutrophil-lymphocyte ratio is higher in contrast with healthy pregnancies (15). Taken together, the maternal immune system is regulated sen-

sitively during pregnancy. Therefore, any imbalance between fetal tolerance and maternal immunity may affect placentation, the outcome and/or the course of pregnancy. Current reports have focused on the immune changes of healthy and pathological pregnancies. In early stages of pregnancy, CBC changes may occur due to interaction between the abnormal fetus and maternal immune system. To our best knowledge, there are no studies investigating the relationship between early prenatal screening and WBC part of the maternal peripheral blood cells. In this study we have retrospectively evaluated (what have you evaluated?) by comparing WBC and sub-parameters of CBC and STS test risks.

Materials and Methods

This study is a retrospective analysis of test results of STS and CBC performed in the Merkezefendi State Hospital Biochemistry Laboratory between January 2011 to September 2015. To determine the risk for trisomy 21, trisomy-18 and NTDs data of maternal serum β-hCG, AFP and uE3 tests, gestational age, mother age and weight were used (Table 1). Gestational age was calculated according to the biparietal diameter (BPD) values obtained by ultrasonography. The hormone levels of maternal serum were evaluated in the Beckman Coulter DXC 600 autoanalyzer using chemiluminescence. WBC, neutrophil, monocyte and lymphocyte levels were evaluated using the Abbott Cell Dyn 3700 automatic cell counter in the CBC samples, drawn into vacutainer 2 mL volume tubes containing 3.6 mg K2 EDTA. The multiples of median (MoM) values of hormone tests were calculated by comparing them with the average of the values of normal gestational population. The calculation of risk analysis was carried out using Benetech PRA v 2.3.0.4 (Benetech Medical Systems Toronto, Ontario Canada).

The cut-off value for the high risk was determined >1/250. Fifty-five cases showed high risk of trisomy 21 and 45 cases expressed high risk of NTD and 55 cases with no risk were evaluated. Trisomy 18 could not be evaluated because only there were 2 cases. The date of ultrasonography, blood collection and gestational age are important variables for prenatal screening. To correctly evaluate cellular changes of maternal peripheral blood, CBC tests were made on the same day and STS were analyzed. Risks occurring due to hormones (for β-hCG 2.5 MoM and above 0.4 MoM and below, for AFP and uE3 0.4 MoM and below) and twin pregnancies have been excluded. Women with high WBC (above the $12 \times 1000/mm^3$) values were not evaluated because of the increased likelihood of infection. Median values and standard deviations of WBC and sub-parameters as well as STS parameters are summarized in Table 1.

All statistical analyzes have been performed using IBM SPSS statistics version 20. All variables were tested for normality by the Shapiro-Wilk method and the results did not show normal distribution (95% CI). Therefore, statistical analysis was chosen to perform nonparametric Mann Whitney U method and was considered significant at $P \leq 0.05$. In addition, we have performed receiver operating characteristic (ROC) curves to evaluate test performance.

Results

There was no significant difference for maternal age and gestational age between all groups. All groups of median for maternal age, gestational age and hormone levels were summarized in Table 1.

The monocyte count of pregnant women with high-risk for trisomy-21 was significantly higher than the pregnant

**Table 1.** The Demographic Data, WBC and STS Hormone Results of All Groups

	Non-risk Group (n=55)	High Risk for Trisomy-21 Group (n=55)	High Risk for NTD Group (n=45)
Maternal age (year)	28±5	32±7	29±6
Gestational age (week)			
	15 (n=4)	15 (n=5)	15 (n=12)
	16 (n=12)	16 (n=10)	16 (n=9)
	17 (n=21)	17 (n=15)	17 (n=6)
	18 (n=16)	18 (n=15)	18 (n=7)
	19 (n=2)	19 (n=4)	19 (n=8)
	20 (n=0)	20 (n=6)	20 (n=3)
AFP (ng/mL)	34.58±10.31	29.82±12.65	107.88±96.45
AFP MoM	0.89±0.26	0.72±0.27	2.59±1.82
β-hCG (mIU/mL)	24613±10481	37783±17196	35085±20390
β-hCG MoM	1.00±0.35	1.55±0.5	1.21±0.49
uE3 (ng/mL)	1.27±0.41	0.9±0.39	1.47±0.77
uE3 MoM	1.21±0.32	0.78±0.26	1.35±0.50
Biparietal diameter (BPD)	38.54±3.64	39.45±4.59	38.06±5.28
WBC count	8.93±1.48	9.38±1.75	9.64±1.55
Neutrophil count	6.57±1.30	6.48±1.67	6.54±1.37
Lymphocyte count	1.75±0.46	1.94±0.49	2.23±0.72
Monocytecount	0.46±0.10	0.89±0.57	0.81±0.58
Neutrophil to Lymphocyte (N/L) ratio	4.08±1.72	3.57±1.40	3.12±0.89
Lymphocyte to Monocyte (L/M) ratio	3.92±1.09	2.93±1.52	3.40±1.34
Neutrophil to Monocyte (N/M) ratio	15.12±4.87	9.54±4.56	10.66±4.79

women with no-risk ($P < 0.001$). The WBC ($P = 0.02$), lymphocyte ($P < 0.001$) and monocyte ($P < 0.001$) counts of pregnant women with high-risk for NTD were significantly higher than the pregnant women with no-risk. No statistical significant difference was observed between the groups in terms of neutrophil count. The neutrophil to lymphocyte ratio (N/L) ($P = 0.027$), lymphocyte to monocyte ratio (L/M) ($P < 0.001$) and neutrophil to monocyte ratio (N/M) ($P < 0.001$) of trisomy-21 group were significantly lower than the control group. The N/L ($P = 0.02$), L/M ($P = 0.034$) and N/M ($P < 0.001$) ratio of NTD group were significantly lower than the control group. The comparison charts of all parameters are shown in Figures 1 and 2.

The area under the ROC curve of monocytes (0.799) and N/M (0.806) ratio were higher than the classical hormone tests of STS (β-hCG: 0.763, AFP: 0.656 and uE3: 0.770) for the trisomy-21 group. The area under the ROC curve of monocytes (0.743), lymphocytes (0.709) and N/M ratio (0.733) were higher than β-hCG (0.629) and uE3 (0.532) for the NTD group, AFP (0.993) remained as the most predictive test for NTD. The ROC curves of all tests are shown in Figure 3.
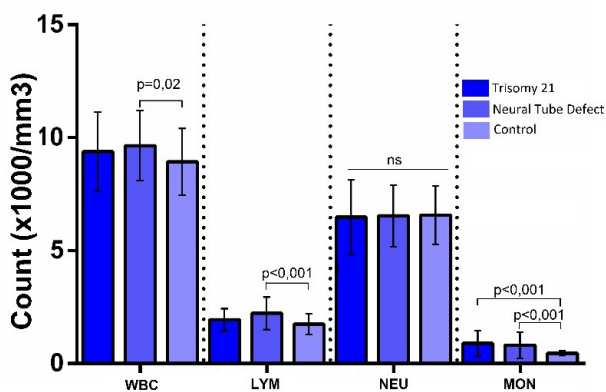
Figure 1. The Comparison Chart of the Counts of WBC and Sub-parameters. Data are presented as mean±SD (standard deviation). WBC, white blood cell; LYM, lymphocyte; NEU, neutrophil; MON, monocyte.
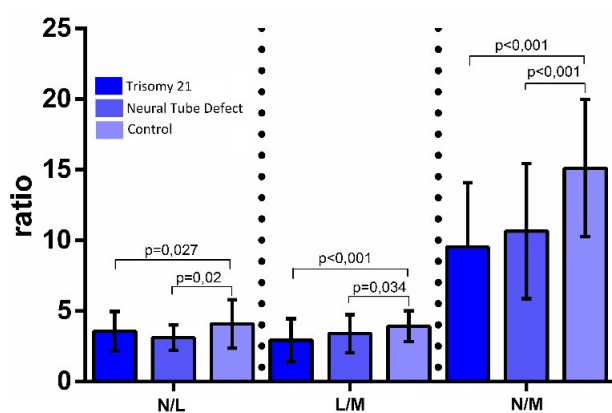
Figure 2. The Comparison Chart of the Ratios of WBC Sub-parameters. Data are presented as mean±SD (standard deviation). N/L, neutrophil to lymphocyte; L/M, lymphocyte to monocyte; N/M, neutrophil to monocyte.

Discussion

We have found statistically significant increases in WBC, lymphocyte and monocyte count in pregnant women with high-risk for NTD but only increase of monocyte count in pregnant women with high-risk for trisomy-21 was observed.

Immune system is specialized in protecting the body against external invaders but during pregnancy, maternal immune system physiologically adapts itself to semi-allogeneic fetus (11,16,17). Some of the physiological adaptations can be monitored easily from maternal peripheral blood (11). The changes of maternal immune response are also seen in pregnancy pathologies such as ectopic pregnancy, pre-eclampsia and molar pregnancy (13-15).

There are many immune assays to determine these changes of maternal immune system. One of the easiest methods is count of the number of cells of different subtypes (e.g. leukocytes, neutrophils, macrophages) in peripheral blood (18). In this study, we have observed the increased count of lymphocyte and monocyte in pregnant women with high-risk for NTD and monocyte in with high-risk for trisomy-21 which may be associated with fetal abnormality. However, for reliable evaluations of CBC it is important to have an adequate number of all types of blood cells in the exact proportions. Furthermore, the normal range for these parameters is quite large and thus small changes are unlikely to have any clinical significance (18). Therefore, the ratio between sub-parameters of WBC may be auxiliary in this case compared to the exact number of these parameters. In this study, we also evaluated the N/L, L/M and N/M ratios and have observed the significant decrease in trisomy-21 and NTD groups compared to control group.

We have observed that the area under the curve of monocyte and N/M ratio were higher than the β-hCG and uE3. The ROC curve is performed for the evaluation of the test performance and to evaluate the performance of a test as "good," the value of area under curve should be higher than 0,8. β-hCG and uE3 are important hormone tests for risk calculation of trisomy-21 (1,2). These findings suggest that monocyte count and/or N/M ratio may be better and cheaper method to increase the sensitivity of risk analysis of trisomy-21. Maternal serum AFP level is a powerful test for risk analysis of NTDs (5,6). We have confirmed that the area under the curve of AFP (0.993) was obviously higher than any of the WBC parameters.

STS may detect only 60%-70% of aneuploidies (trisomy 21 and 18) with 5% false positive result (3,4). Amniocentesis and chorionic villus sampling are invasive diagnostic tests which allow examination of the fetal karyotype and/or genotype (19). The placental samples are obtained by transabdominal or trans-cervical biopsy. However, in 1%-3% of cases there is a risk of pregnancy loss (19-21). For this reason, advanced diagnosis tests are performed only in high-risk pregnancies. To prevent pregnancy losses caused by the invasive process, a reliable method for non-invasive diagnosis of fetal anomalies is of a critical importance. In this study, we showed the significant differences
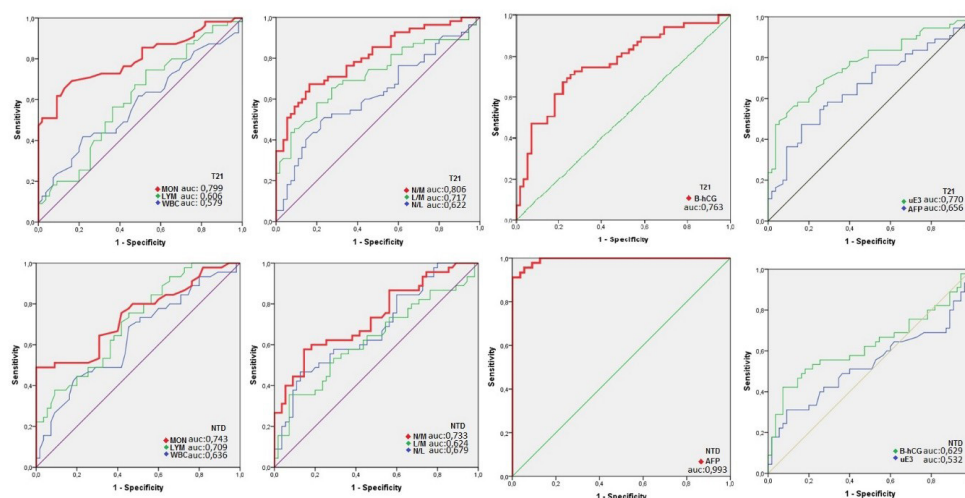
Figure 3. The Receiver Operating Characteristic (ROC) Curves of All Tests. WBC, white blood cell; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; N/L, neutrophil to lymphocyte; L/M, lymphocyte to monocyte; N/M, neutrophil to monocyte

in peripheral blood WBC and sub-parameters of pregnant women who have high-risk for an abnormal fetus. These findings may be useful for increasing the predictive value of prenatal screening test. However, any infection can easily affect peripheral blood WBC count. We believe that the specific parameters of the interaction between maternal immunity and abnormal fetus should be investigated in more detail.

There are some limitations in this study. The results of advanced diagnostic tests (amniocentesis and chorionic villus sampling) of pregnant women with high-risk for trisomy-21 and NTD could not be reached. This prevented us from checking the accuracy of test results of STS. CBC tests usually are not requested from laboratories at the same time with STS test by the clinicians in our hospital. Therefore, although numerous tests were carried out between 2011 to 2015, a limited number of test results were evaluated.

Conclusion
In conclusion, the interaction of maternal immune system with abnormal fetus may change the compositions of peripheral blood WBC and sub-parameters. Some of these changes may be useful in determining the predictive sensitivity of STS test. Further prospective studies are needed to confirm these findings.

Ethical issues
Ethical approval has been obtained from Celal Bayar University, Faculty of Medicine, Ethics Commissions with dedicated number ''14701/2015 20478486-23.''

Conflict of interests
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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