

## Stereological Changes of Human Placenta in Systemic Lupus Erythematosus Compared with Healthy Controls

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Article information	Abstract
<p>Article history: Received: 31 Jul 2012 Accepted: 30 Aug 2012 Available online: 2 May 2013 ZJRMS 2013; 15 (7): 50-54</p> <p>Keywords: placenta stereology systemic lupus erythematosus</p> <p>*Corresponding author at: Department of Biology, Fars Science and research branch of Islamic Azad University, Shiraz, Iran E-mail: nahidnourzaei@yahoo.com</p>	<p><b>Background:</b> Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can cause changes in the placenta. In this study, quantitative changes of placenta were investigated using stereological methods.</p> <p><b>Materials and Methods:</b> In this case-control study, 10 placentas from systemic lupus erythematosus pregnancy (antinuclear antibody&gt;10), and 10 placentas from normal uncomplicated pregnancy were obtained from Imam Ali Hospital. Volume of placentas was estimated using Cavalieri's principle. 3 full-thickness columns of each placenta were taken using systematic uniform random sampling (SURS). After fixation in modified Lillie's solution, they were cut into 5 mm slices. 5-7 sections selected from each slice using SURS and stained by Masson's trichrome. Then stereological analyses were done on 8-10 SURS fields of each section. Placental volume, absolute volume and volume density of chorionic villi, intervillous space, syncytiotrophoblast, fibrin and blood vessels in chorionic villi were estimated in both groups. The Mann Whitney-U test was employed to determine statistically significant differences between the means. Significant level was set at <math>p&lt;0.05</math>.</p> <p><b>Results:</b> Total volume and volume density of fibrin and total volume and volume density of blood vessels significantly increased in SLE group in comparison with control group (<math>p&lt;0.01</math>). Volume density of syncytiotrophoblast increased 50% in SLE group in comparison with control group, this increase was statistically significant (<math>p&lt;0.01</math>).</p> <p><b>Conclusion:</b> Results showed that systemic lupus erythematosus disease can cause significant changes in the structure of placenta that may be influential on the evolution and survival of fetus.</p>

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### Introduction

Placenta functions as an interface between mother and fetus during pregnancy and is essential for the fetal growth and development [1-3]. Human placenta firmly attached to the uterine wall and consists of chorionic villi [4]. Placenta basically functions through chorionic villi [5]. Therefore, any changes in the placental vessels and chorionic villi might impose serious side effects on the developing fetus [1, 2].

Systemic Lupus Erythematosus (SLE) is an autoimmune disease in which the organs, tissues and cells are subject to severe damages caused by immunity mediators, autoantibody productions as well as formation of immunity complexes and their deposition in tissues [6]. In other words, it is a disease of various clinical presentations and its main characteristic is production of antinuclear antibody (ANA) [7]. The disease is ten times more common in women than men [8, 9]. This difference is due to female sex hormones including estrogen and prolactin [10]. The increased secretion of female hormones during pregnancy and its recurrence might endanger the lives of both mother and the fetus [10].

Typical symptoms of the disease include arthritis and fatigue, yet more serious forms of the disease spectrum may lead to nephritis, neurological, anemia and thrombocytopenia [11, 12].

More obstetric complications are reported in women suffering from SLE. Normal pregnancy-related complications include high blood pressure during pregnancy, bleeding before delivery, preeclampsia, preeclampsia with seizures, hemolysis, elevated liver enzymes, low platelet count (HELLP), gestational diabetes, intrauterine growth retardation (IUGR), premature, miscarriage and stillbirth, deep vein thrombosis as well as pulmonary embolism [13-15].

In recent years, microscopic measurements have been increasingly used both in studies and treatments; meanwhile, new methods have been developed [16]. During last two decades, stereology has been considerably improved, so that it is the first choice for obtaining three-dimensional data from two-dimensional images [17].

In histological methods, we always face to two-dimensional sections or their microscopic images.

Relying on mathematical knowledge and statistics, stereology makes it possible to extract three-dimensional data from two-dimensional images [18, 19]. Stereology helps measurement of some parameters including number, size and volume by the images of two-dimensional sections [18-20]. As an advantage, stereology provides the opportunity of generalizing data to entire structure, by proper and systematic sampling.

Due to the prevalence of pregnancy complications and changes in the placental structure in patients suffering from SLE, the stereological changes of placenta in the SLE patients in comparison with healthy controls were investigated.

## Materials and Methods

In this case-control study, 10 placentas from mothers with SLE were obtained immediately after delivery, in Imam Ali Hospital, Zahedan, Iran. The control group included ten healthy mothers with no underlying disease or pregnancy complications who had been age-matched with the patient group. Smokers, addicted mothers as well as mothers with fetal malformations and chronic diseases were excluded from the study. Maternal parameters including age, weight, height, gestational age, number of pregnancies, number of abortions, number of deliveries, socio- economic status and the history of obstetric and gynecologic diseases, and fetal parameters including sex, birth height and weight; and also placental parameters including size and weight were recorded in the information form. All participants completed a written informed consent form.

The placentas were removed by a standard method. The umbilical cord was clamped immediately after the childbirth to ensure the placental blood drainage. The placental weight was measured with a digital scale of 0.01 gram precision, the placental thickness was measured with a digital caliper with 0.01 mm precision in three points and then the mean was applied for the measurement of the placental volume. The placental volume was determined using the Cavalier's point-

counting method. Then, using the systematic uniform random sampling (SURS) three full-thickness columns of tissues from each placenta were selected and fixed in modified Lillie's solution. Then the columns were cut to 5 mm slices and 5-7 SURS selected slices from each column processed by routine histological method and embedded in paraffin Wax. The tissue slices were serially sectioned to 4  $\mu$ m thickness. Then 5-7 SURS selected sections of each slice, stained with the Masson's trichrome technique. In each section, 8-10 SURS selected microscopic fields analyzed using Cavalieri's point counting method. The images of the selected fields transferred to a computer using a digital photomicroscope. Then the stereological grid containing organized points were placed on the tissue sections and the absolute volume and volume density of placental villi, intervillous space, villous surface fibrin, villous core vessels, and syncytiotrophoblast were estimated as previously described [21]. Data analysis was performed using SPSS-18 and comparison between the groups performed using nonparametric statistical test of Mann-Whitney *U* test.  $p < 0.05$  was taken as the significant level.

## Results

Table 1 represents stereological data of SLE patients and the control group. These data indicate that since both groups were matched in terms of age and gestational age, no significant difference could be identified between them. The average placental volume in the lupus group had a 19% reduction compared with the control group, but this difference was not statistically significant ( $p=0.128$ ). The umbilical cord diameter in lupus group was reduced and the reduction was statistically significant ( $p < 0.05$ ) compared to controls. The absolute and relative volume of intervillous space in lupus group was reduced compared to the control group, but this difference was not statistically significant ( $p=0.131$ ). The absolute and relative volume of chorionic villi in lupus group was increased compared to the control group but it was not statistically significant ( $p=0.275$ ).

**Table1.** Comparison of stereological parameters of placenta in patients with systematic lupus erythematosus (SLE) compared with the control group (C)

Groups		SLE	Control	Percentile differences (%)	<i>p</i> -Value
Stereological Parameters					
Placental weight (g)		604 $\pm$ 33.11	550 $\pm$ 42.16	9.9	NS
Placental volume (cm <sup>3</sup> )		395 $\pm$ 48.20	489 $\pm$ 30.76	-19.22	NS
Cord diameter (mm)		8.3 $\pm$ 0.77	11.4 $\pm$ 0.84	-27.2	0.015*
Intervillous space	Total volume (mm <sup>3</sup> )	134 $\pm$ 15.24	165 $\pm$ 12.35	-18.9	NS
	Volume density (%)	33.9 $\pm$ 1.11	36.2 $\pm$ 1.72	-6.35	NS
Chorionic Villi	Total volume (mm <sup>3</sup> )	261.6 $\pm$ 34.43	193.8 $\pm$ 23.75	35.23	NS
	Volume density (%)	66.1 $\pm$ 1.11	63.8 $\pm$ 1.72	3.60	NS
Fibrin	Total volume (mm <sup>3</sup> )	3.8 $\pm$ 0.57	32.6 $\pm$ 4.80	5.55	0.0001**
	Volume density (%)	1.08 $\pm$ 0.19	6.8 $\pm$ 0.57	-84.11	0.0001**
Blood vessels	Total volume (mm <sup>3</sup> )	119.8 $\pm$ 13.81	101.4 $\pm$ 11.11	18.14	NS
	Volume density (%)	30.8 $\pm$ 0.98	21.8 $\pm$ 1.42	41.28	0.0001**
Syncytiotrophoblast	Total volume (mm <sup>3</sup> )	94.0 $\pm$ 11.28	99.2 $\pm$ 16.5	-5.23	NS
	Volume density (%)	24.0 $\pm$ 0.76	16.0 $\pm$ 1.12	50	0.0001**

The absolute volume of fibrin in lupus group had a 5% increase compared with the control group and this increase was statistically significant ( $p=0.001$ ). The relative volume of fibrin in lupus group had an 84% reduction compared with the control group and this reduction was statistically significant ( $p=0.001$ ). The absolute volume of blood vessels in lupus group was increased compared with the control group and this increase was not statistically significant. The relative volume of blood vessels in lupus group had a 41% increase compared with the control group and this difference was statistically significant ( $p=0.0001$ ). Also the absolute volume of the syncytiotrophoblast in lupus group was reduced compared with the control group, but the reduction was not statistically significant. The relative volume of syncytiotrophoblast in lupus group had a 50% increase and this increase was statistically significant ( $p=0.0001$ ).

## Discussion

In this research, the volume of blood vessels, syncytiotrophoblast and villous surface fibrin were increased in the placentas of those mothers suffering from lupus, compared with the control group. Although no quantitative and stereological studies have been yet carried out on microscopic parameters of the placenta in SLE patients, extensive stereological studies have been accomplished on other diseases affecting placenta, such as preeclampsia [22], diabetes and gestational diabetes [23, 24], anemia [23, 25] and hypoxia [23].

In this study, no statistically significant difference was identified between the placental villi volume and intervillous spaces. In line with our study Mayhew studied the placentas of preeclampsia patients (PE) by the stereological method and found no significant difference between both groups in terms of placental villi volumes [22]. But the studies of Ducray et al. using an image analysis method, on the PE patients' placentas indicated that there was an increase of the placental villi volume and also significant reduction of intervillous space volume [26].

Saga et al. studied the effects of height on the morphology of human placenta, the terminal villi and the syncytial knots on 40 pregnant women at about 1800 feet above sea level. They indicated that the terminal villi were reduced, but the intervillous space was increased [27]. The diversity of the results with our study could be due to their living at high altitudes which resulted in more severe hypoxia compared with the conditions of hypoxia in present study. Hypoxia probably causes structural changes in placenta and could influence the placental transportation and hemodynamic processes. The placental weight showed no significant difference between two groups of our study. Hong et al., in their pathological studies in conducted on the placenta of 18 SLE patients and 23 healthy mothers, reported that the average placental weight and placental villi was significantly reduced compared with the control group.

They attributed this to high concentration of plasminogen activator inhibitor (PAI-2) and low concentration of placental thrombomodulin which would lead to pre-thrombotic state and the excess deposition of fibrin in placental villi [28].

Magid et al. carried out some studies on the placenta of SLE patients who had antiphospholipid (APL) and reported the placental pathological changes of the SLE patients to be shown as the placental weight loss, placental severe damages and thrombosis phenomenon [29]. Surita et al. studied on about 76 autoimmune patients in Brazil, 67 of them were SLE patients. They showed that the placental weight in patients with kidney problems was lower than the others. They argued that the existence of C3, IgM, IgA and IgG on the villous wall surface of capillaries could damage the placenta [30].

The volume of blood vessels in SLE group was increased compared with the control group which might be the result of the increased need for blood and compensatory response to multiple thromboses and also in response to the decrease in perfusion caused by the tissue ischemia.

On the other hand, in researches of Maly et al. on preeclampsia patients (PE), the blood vessels volume was found to be about 1.6 less than the control group. They applied a descriptive optical microscopic method to achieve the results which had less accuracy than the stereological method [31].

In those studies, the syncytiotrophoblast was increased compared with the SLE group. Levy et al. studied on the placental pathology of antiphospholipid syndrome patients in Brazilians and showed that the syncytial cells had been increased [32].

In the present study, the volume of villous surface fibrin was significantly increased in SLE group compared with the control group. Kanfer had previously achieved the same results in a quantitative study by histomorphometric analysis on PE patients' placenta [33]. Preeclampsia is a frequent complication of pregnancy in SLE, occurring in approximately 13 percent of patients [34]. Immunohistochemical studies by Costa et al. showed that the villous surface fibrin had been increased in SLE and PE groups [35]. The results of the previous studies indicated that SLE imposes some changes to microscopic and macroscopic parameters in placenta such as thrombosis, vascular problems, fibrin increase and the blood clotting phenomenon, which increased the risk of spontaneous abortion and fetal death, due to the placental infarction and decrease of perfusion to the fetus [36].

According to the results of this study, it can be concluded that SLE disease imposes some changes in placental structures including increase in blood vessels volume, syncytiotrophoblast volume and the villous surface fibrin volume, all of which might eventually lead to the placental malfunction and subsequently fetal abortion.

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### Authors' Contributions

Z Heidari and H Mahmoudzadeh-Sagheb conceived and co-designed the study, supervised all the experimental design, analyzed the results, and drafted the manuscript.

Z Zakeri contributed in study design, supervised the sample collection of SLE patients and healthy controls and participated in the interpretation of the results. N Nourzaei contributed in literature review, sample collection, histological procedure and drafted the manuscript. All authors read, modified and approved the final version of the manuscript.

### Conflict of Interest

The authors declare no conflict of interest.

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