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Evaluation the Expression of Endoglin (CD105) & IL-3 Level of Pregnancy Women with Antiphospholipid Syndrome

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Abstract

This study designed to describe the histopathology changes of human placenta for pregnancies women with APA and normal pregnancies, also to estimate placental Immunohistochemical staining (IHC) expression patterns of endoglin in pregnancies with APA compared to normal pregnancies and quantify IL-3 serum level for both groups by using ELISA test. The histopathology results showed that arteries with degenerated media were associated with inflammatory cells and areas of hemorrhage were associated with moderate placentitis with inflammatory cells and fibrosis; also congested capillaries and blood vessels. The immunostaining results showed decrease in expression of endoglin in PAS pregnancy compared than healthy pregnancy with significant difference (43.3% versus 75 % ; $p \leq 0.05$) . Some arteries with degeneration of sub region with hyalinization and congestion. The evaluation of the serum levels of IL-3 were showed the elevated level in healthy pregnancy (599.15 ±SE68.59 *pg/ml*) compared to APS pregnancy (264.96± SE 38.28 *pg/ml*) with significant difference ($P \leq 0.05$)**.**

Key words: CD105/Endoglin, APS.

الخلاصة

صممت الدراسة لوصف التغيرات النسيجية في مشيمة الإنسان للنساء الحوامل الموجبة لاختبار الفسفور الشحمي ونساء حوامل صحيحات وكذلك تقدير التعبير المناعي للمعلم CD105 وتقدير مستوى تركيز الانترلوكين -٣ في أمصال مرضى متلازمة الفسفور ألشحمي والحوامل الصحيحات كعينات سيطرة ، استخدم التصبيغ المناعي لتقدير مستوى التعبير المناعي الكيمونسجي لمعلم الـ CD105 و استخدم اختبار الاليزا المباشر لتقدير مستوى تركيز البروتين في ألامصال . وقد بينت التغيرات النسيجية وجود بيئة من الشرايين المنحلة مرتبطة مع الخلايا الالتهابية ومساحات نزفية مرتبطة مع التهاب المشيمة المعتدل مع تليف وخلايا التهابية ، كما أوجدت الدراسة وجدت الدراسة أن مستوى التعبير المناعي الكيمونسجي لمعلم الـ +CD105 كان مرتفع نسبيا مع فرق معنوي احصائي في مرضى متلازمة الفسفور ألشحمي مقارنة مع النساء الأصحاء ($p \leq 0.05$; 75% versus 34.3%). أن مستوى تركيز الانترلوكين -٣ في المصل قليل إحصائيا في مرضى متلازمة الفسفور ألشحمي مقارنة بالنساء الأصحاء وكانت القيمة الإحصائية ($P< 0.05$) بين المجاميع.

Introduction

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies in the maternal circulation. These antibodies are associated with arterial and venous thromboses and with adverse obstetric outcomes such as recurrent fetal loss [1].Mechanisms suggested to explain APS include thrombosis, vascular and endothelial inflammation, and an imbalance of angiogenic and antiangiogenic placental factors such as endoglin and soluble fms-like tyrosine kinase 1 (sflt-1) [2, 3]. In APS, anti-endothelial cells antibodies lead to endothelial cell injury and apoptosis. The underlying pathophysiology of this disease suggests an imbalance of an angiogenic substances associated with endothelial dysfunction [4,5].CD105/Endoglin is a Type I membrane glycoprotein located on cell surfaces and is part of the TGF beta receptor complex. This protein has been found on endothelial cells, activated macrophages, fibroblasts, and smooth-muscle cells. Endoglin is highly expressed on cellular membranes of the vascular

endothelium and on the syncytiotrophoblast [6-9]. It is involved in angiogenesis and has a major role in maintaining vascular tone [10-11]. The placenta is an organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply. "True" placentas are a defining characteristic of eutherian or "placental" mammals, but are also found in some snakes and lizards with varying levels of development up to mammalian levels [12]. Advances in immunology, the study of the body's defense systems, enable us to understand how during pregnancy the mother's immune system is altered so that the fetus is not rejected by her body and allows the fetus to grow. The immune system is comprised of white blood cells, also known as leukocytes, which make a variety of antibodies. Some of the antibodies protect us and others are harmful to our bodies. Some of the immune issues that are important to the reproductive system are: Antiphospholipid antibodies, antinuclear antibodies, natural killer cells and cytotoxic B-cells (autoimmune), blocking (protective) antibodies (alloimmune).When the immune system is the cause of miscarriage, the chances of mother having a successful pregnancy without treatment after three miscarriages is 30%, after four miscarriages 25%, and after 5 miscarriages 5%,with proper treatment ,overall success is 80%, phospholipid molecules are normal components of all cell membranes. Some also have glue like properties and allow cells to fuse. Antibodies to Phospholipid molecules can, therefore, cause problems. Specifically, they can damage the inside of the blood vessel wall. This allows blood cells to stick to the site of the injury and cause blood clots (Figure- 1) [13]. The formation of the normal placenta involves the fusion of small cells called cytotrophoblast into giant cells known as syncytiotrophoblasts. The syncytiotrophoblasts play a key role in the regulation of nutrients going to the baby. With each pregnancy loss, there is a 10% chance that the mother will develop an antibody to a phospholipid molecule. Most women with antiphospholipid antibodies are not sick. However, some have underlying autoimmune tendencies and should be appropriately evaluated. Women with underlying autoimmune diseases may have antiphospholipid antibodies even before they ever become pregnant [13] .Many cytokines from the colony stimulating factor family, like GM-CSF and IL-3, were shown to be positive factors for implantation and to promote placental development and fetal growth [14,15] . IL-3 was shown to play a role in the regulation of placental growth. The proliferation of mouse unfractionated placental cells was stimulated *in vitro* by the addition of IL-3 [15,16] . The placenta by itself is capable of synthesizing IL-3 as well as G-CSF, M-CSF, and GM-CSF, and thus by an autocrine loop to regulate its own expansion [17] .These characteristics of IL-3 may explain its ability to induce fetal development in the APLS-induced mice. Moreover, IL-3 was shown to stimulate the activity of the urokinase-type plasminogen activator of murine bone marrow derived macrophages and peritoneal macrophages following administration *in vivo* [18].

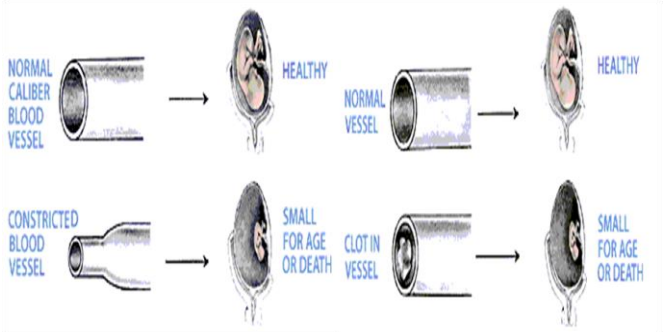

Figure 1 : Affect Antiphospholipid antibodies and cause clot in vessels, Constricted blood vessels impair blood supply to the fetus and placenta resulting in complete fetal demise or growth retardation [13].

Subject, Material and Methods

Thirty patients of women with recurrent spontaneous abortions positive for primary antiphospholipid antibody , and 20 normal pregnancy cases as control with an age ranged from 20 to 40 years, were included in this study, the patient samples (Serum & placenta) were collected from Al-Hussain Teaching Hospital and privat laboratories in Thi-Qar during the period from January to September 2015. The diagnosis was made by the consultant medical staff, which was based on a history inspection, clinical examination and evaluation of sex hormone profiles. The patients women with a history of three or more pregnancy loss, and this loss occur in first and/or second trimester pregnancy loss (< 22 week of gestation) . For each case, one representative section was stained with Hematoxylin and Eosinand the histopathological diagnosis was revised, while other sections were put on positive charged slides and stained immunohistochemically for endoglin (CD105). Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from

Novocastra, Newcastle, UK, RE7150-K , the slides were deparaffinized, rehydrated then blocked. All of the slides were treated with anti-CD105 monoclonal antibody, dilution1:100 (Dako, Denemark), then incubated with a post primary block solution for 30 minutes. In the next step the slides were rinsed gently in PBS 2× 5 and tissue sections incubeted with a secondary antibody Novolink TMpolymer mouse and rabbit immunoglobulins) for 30 minutes, washed in PBS 2× 5 with gentle rocking. After washing, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin. Slides washed, dehydrated then mounting, and examining under light microscope at 10X,20X,40X magnification .The blood samples of 4-5 ml collected by venipuncture, using plastic disposable 5ml syringes, from all patients and control groups. Blood samples allowed to clot at room temperature ,then centrifuge for 15 minutes at approximately 500 rpm to obtain of unhemolyzed cell-free serum. Serum samples stored aliquots at -20 ºC until use for the measurement of IL-3 levels [11]. IL-3 concentrations were quantitatively determined in sera of patients and healthy control subjects by means of ELISA (Enzyme Linked Immunosorbent Assay) using ready kits manufactured by USBiological company (USA) .

Result and Discussion

Histopathological aspect

Most the histopathological cross sections of human placenta with APA showed that arteries with degenerated media were associated with inflammatory cells and areas of hemorrhage were associated with moderate placentitis with inflammatory cells and fibrosis; also congested capillaries and blood vessels. Some arteries with degeneration of sub region with hyalinization and congestion . Prominent langerhans giant cells also blood vessels with hyper plastic thickened media with nearly occluded lumen .Others with thickened media associated with inflammatory cells and reduced lumen degeneration and hyalinization (Figures 2,3,4). All this compared with normal placenta (Figure 5).The results agreed with Stone *et al.* (2006) [19] .In pregnancy, circulating APAs are associated with histopathologic changes in the placenta that reflect decreased uteroplacental perfusion (which include villous infarction, decidual vasculopathy, decidual vascular thrombosis) and "accelerated" villous maturity the resulte and others [19,20] .Although traditional experimental models have emphasized the role of thrombosis in placental tissue, Some studies demonstrate that the trophoblastic basement membrane is a particular target for APAs, which suggests that these antibodies may be directed specifically at the placenta. These and other pro-inflammatory factors may also contribute to the characteristic pathologic changes that are observed in the placentas from these patients [19,21] .

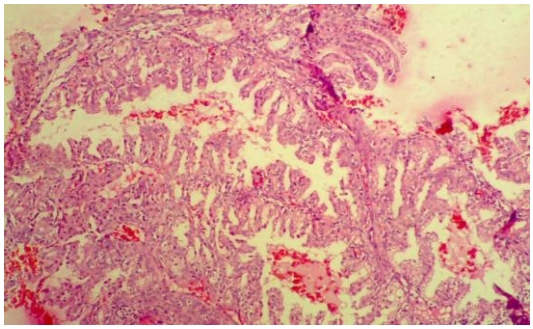

Figure (2): Histopathological cross section of human placenta with antiphospholipid antibodies (H&E stain 10×) .

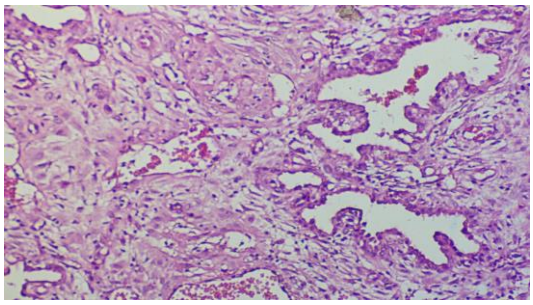

Figure3 : Histopathological cross section of human placenta with APA (H&E stain 40×).

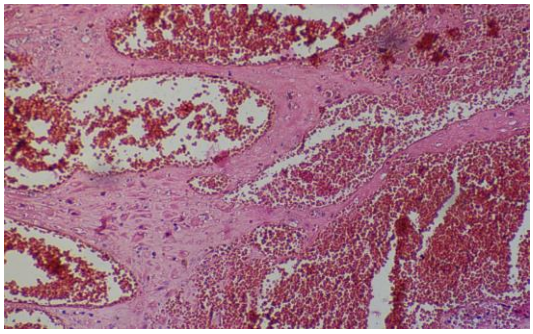

Figure 4 : Histopathological cross section of human placenta with APA (H&E stain 40 X)

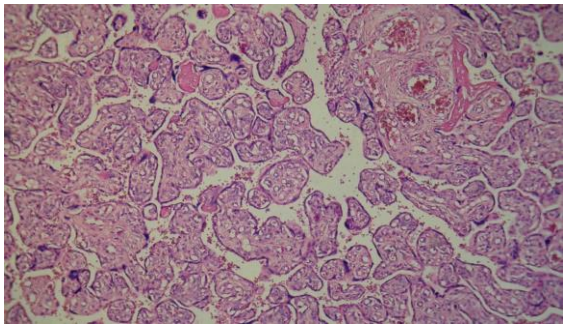

Figure 5 : cross section of normal human placenta (H&E stain 10x)

Immunochemistry: we evaluated the expression of endoglin (CD105) in placenta of women with PAS and healthy pregnancy, the results showed decrease in immunohistochemical expression of endoglin in PAS pregnancy compared than healthy pregnancy with significant difference (43.3% versus 75 % ; $p \leq 0.05$) (Figure 6) (Table 1) .Impaired endometrial differentiation in decidual phenotype as well as endometrial angiogenesis inhibition by aPL has also been advocated [22] . Only if an adequate blood supply is provided by the maternal blood vessels, normal placental growth and development will continue. In case of thrombophilia related fetal loss, where there is a compromised blood supply, it will not be surprising that the placenta exhibits significant pathological changes [23]. It is known that endoglin plays a key role in the vascular system development and morphogenesis [24]. In our study infiltration of (CD105+) in healthy pregnancy were the most abundant in Erythroid precursors , Syncytiotrophoblast and Vascular smooth muscle cells, these results compatible with Shchyogolev et al. (2012) who found that the intensity of endoglin and VEGFR1 expression was maximum in the syncytiotrophoblast and extravillous cytotrophoblast cells in severe pre-eclampsia [25].

Table (1): CD105 expression in placenta groups

Study groups		CD105 EXPRESSION		Total	Chi-square	P value
		positive	negative			
APS	No	13	17	30	4.884	* 0.02711
	Percentage%	43.3	56.7	100		
HEALTHY	No	15	5	20		
	Percentage%	75	25	100		
Total	No	28	22	50		
	Percentage%	56	44	100		

* Significant at ($P \leq 0.05$)

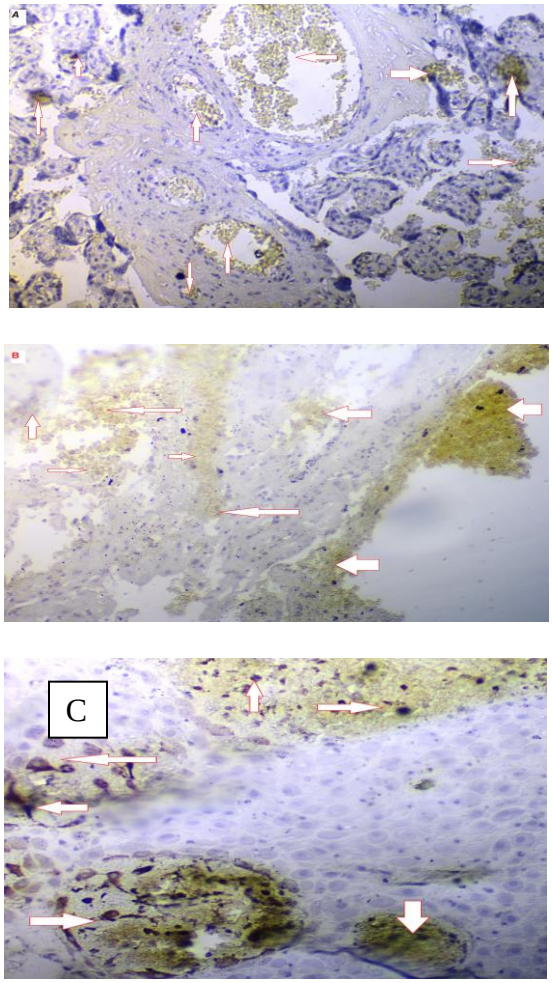

Figure (6:A,B): **A**: Immunohistochemical tissue expression of CD105 in healthy pregnancy showing positive expression of Cd105 , B&C : showing positive expression of CD105 in APS pregnancy .

Sera Levels of Interleukin 3 (IL-3)

There was an elevated mean serum level of IL-3in sera of healthy pregnancy (599.15 ±SE68.59 pg/ml) Table (2), when compared to sera IL-3 levels of APS pregnancy (264.96± SE 38.28 pg/ml), with a statistical significance($P \leq 0.05$). IL-3 may be effective in prevention of recurrent fetal loss in APLS. This was in agreement with Chaouat et al. who found that treatment with IL-3 prevents abortions [15] This results agreed with the fact that IL-3 were shown to be positive factors for implantation and to promote placental development and fetal growth [14,15].

Table 2: Sera levels (mean ± S.E.) of IL-3 in APS patient and control.

IL-3 Mean Serum Level ± S.E. (pg/ml)		
Groups	**Total No. 50**	**Mean ± S. E.**
Control	20	599.15 ± 68.59
APA patient	30	264.96 ± 38.28

The primary pathology observed thrombosis. Some of the other pathological features observed were placental infarction, decidual vessel thrombosis, chronic villitis and excessiveperivillous fibrin deposition. Multifocal uteroplacental thrombosis was also a significant feature in patients with thrombophilia. Many physiological changes occur in the uterine wall during pregnancy in order to allow the foetus to grow. These include dilation and enormous growth of the spiral arteries, destruction of the spiral artery endothelium and decidual invasion by trophoblast.

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