High Expression of P53 protein in Recurrent Pregnancy Loss Could Play a Role in the Pathology!!

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Summary:
Background: Recent data provide evidence that p53 plays a critical role in mediating pregnancy by regulating steroid hormone activation
Objective: localization of semi quantitation tof por protein at the materno-fetal interface, in patients with recurrent pregnancy loss.
Methods: Immunohistochemistry analysis of p53 protein using paraffin embedded sections of curate samples obtained from 40 women, who where divided into three groups: 24 women with RPL, 10 women with abortion for the first time, and 6 women with induced abortion.
Results: The mean value of the expression of p53 protein in the RPL group was (65.8±2.16), which is significantly higher than that of the second group (48.0±2.81), and the third group (50.0±4.66), (p=0.000).
Conclusion: High expression of p53 protein in women with RPL may have a role in accelerating placental apoptosis leading to failure of pregnancy.

Key wards: p53, recurrent pregnancy loss (RPL).

Introduction:
The p53 tumor suppressor gene is a well-known factor regulating apoptosis in a wide variety of cells and tissues. Alterations in the p53 gene are among the most common genetic changes in human cancers. In addition, recent data provide evidence that p53 plays a critical role in mediating pregnancy by regulating steroid hormone activation. In recurrent pregnancy loss (RPL), causes and associations are much debated as the exact pathophysiological mechanisms are unknown. (1,2) Recurrent miscarriages, defined as at least three consecutive miscarriages, are an etiological enigma (3). Presumed etiological factors include endocrine dysfunction such as hypothyroidism and luteal phase inadequacy (4,5), genetic (6), uterine anatomical abnormalities (7), and infectious disorders. In about 50% of the cases, there is no apparent cause (8). However, immunological factors are thought to account for many of these unexplained miscarriages (9). In cases of idiopathic recurrent miscarriage, gene polymorphisms have been proposed as susceptibility factors, increasing the chances of miscarriage in otherwise healthy women (10,11). Pregnancy is exquisitely dependent upon signals exchanged between these cells (12). Abnormalities of placental vasculature may result in a number of gestational pathologies that could lead to pregnancy loss (12,13), intrauterine fetal death (IUD), intrauterine growth restriction (IUGR), placental abruption, and pre-eclampsia (14). The p53 tumor suppressor gene encodes a multifunctional transcription factor that is activated by stressful stimuli, including DNA damage and hypoxia. It is a well-known factor regulating apoptosis in a wide variety of cells and alterations in p53 are among the most common genetic changes in human cancers (1). However, beside its role as a tumor suppressor gene, p53 plays a critical role in regulating angiogenesis (15,16). Alterations in the p53 gene products have been shown to be a potent inducer of angiogenesis via the Hypoxia inducible factor 1 (Hif1) and vascular endothelial growth factor (VEGF) pathways (17). In this study we tried to find out any association between the expression of P53 protein in women with RPL in situ (at the materno-fetal interface), trying to explain the underlying pathological mechanisms in this disorder.

Patients, materials and methods:
This study was conducted in April 2007. Patients were collected from Al-Kadhmiya and Al-Ulwiya teaching hospitals, and then divided into three groups; Group A: comprised 24 pregnant ladies presented with abortion during the first trimester, all of whom gave a history of previous 3-6 consecutive first trimester abortions. In
all of them there were no apparent medical diseases, nor family history of genetic diseases or uterine anatomical anomaly. Also all of them were confirmed by laboratory tests to be negative for acute infection with rubella, CMV and toxoplasmosis. Group B: comprised 10 pregnant ladies presented with abortion during the first trimester and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness, and Group C: consisted of 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indications under approved consent of two senior gynecologists and a physician (as control group). Curate samples of the materno-fetal interface were taken from all these women at the end of evacuation curate operation then embedded in paraffin and confirmed by a pathologist, and then subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark).

Immunohistochemistry procedure: the IHC procedure was performed according to the manufacturer instructions. For more details refer to the immunohistochemistry procedure in reference (12).

Evaluation of the immunohistochemistry signal: The expression of P53 was measured by counting the number of positive decidual and trophoblastic cells, which gave a brown cytoplasmic staining under the light microscope (18, 19). The extent of the immunohistochemistry signal in the villi was determined in 10 fields (X100 magnification). In each field the total number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was graded as 3, (75–100%); 2, (25–75%); or 1, (<25%). The total staining score was divided by the number of whole villi per field in 10 fields. These scores (between 1 and 3) were added for each field, and a score between 10 and 30 was gained for each sample (20), and to be simplified as percent, the percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields as advised by Hennessy et al (20). The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers. Negative controls were obtained by omitting the monoclonal antibody and using phosphate buffer saline to verify the signal specificity. Positive control signal was obtained using normal healthy ovarian tissue.

Statistics: ANOVA test was used to determine the differences in the expression of P53 protein among the three groups. Values of p<0.05 were considered as statistically significant.

Table 1. The expression of P53 protein among the studied groups

<table>
<thead>
<tr>
<th>P53 protein</th>
<th>n</th>
<th>Mean ± S.E.</th>
<th>Min. Value %</th>
<th>Max. Value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>24</td>
<td>65.8±2.16</td>
<td>50</td>
<td>85</td>
</tr>
<tr>
<td>Group B</td>
<td>10</td>
<td>48.0±2.81</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Group C</td>
<td>6</td>
<td>50.0±4.66</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>59±2.08</td>
<td>40</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 2. The significance of differences in the expression of P53 protein in between the groups

<table>
<thead>
<tr>
<th>P53 protein</th>
<th>Among the groups</th>
<th>Between group A and B</th>
<th>Between group A and C</th>
<th>Between group B and C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P Value</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.755</td>
</tr>
</tbody>
</table>

P53 protein expression showed heterogeneous brown cytoplasmic staining involving the trophoblasts, both cyto- and syncytiotrophoblasts in the three groups of women but it was more significant and obvious in the recurrent loss group (Fig1).

Discussion:

Our findings of an increase in the expression of P53 protein in women with RPL are in agreement with other recent studies showing important regulatory effect of P53 protein on placental function and over-expression of p53 might cause a pathological effect.
and failure of the gestation (1, 18, and 21). This finding might be explained by the facts that fetal growth and development depend on intact placentation function. Maintenance of placentation structure and differentiation is essential for the provision of adequate gas, nutrient and waste exchange between the fetus and its mother. Placental trophoblast and placentation reorganization are ongoing processes during pregnancy. Therefore, apoptosis and cell proliferation is frequently observed during pregnancy in blood vessel cells, and trophoblasts of the placenta. Imbalances in these highly regulated processes of tissue or cell differentiation caused by an increased number of cells arrested at the G1 checkpoint, might to some extent cause inadequate supply of nutrients, gases or waste exchange between mother and fetus, leading to preterm abortion (1,12,13). A second possible explanation for the observed increase of p53 in RPL women might be their higher potential for apoptosis. Because placentation development is a dynamic process of cell proliferation and cell degradation, the high level of apoptosis in these patients might lead to misguided growth of cells or tissues determined to be degraded by intrinsically apoptotic stimuli (1,22,23). To have more support of our finding, recently, it was shown that p53 is a potential mediator of pregnancy by estrogen and progesterone activation (24). Dysfunction of p53 may lead to accumulation of cytoplasmic p53 which in turn may lead to immunity to abnormally expressed p53 as revealed by autoantibodies in the blood. Alterations in the p53 gene product may be caused by polymorphic sites within the gene. It was hypothesized that the polymorphism in the p53 gene is associated with RPL by influencing p53-mediated function during pregnancy (1). In addition, p53 protein expression was found to be significantly associated with the expression of Bc12 (the antiapoptotic proto-oncogen) (unpublished data). Over-expression of Bc12 suppresses apoptosis by preventing the activation of caspasas that carry out the process (25). While P53 is well known as a tumor suppressor, it is also a transcription factor that induces apoptosis mainly through inducing the expression of a batch of redox-related genes (22) and the down-regulating Bel-2(23). This might explain the over expression of p53 in women with RPL to counteract the anti-apoptotic effect of Bc12, and to increase placental apoptosis in a pathological manner.

Conclusion:
Highly expressed apoptotic P53 protein in RPL could indicate abnormality in the programmed death of the placental cells in these patients and subsequently placental dysfunction leading to failure of gestation.

References:
16- Yuan A, Yu CJ, Luh KT et al. Aberrant p53 expression correlates with expression of vascular...
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