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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF PRODUCT OF CONCEPTION USING P53 AND Ki67 AMONG WOMEN IN BAYELSA STATE SOUTH-SOUTH NIGERIA

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ABSTRACT

Background; Products of conception, abbreviated poc, is medical term used for tissue derived from the union of an egg and a sperm. It encompasses an embryonic gestation (blight ovum) which does not have a viable embryo. The prognostic indicators based on currently available clinical and histopathological variables such as villous size, outlines, stroma and hydrops etc. However, these indicators are still inadequate. **Objectives;** To characterize the various moles (complete and incomplete moles) using various genes expressed and to determine the extend of percentage expression of the individual genes. **Materials and Method;** A total of fifty (50) slides of product conception tissues were collected of different age's and ethnicity, the histological characterization and morphological evaluation were defined using the routine (Haematoxglin and Eosin) staining method into 20 complete moles and 30 incomplete moles. Immunostaining was done for P53 and ki67 using the manufacturer's antibodies. **Result;** The result are presented in photomicrograph and tables. the expression of p53 gene in product of conception. A total of 50 cases are used for the study 20 (66.7%) complete moles with 9 (18.0%) positive expression and 11 (22.0%) negative expression. 30 (60.0%) incomplete moles with 5 (10.0%) positive and 25 (50.0%) negative expression the expression of ki67 gene in product of conception 20 (66.7%) complete moles with 0 (0.0%) positive and 20 (66.7%) negative expression,30 (60.0%) incomplete moles with 0 (0.0%) positive expression and 30 (60%) negative expression. **Conclusion;** This study shows that based on the percentage of expression, p53 gene has a good sensitivity and with good prognosis as oppose to ki67. Therefore P53 immunohistochemical stain should be incorporated together with haematoxylin and eosin for differentiation of complete and incomplete moles.

KEYWORDS: p53, ki67, immunohistochemical, product of conception, complete moles, incomplete moles.

INTRODUCTION

Products of conception, abbreviated poc, is medical term used for tissue derived from the union of an egg and a sperm. It encompasses an embryonic gestation (blight ovum) which does not have a viable embryo. The presence of product of conception excludes an ectopic pregnancy. Also retained products of conception has to do with products of conception remains in the uterus after child birth, medical abortion or miscarriage (Medilexicon, 2014). Various studies indicates that microscopic examination of product of conception is common task of many surgical pathologist. It is well known that on some occasions, it is difficult to distinguished degenerative changes in non-molar placenta (so called) "hydropic abortion") from partial hydatiform mole. Howat *et al.,* (1993), Attested to the fact that found 5 to 7 pathologist could reach diagnostic agreement in only 70% (35 to 50) of hydropic placenta when employing histologic criteria alone, underscoring the imperfect nature of the histologic criteria employed for distinguishing these entities. Particularly difficulties were encountered in the identification of partial mole. Another study by Takahashi *et al.,* 1990, employing restriction fragment linked polymorphism analysis discovered that 20% of 10 hydropic placenta specimens were misclassified using histopatholgic criteria. Because of differences in DNA ploidy analysis by flow cytomentry can assist in the accurate classification of these specimens, although those techniques may not be readily available in some location. In 1996 schammel

and Bocklage investigated the utility of p53, PCNA, and Ki67 immunostains in distinguishing hydropic molar from nonmolar placentas.

P53 has been described as 'the guardian of the genome' because of its role in conserving stability by preventing genome mutation. Hence Tp53 is classified as tumor suppressor gene (Kern *et al.,* 1999).The ki-67 protein (also known as MKi67) is also a cellular marker for proliferation (Seckl *et al.,* 2000). It is strictly associated with cell proliferation. Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of ki-67 positive tumor cells (the ki-67 labeling index) often correlated with the clinical course of cancer.Thus the study tends to explore the characterization of P53 and ki-67 immunostains in genes expressed in product of conception.

MATERIALS AND METHODOLOGY

Study Area

The study was performed at Niger Delta University Teaching Hospital Okolobiri Bayelsa State. South South Nigeria The hospital serves as a referral centre in Bayelsa State and equally serves as a training institution for medical and allied medical science students of the state own university. Bayelsa State is located within lat. 4.15IN and lat. 5.23 south and long. 5.221 and 6.51 East of the equator, bounded by the Atlantic ocean by the south of Nigeria. Bayelsa state has the highest collection of the Ijaw tribes in Nigeria. The state is a second largest producer of crude oil in Nigeria and has the largest gas reserve and oil well. Her major occupation is fishing and civil service.

Ethical Clearance

Ethical cleareance was sought and obtained from the ethics committee of Niger Delta University Teaching Hospital Okolobiri Bayelsa State**.**

Sample Collection

Paraffin embedded tissue blocks were collected from the Department of Histopathology, under the supervision of the laboratory scientist in charge at the Niger Delta University Teaching Hospital Okolobiri from January 2011 to December 2015 was used for this work.

Sectioning Of Block

The blocks are sectioned, using a microtone to section the tissues into 3-5 microns that is suitable for preparing a slide out of it.

Slide Preparation

The tissues are prepared on a slide by adhering the tissues to the slide properly by picking it from the hot water bath.

Sample Size

A total of fifty (50) slide of product conception tissues were collected of different age and ethnicity.

Methodology

The histological characterization and morphological evaluation were defined using the routine (Haematoxglin and Eosin) staining method. Immunostaining was done for P53 and ki67 using the manufacturer's antibodies.
Colour development and background staining was visualized using the 3,3-diaminobenzadine chromogen and haematoxylin counter stain, respectively. Appropriate negative controls for immunostaining were prepared by eliminating the primary antibody step.

The samples for the study were randomly assigned into four groups of individuals and the extend of the percentage of genes of individual gene. The different histochemical stains are used to stain each groups to characterize the various moles and gene expressed.

RESULT

A total of 50 cases of product of conception are used for this study comprising of 20 complete mole and 30 incomplete moles. The results are presented in photomicrograph and tables.

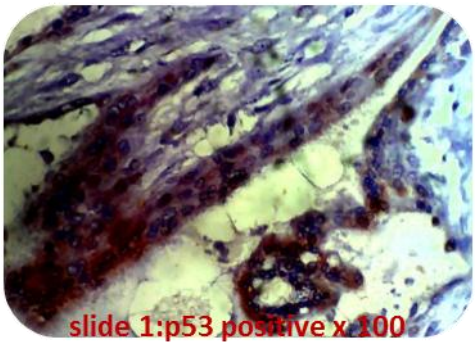
Table 4.1: P53 expression in product of conception.

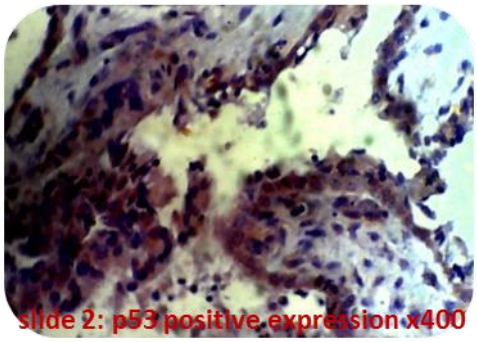
	Number examined	Positive	Negative
1. Complete	20(66.7%)	9(18.0%)	11(22.0%)
2. Incomplete	30(60.0%)	5(10.0%)	25(50.0%)
Total	**50(100.0%)**	**14(28.0%)**	**36(72.0%)**

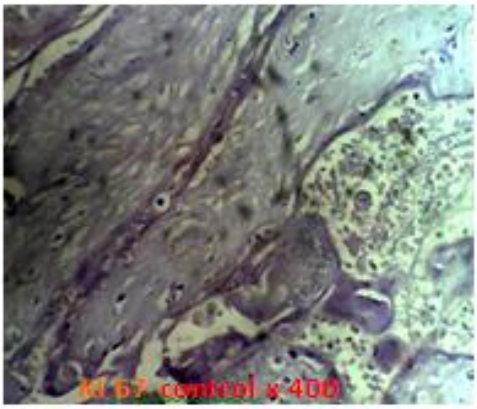
Chi square $x^2 = 0.3125$

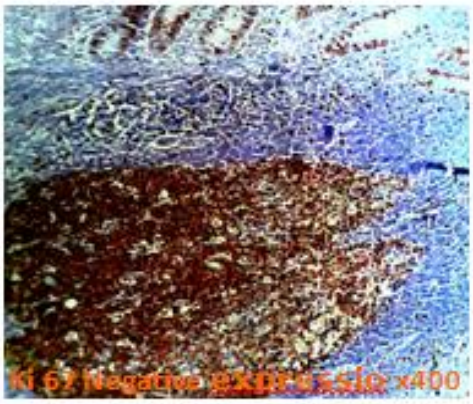
Table 4.2 Ki67 expression in product of conception.

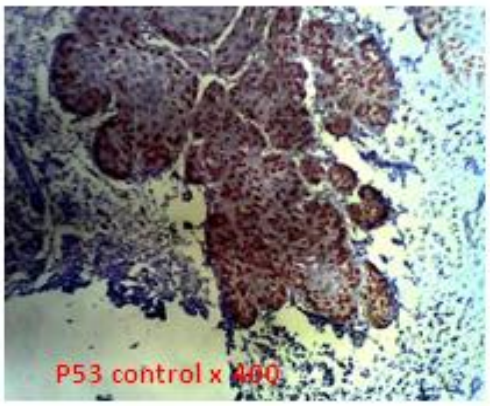
	Number examined	Positive	Negative
1. Complete	20(66.7%)	0(0.0%)	20(66.0%)
2. Incomplete	30(60.0%)	0(0.0%)	30(60.0%)
Total	**50(100.0%)**	**0(0.0%)**	**50(100.0%)**

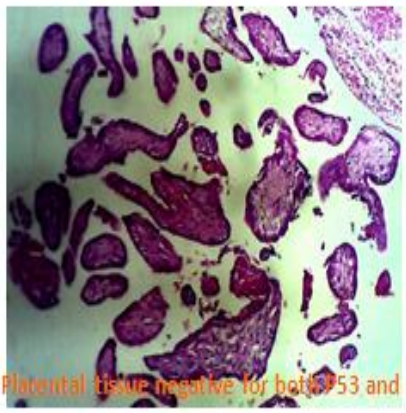

slide 1:p53 positive x 100

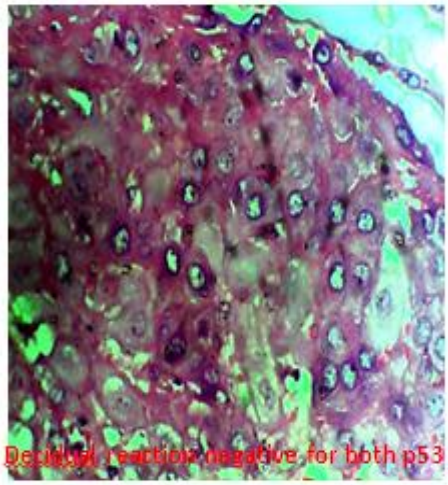

slide 2: p53 positive expression x400


Ki 67 control x 400


Ki 67 negative expression x400


P53 control x 400


Placental tissue negative for both P53 and Ki67


Decidual reaction negative for both p53 and Ki67

DISSCUSSION

This studies is majorly concerned with characterization of genes expressed in product of conception, abortions and miscarriages. The prognostic indicators based on currently available clinical and histopathological variables such as villous size, outlines, stroma and hydrops; cistern formation, stromal karyorrhectic debris, villous vessels, trophoblast pseudo inclusion, trophoblast hyperplasia, extravillous trophoblast, implantation sites also alongside are vagina spotting or bleeding, pain or cramp in the abdomen or lower back, fluid or tissue are also passed from the vagina, subsequent preterm delivery and also a recurrent spontaneous miscarriage, hormonal receptor status are also used to predict a patients clinical outcome in certain situations (Jurkovic *et al*., 2013).

However, these indicators are still inadequate in that within a given patients population with a specific predicted risk of recurrence, there are patients whose actual clinical outcome does not match that predicted by the indicator. Therefore attempts have been made to use molecular profiling to create more accurate prognostic indicators to address these issues of discrepancies (Bagshawe *et al*., 1990: Rice *et al.*, 1990) and thus the important of this study in Niger Delta University Teaching Hospital Okolobiri Bayelsa State.

It has been suggested that gestational trophoblastic disease can be classified according to their gene expression profiles into three main groups: Complete mole, partial mole and chronocarinoma. Most importantly, these groups may have prognostic and predictive implications (Howat *et al.*, 1993).

However, several attempts have been done to classify Gestational trophoblastic disease on the basics of IHC Surrogate and the various subtypes (Bashawa *et al*., 1990).

Consequently in this present study p53 expressed 18.0% for the complete mole and 10% for the incomplete mole thus a total of 28.0% positive expression.

The level of expression (28.0%) when compared statistically using the chi square x^2 gave a value of 0.3125 which is insignificant. From the present study it can be deduced that in product of conception there is mutation of tumor suppression gene (p53) in a minimal pattern. However this minimal pattern of mutation (18.0%) can also be used to differentiate between complete and incomplete mole (10.0%), and this minimal pattern of mutation is more in complete mole.

Although several investigators have studied the expression profile of these two protein together, only Hussein revealed a correlation between the two proteins in complete hydatidiform moles (Hussein *et al.*, 2009). The finding confirms the speculated association between BCL-2 and P53, and suggests that the increase is p53 expression is likely an attempt to prevent excessive trophoblastic proliferation in complete hydatidiform moles, partly through modulation of regulators like BCL-2. However no correlation could be found between the clinical progress for patient with complete hydatidiform moles and the p53 (p= 0.35) or BCL -2 protein) P = 0.25) expression, probably due to the inadequate number of accessible patient follow ups. Hence it can be inferred that although the differential contribute to the pathogenesis of the disease, Neither of the proteins has independent of independent prognostic significance (Hussein *et al.*, 2009).: ki67 is considered a useful marker of cell prodiferation. An increase of the ki67 indicates an increase of cell mitotic activity and cell proliferation (Taylor *et al*., 2003). In a study done by Cheville et al., growth fraction (number of positive cells/total number of cells) of ki67 in cytotrophoblast cells was useful in separating complete mole from partial moles but not partial mole from hydropic abortion. (Cheville *et al.*,1996). In his study growth fraction on stromal cells did not differ among these three entities. These findings correlates with present results that growth fraction of ki67 in syncytotrophoblastic cells in separating partial moles from hydropic abortion but not complete mole from partial moles and the distribution of ki67 in cytotrophoblastic cells was also useful in separating these three entities. Also the expression of ki67 in this present study showed 0.0% positive expression for both the complete and incomplete mole. Suggestive of the fact that from this present study there is no cell proliferation activity in both complete and incomplete mole.

CONCLUSION

This study therefore has suggested that based on the percentage of expression, it can be infered that p53 gene (28.0% positive and 72.0% negative) in 50 cases of complete mole has a good sensitivity and with good prognosis, and ki67 (0.0% positive and 100.0% negative) in 50 cases of incomplete mole of poor sensitivity with a poor prognosis of cases in product of conceptions. Therefore P53 immunohistochemical stain should be incorporated together with haematoxylin and eosin for differentiation of complete and incomplete mole.

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