

Detection of Fungal and Parasitic Infection in the Product of Conception in Khartoum State

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Abstract:

Candida guilliermondii is saprophytic opportunistic pathogenic yeast considered to have low virulence. It is related to local or systemic host immune system, that disease from massive contamination of uterus during gynecological procedures, leading to ascending of infection up through the cervix to placenta then abortion induced. This study aimed to detect the presence of fungi and parasitic infections in the product of conception and abortion, analyzed by blocks of enrolled married women with age ranging between 18 and 45 years. Simple Random sampling diagnosed product of conception. Hundred formalin fixed paraffin blocks (FFPB) were cut by rotary microtome, and then stained by special stain PAS, Crocott hexamine silver and Giemsa for detection of *Candida*. Hospital based study. In Khartoum North Teaching Hospital during period Dec2014 – Aug2015. Characteristics of aborted females, the data collected from record from the lab. The data were analyzed using SPSS program. The abortion increase in number 7% in the women has first time abortion, women has second time abortion 58%, 33% for women that third time abortion and 2% for women has fourth time abortion. The candida was detected in 9 (9%) sample and negative 91(91%) samples. This result showed insignificant statistical association ($P= 0.04$). Conclusion: This

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study concludes that there is association between Candida infection and abortion.

Key words: PAS periodic acid Schiff's reagent, Giemsa stain, Grocott hexamine silver.

INTRODUCTION:

Abortion defines an "abortus" as a fetus or embryo removed or expelled from the uterus during the first half of gestation __ 20 weeks or in the absence of accurate dating criteria , fetuses weighting less than 500g are usually not considered as births ⁽¹⁾ Recently , the ratio of abortion increase due to many factors. In this study we will try to detect some of those factors and to investigate the relation between the type's fungi and parasite and abortion occurrence. Many studies reported the direct relationship between different types of fungi and some parasitic agents like toxoplasma.

Epidemiology the fungi in Sudan cause many diseases, in the analysis the incidence in frozen equine semen show Candida in 57.1% of cases, 42.8 % of Candida incospicua,14.3% Candida guilliermondii that due to transmission by artificial semen from males to females ⁽²⁾ Males are asymptomatic carrier ⁽³⁾ Other study of 53 cases of fetal Candida infection seventeen associated with IUCD in situ associated with delivery in earlier gestational age when compared to cases not associated with an IUCA (23.3+/-4.9 VS 31.6+/-7.0, P) 0.001) 77% of fetal candidal associated with ICUD were systemic (heart , brain ,liver, gastrointestinal gland and lung) compared to 33% of cases not associated with IUCD ⁽⁴⁾.

Recently, the ratio of abortion increase due to many factors. In this study we will try to detect some of those factors and to investigate the relation between the type's fungi and parasite and abortion occurrence.

Causes may be due to genetic, immunocompromise disease, rhesus incompatibility and Infectious disease like bacteria, fungi, and parasite.

Fungi infection:- we have different type of fungi but some is very important.

Candida guilliermondii is saprophytic opportunistic pathogenic yeast considered to have low virulence. It is widely distributed in nature isolated from soil, insect, plant, seawater, saprophyte human, skin and mucosal surface (uncommon causative agent of disease). *Candida albicans* species is non *Candida guilliermondii* virulence ⁽⁵⁾.

It is related to local or systemic host immune system, That disease from massive contamination of uterus during gynecological procedures, leading to ascending of infection up through the cervix to placenta then abortion induced ⁽⁶⁾ .

The fungi from focal origin or skin are source of causing disease of reproductive system. Those are some types of fungi that can identify from urine in culture with *Candida* species and *aspergillums* species. *Candida albicans* is the first pathogen isolated from the reproductive tract ⁽⁷⁾.

In different study, some types of *Candida* spp cause endometritis or abortion like: -*Cryptococcus* and *Aspergillus* was reported ⁽⁸⁾.

In the Arab mare, *Candida guilliermondii* is the most fungi spp that can cause abortion reported, more than other type of fungi.

Sometimes parasite can cause abortion like *trichomonas vaginalis*.

MATERIAL AND METHOD:

This was descriptive cross sectional study – Hospital based study, in Khartoum North Teaching Hospital during period Dec2014 – Aug2015, characteristics of aborted females, the data

collected from record from the lab. Simple Random sampling diagnosed product of conception.

Slides preparation:

One section of 4µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for special stain which taken in frosted end slide and dried in oven in 65 °C.

Laboratory Methodology:

Three sections from each block with thickness 4 µm were be cut by rotary microtome.

We used three types of special stain:

1- PAS (periodic acid Schiff's reagent):-

Sections were dewaxed in xylene, then hydrated through graded alcohols (100%, 90%, 70%) then to tap water, stained the sections with periodic acid for 5 minute, then washed by tap water, then covered by Schiff's reagent for 20 -30 minute, then put the sections in jar under tap water for 5-8 minute, after that stained by Mayer's for 5 minute, then washed, dehydrated through alcohols (70%, 90%, 100%), then blotted by filter paper, cleared the slides, and mounted in D.P.X mounting media.

2-Grocott hexamine silver:-

Sections were dewaxed in xylene, then hydrated through graded alcohols (100% , 90% ,70%) to water then oxidized in 5 % aqueous chromium trioxide (chromic acid) for 1 hour, then washed in tap water, then rinsed in 1% sodium metabisulphide, then washed in tap water for 5 minute then rinsed in distilled water , then placed in preheated (56°C) incubating solution in dark , up to 1 hour , then rinsed well in distill water then fixed in 3% sodium thiosulphate for 5 minute, then covered with

counter stain 15-30 seconds then blotted, dehydrated, cleared and mounted in D .P.X mounting media.

3-Giemsas:-

Sections were dewaxed in xylene, then hydrated through graded alcohols (100%, 90%, 70%) to water, then rinsed in pH 6.8 buffer distilled water, then stained in working Giemsa stain for 4 hours, then rinsed in distilled water, then rinsed in 0.5% aqueous acetic acid until section is pink, then washed in tap water, then blotted until almost dried, dehydrated rapidly through alcohols, cleared in xylene and mounted in D.P.X mounting media.

RESULT INTERPRETATION:

Results obtained were detected by researcher and confirmed by experienced histopathology's.

Negative results: that mean not found of my target but the processing in microtome may be out in trimming.

Positive result: samples containing my target appear by stains.

Data analysis:

All information about the study population was entered a computer as well as obtained results. The data was analyzed using SPSS (Statistical Package of Social Science) software program version and Chi square test were calculated.

RESULTS:-

This study was done in 100 samples in age between (18-45) I was divided age into two groups less than 30 and above 30 years old, high percentage in above 30 and low percentage in less 30 years old.

The abortion increase in number 7% in the women has first time abortion, women has second time abortion 58% , 33% for women that third time abortion and 2% for women has fourth time abortion , no any parasite infection found . That positive 9% of Candida. See the tables below:-

Frequencies:

Table (1) distribution of abortion among population.

Abortion	frequency	percent
1	7	7.0
2	58	58.0
3	33	33.0
4	2	2.0
Total	100	100.0

Table (2)distribution of PAS stain among study samples

PAS	Frequency	Percent
negative	93	93.3
positive	7	7.0
total	100	100.0

Table (3) distribution of Giemsa stain among study samples.

Giemsa	frequency	Percent
negative	98	98.0
Positive	2	2.0
Total	100	100.0

Table (4) distribution of sliver stain among study samples.

GHS	Frequency	percent
negative	99	99.0
positive	1	1.0
Total	100	100.0

Table number (5) final result percentage of 3 types of stain with Candida

Result	Frequency	Percent
negative	91	91.0
positive	9	9
Total	100	100.0

Table (6) Cross tabulation demonstration the distribution of all stain (PAS, Silver. Giemsa) detection of Candida among aborted women

Total	All result	
	Positive	negative
100	93	7
33.3%	31.0%	2.3%
100	98	2
33.3%	32.7%	0.7%
100	99	1
33.3%	33.0%	0.3%
300	290	10
100.0%	96.7%	3.3%

P value equal .040

DISCUSSION:

Candidacies detection rate is still low in Sudan and the technical issues is the most important factor, since the yeast cannot be diagnosed by routine stain in histopathology lab. It found in high rate in cold country due to humidity. In this study the age of the study population ranged between 18 to 45 years with mean age of 40 years.

The study results showed , there is marked increase in abortion cases among females of age group more than 30, the majority of cases diagnosed in age over 30, that may be due to different genetic and environmental factors especially pathogens and genetic factors.

Concerning this study I found Candida 2% of 100 sample. Candida- associated abortion occurs rarely but is often associated with the presence of an intra-utrine conception device (IUCD) that may ascend infection from contaminated external genitalia. ⁽⁹⁾.

Abortion percentage of known pregnancies was 21% worldwide, with 26% in developed countries and 20% in developing countries ⁽¹⁰⁾.

Abortion rate may be expressed as the average number of abortion women has during her reproductive years this was referred to as total abortion rate (TAR). Abortion rate depends

on stage of pregnancy and method practice. In 2003; the centers for disease control and prevention (CDC) in the U.S, reported that percentage of less than 6 weeks gestation abortion is (18%) at 7th week, (15%) at 8th week, (18%) at 9-10th week, (9.7%) at 11th week, (4,11%) at 16-20th week, (1.4%) more than 21th week⁽¹¹⁾.

I used three types of stain Periodic acid Schiff's reagent PAS, Giemsa stain and Grocott's hexamine silver stain .PAS demonstrated only living and GHS for both living and dead fungi. Giemsa stain available in all the department of the lab and easy to use in different concentration.

PAS work in polysaccharides and mucosubstane and found in the histopathology lab and not expensive and cannot take time in work.

GHS expensive and need long time for preparation and long time for working.

Final diagnosed was severing diffuse necrotized placentitis with intraregional yeast, associated with fetal pneumonia and hepatitis.

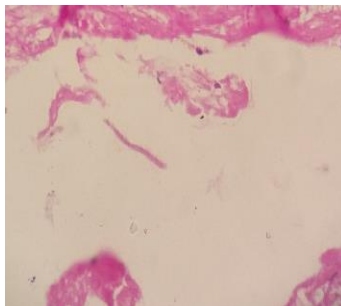


Figure (1) show Candida stained by PAS stain by high power 40x Final diagnosed was severing diffuse necrotized placentitis with intraregional yeast.

Limitations: -

In the techniques the component of Grocotte sliver stain very expensive but the university staff helps me in preparation of stain. I was found CMV.

Recommendations:

All sample of the product of conception must be use one of special stain that help in treatment of abortion. Must be avoiding the multiple partners.

Acknowledgment:

I do acknowledge the effort of Khartoum bahri hospital lab, staff of histopathology at faculty of medical laboratory science AL-Neelain University, and also my supervisor Abu Elgasim Abas Awad Elkareem.

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