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Correlation of Aspirated Peritoneal Fluid Findings at Laparoscopy &Tubal Pathology In Infertile Women

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Abstract: Background: Infertility remains a major clinical and social problem, the majority being residents of developing countries; the most common cause of infertility is tubal blockage. Objective: A hospital based prospective study was conducted at the Minimal Access Gynecology Surgery (MAGS) unit at Omdurman Maternity hospital from June 2007-_August 2008 to look into peritoneal fluid bacteriological and cytological findings in infertile women and correlate the results to the tubal pathology. Method: The study included 205 infertile women attending this centre. The peritoneal fluid was aspirated from cul-de-sac at laparoscopy and sent to the laboratory. Results: Tubal blockage was found in 90(43.9%) women. The bacteriological studies showed sterile aspirate with no growth while the cytological studies revealed inflammatory cells in the fluid mainly macrophages, lymphocytes and plasma cells. The incidence of tubal blockage was found to be significantly higher in patients with inflammatory cells indicating chronic inflammation. Coclusion: Cytological studies of peritoneal fluid are of value when correlate to tubal pathology in infertile women, while cytological and bacteriological studies at the time of laparoscopic procedures are of no diagnostic value. Conclusion: The association between tubal pathology and Cytological studies of peritoneal fluid was confirmed but did not add clinically valuable information during the diagnostic work up of infertile patient. Synopsis: Cytological studies of peritoneal fluid are of value when correlate to tubal pathology in infertile women, while sterile peritoneal fluid in cul-de-sac does not exclude PID.

Keywords: Peritoneal fluid, Tubal Pathology, laparoscopy.

INTRODUCTION

Infertility is defined as inability of a couple to conceive naturally after one year of regular unprotected sexual intercourse. It remains a major clinical and social problem, affecting perhaps one couple in six (Kamel, 2010). Worldwide more than 70 million couples suffer from infertility, the majority being residents of developing countries. Negative consequences of childlessness are experienced to a greater degree in developing countries when compared with Western societies. Bilateral tubal occlusion due to sexually transmitted diseases and pregnancy-related infections is the most common cause of infertility in developing countries (Ombelet, 2008).The exact prevalence of infertility in developing countries is unknown due to a lack of registration and well-performed studies (Ombelet, 2008).

Pelvic inflammatory disease (PID) is defined as an acute or chronic condition in which inflammation of the cervix, uterus, fallopian tubes, or ovaries, often caused by sexually transmitted microorganisms and characterized by fever, abdominal pain, and discharges of pus, may result in sterility (Cohen, 2005). Tubal pathology accounts for 14-36% of female infertility. (Dabekausen, 1994) Diagnostic laparoscopy with dye test remains the gold standard for the accurate assessment of tubal patency (Corson, 1977). Laparoscopy allows pelvic adhesions and endometriosis to be detected and treated. However, it is usually performed under general anesthesia and can be associated with risks of bleeding and injury to internal organs such as the bowel.

Fitz-Hugh Curtis syndrome is usually caused by gonorrhoeae (acute gonococcal perihepatits) or Chlamydia, which causes a thinning of cervical mucous and allows bacteria from the vagina into the uterus, oviducts, causing infection and inflammation. Some organisms travel trasperitoneally from the fallopian tubes to reach the liver surface (Anonymous, 1999).

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The Centre for Disease Control and Prevention (CDC) had adopted an approach to maximize diagnosis by using minimal criteria and by urging providers to maintain a low threshold for diagnosis and treatment.

A large multicenter U.S study found that cervical movement tenderness as a minimum clinical criterion increase the sensitivity of the CDC diagnosis criterion from83%to95%.However even the modified 2002 CDC criterion does not identify women with sub clinical disease (Blenning, 2007).

Ultra sound, CT and MRI findings in the early stages of PID do not show any significant changes. However in the later stages signs include endometrial thickening with or without endometrial fluid and gas, ovarian enlargement with indistinct ovarian borders, uterine enlargement with indistinct uterine contours and free intraperitoneal fluid (Hajenius, 2000; Money, 1997).

The free intraperitoneal fluid may be classified as transudates or exudates. Exudates are usually resulting from increased capillary permeability, lymphatic resorption associated with injury or inflammation of the peritoneum which include infectious diseases caused by viruses, bacteria or fungi. Infections may originate in the peritoneum, contamination during surgery, or may spread to the peritoneum from other places in the body like lower genital tract gives rise PID (Jenkins, 2000).

Laparoscopy as the gold standard for diagnosis of PID correctly identifies women with confirmed diseases in 65-90% of cases (Lauren Nathan, 2003). This is the first study done in the Sudan to look into peritoneal fluid bacteriological and cytological findings in infertile women and correlate the results to tubal pathology.

MATERIAL AND METHODS

This is a hospital based prospective study conducted during the period from the10th June 2006 to 28th August 2008. The study included 205 infertile women from those attending the infertility clinic at the minimal access gynecological surgery (MAGS) Center at Omdurman Maternity Hospital. Patients were usually referred to MAGS by gynecologists from our own hospital, other local hospitals, Family Planning Clinics and the private sector. A thorough infertility work-up was conducted. Standard protocol of history taking, physical examination and investigation including conventional semen analysis and hormonal assessment were performed.

Only women who were advised to undergo laparoscopy to assess tubal patency were included. Those with absolute contra indications for laparoscopy, or who were in need for IVF/embryo transfer treatments because of severe male factors were excluded from this study. Every patient was counseled and gave an informed consent prior to participating in the study, which was approved by the Ethic Committee, Faculty of Medicine, ALziem ALazhari University.

Blood was taken for Hemoglobin and blood grouping on the day of admission into the hospital. Laparoscopy and dye test were performed as day surgery in the standard manner under general anesthesia. The free fluid in cul-de-sac was aspirated in two sterile containers. One container was kept in liquid nitrogen (- 60C) to preserve Chlamydia trachomatis if present until it reached Soba Research Laboratory Center where Chlamydia was searched for by means of direct immunoflourecent microscopy and the other container was sent to the laboratory in Ahamed Gasim hospital for culture and cytology as detailed below.

Pelvic adhesions peritubal, periovarian, massive pelvic adhesions, frozen pelvis and periportal adhesions were reported. Endometriosis if present was graded according to the revised American Society for Reproductive Medicine classification of endometriosis into four stages: minimal, mild, moderate and severe (15-S.Arukumaran, 2006).

Tubal patency was confirmed with free spillage of Methylene blue dye from the fimbrial end. Tubal pathology could either be distal obstruction which was diagnosed when there was no spillage of dye from the fimbrial end with or without hydrosalpinx. Proximal obstruction was considered only when there was no filling of the tube and absence of hydrosalpinx. Pathology on one side was considered to be abnormal.

Fluids were cultured on Thayer-Martin chocolate blood agar and blood agars under aerobic and anaerobic condition .Gram stains were performed to see presence of microbial morphology.

For cytological studies the fluids were centrifuged, and then the sediment smeared into three slides, stained by H&E stain covered by cover slide and examined under the microscope.

The sample size was determined using the following equation:

$$n = \frac{Z \times P \times Q}{D} = \frac{1.96 \times 10 \times 90}{25} = 139$$

The data was analyzed using statistical Package for the Social Science (SPSS) and summarized using the percentage and X^2 at 0.05 level when the difference between two Proportions were tested.

Results:

A total of 205 consecutive women aged 20-45 years (median 33.0 years) were recruited. The duration, of infertility, ranging 1-21 years. Some patients had more than one pathology.

Tubal blockage was found in 90(43.9%) women, pelvic adhesions in 30 (14.6%) and peri portal adhesions in 3 (1.5%). Out of the 205 women 50 (24.4%) women showed secondary infertility 22 of them the cause is tubal blockage as shown in table 1. 11 out of the 22 had normal spontaneous vaginal delivery 5 had Caesarean Section (C/S) and 6 had previous abdominal surgery. The remaining 155 (75.6%) women had primary infertility, 68 (44%) out of 155 had tubal blockage..

Fig 2 shows types of tubal blockage 57 (63.3%) had distal block while 33 (36.7%) had proximal blockage which was associated with uterine Fibroid in 10 patients.

Out of the 30 patients who showed pelvic adhesions, 8 (26.7%) had previous abdominal surgery, 3 had C/S, 3 had appendesectomy and 2 had myomectomy and this might explain their pelvic adhesions (Table 1). The other 22 patient (72.3%) one had endometriosis, 2 had fibroid and 19 might be due to PID.

We reported 3 patients with Fitz-Hugh -Curtis syndrome; 2 had massive pelvic adhesions and tubal blockage while one patient had normal laparoscopic findings as shown in Table 2.

Table 3 showed incidence of pelvic adhesion & block tubes in the two groups of women with or without previous abdominal surgery. The comparison revealed that there was no statistically significant in both groups using X^2 =0.68 & 1.5 respectively at $p<0.05$.

The peritoneal fluid was sterile in the 170 samples so we added cytological studies. The cytological results are shown in figure 3.

In 12 (7%) patients out of 170 the predominant cells were neutrophils which indicates acute inflammatory reaction, 5 (41%) of these had tubal blockage, 7(59%) with patent tubes and there was no pelvic adhesion in this group. In 24 (14.1%) patients the fluid was clear, 14(58%) of these 24 patients had patent tubes, while 10(42%) had tubal blockage; and there was no pelvic adhesion. In remainder 134 (78.8%) patients the predominant cells were macrophages, lymphocytes and plasma cells which indicate chronic inflammation induced by intra-cellular organisms, such as Chlamydia trachomatis (figure 3). 69 (51.5%) patients out of134 showed patent tube while 54 (40%) showed tubal blockage and 11(8.5%) with pelvic adhesion (figure 4).

A comparison of presence of chronic inflammatory cells in the two groups of women with block and patent tubes revealed a statistically significant increase in the group of women with block tube using X^2=0.04 ($P<0.05$) as shown in table 4.

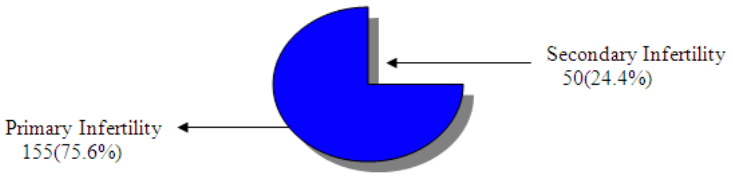

Fig. 1: Types of infertility.

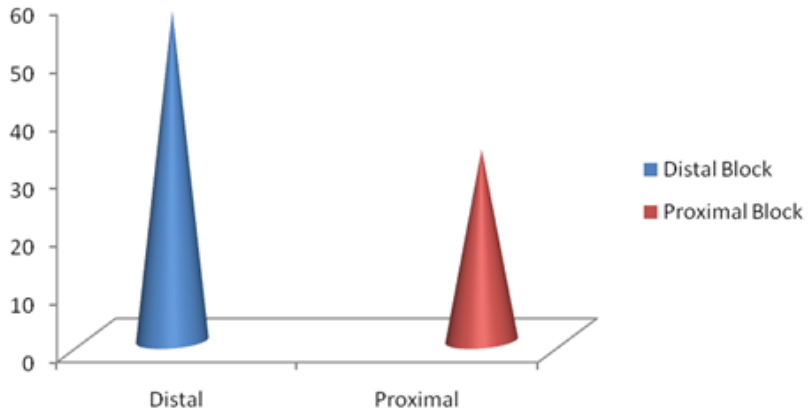

Fig. 2: Types of tubal blockage.

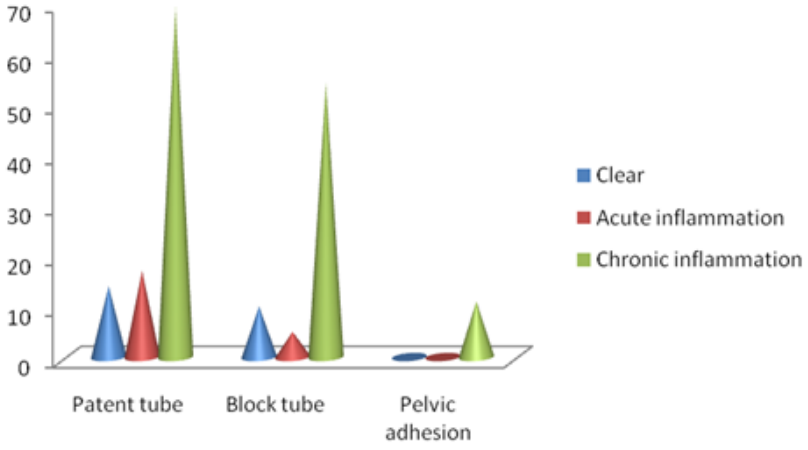

Fig. 3: InflammatorycellsLaparoscopicFindings.

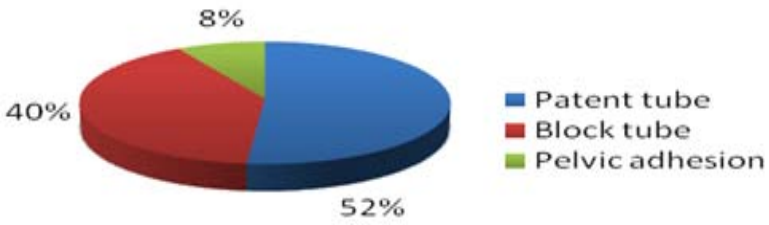

Fig. 4: Chronic inflammatory cells and laparoscopic findings.

Table 1: Age &Type of Infertility:

Age group	No. of patient with primary infertility	No. of patient with secondary infertility	Total no. of patient
20-24	22(10.7%)	4(1.90%)	26
25-29	35(17.1%)	20(9.80%)	55
30-34	41(20%)	20(9.80%)	61
35-39	51(24.9%)	2(0.6%)	53
40-45	6 (3.3%)	4(1.90%)	10
Total	155(76%)	50(24%)	205

Mean = 33 years +SD
179 (87.3%) patients out of 205 were over 25years old.

Table 2: Laparoscopic findings

	No.	%
Normal laparoscopic findings	36	17.6
Tubal Blockage:	90	43.9
*Distal	57	27.9
*Proximal	33	16.0
Pelvic adhesion:	30	14.6
*Massive pelvic adhesion	20	9.8
*Peritubal adhesion	10	4.8
Uterine fibroid:	22	10.3
*Block tubes	10	4.9
*Patent tubes	12	5.4
Periportal adhesion(Fitz-Hugh -Curtis syndrome):	3	1.5
*Block tube and pelvic adhesion	2	0.9
*Patent tubes	1	0.6
Other findings:	70	9.53
*poly cystic ovaries	56	2.73
*Streak ovaries	3	1.5
*Infantile uterus	2	0.9
*Endometriosis	9	4.4

Table 3: Pelvic adhesion and tubal blockage in patients with or without previous abdominal surgery

Abnormality	No. of patients with previous abdominal surgery	No. of patients without previous abdominal surgery
Pelvic adhesion	8	22*
Tubal blockage	15	75*

*p < 0.05 not significant

Table 4: The cytological studies of the peritoneal fluid in women with block and patent tube:

The state of the tube	No. Of patients with Chronic inflammation	No. Of patients with clear fluid
Block tube	54**	10
Patent tube	69	14

**p < 0.05

Discussion:

Bilateral tubal occlusion due to sexually transmitted diseases and pregnancy-related infections is the most common cause of infertility in developing countries, a condition that is potentially treatable with assisted reproductive technologies (ART). New reproductive technologies are either unavailable or very costly in developing countries (Ombelet, 2008).

Tubal blockage was found in 90 (43.9%) of our study population, 57 had distal blockage while the remainder 33 had proximal blockage .10 out of 90 patients had uterine fibroid which might explain their tubal blockage because uterine fibroid gives rise to infertility when it interferes with uterine cavity and blocks the tube (Arukumaran, 2006). Gonorrhea and Chlamydia are the most common causes of PID, up to 70% of Chlamydia infections are asymptomatic and remain unnoticed and untreated (Cohen, 2005). Co infection with both of them occur frequency. In many studies, half to two thirds of women with proved PID are not infected with gonorrhea or Chlamydia. Anaerobic bacteria and facultative bacteria have frequently been isolated (Lardenoije, 2007). In this study search for Gonorrhea, Chlamydia G. vaginalis, aerobic and anaerobic bacteria from the fluid aspirated from culde-de-sac of infertile women at laparoscopy were studied but no organisms were isolated. This result might be explained by the fact that if there are no organisms it does not rule out an infection; they may be present in small number or their growth may be inhibited because of prior antibiotic therapy (2008).

The organisms that give rise to acute PID are usually isolated in the first 48 hours but sometimes one third of these organisms can not be isolated in this period, (Gebreselassie, 2005) also in chronic PID micro organisms are not usually present (Anorlu, 2005).

In the WHO study 76.2% of African women with infection-related infertility cases had a history of either postpartum or post abortion complications [(18)] Secondary infertility was reported in 50 (24.4%) out of 205 of infertile women; tubal blockage was found in almost 50%.

Two out of three patients of our study population who showed perihepatitis syndrome had massive pelvic adhesions and tubal blockage. The same results were reported by Money et al who found that patients with perihepatitis plus salpingitis did not have distinctive clinical or microbiological findings from those with salpingitis alone, but they did have a higher prevalence of moderate to severe pelvic adhesion (70% against 30%). (Money, 1997).

Previous laparotomy may give rise to adhesions especially if it was complicated by infection (Arukumaran, 2006). We had 23 patients with previous scars; they had neat skin scars and no history of post operative infection; pelvic adhesions were not statistically significant $X^2c=0.68(P<0.05)$. This is probably due to their previous pathology which indicated the operation or that the operation gave rise to peritoneal irritation and subsequent adhesion (Emmert, 2000).

Acute reactions are seen when the stimulus to the inflammation is transient as in physical trauma, burns and micro biologic infections that are rapidly eradicated by the defensive forces of the body. The acute response is characterized principally by vascular and oxidative changes. The white cells that participate in the acute reaction were principally neutrophils and macrophages. The oxidative changes give rise to free fluid in cul-de-sac. The amount of the fluid can be large, moderate or absent so we were able to aspirate 170 (83%) samples of the 205 patients. Chronic inflamed tissue is characterized by the infiltration of mononuclear immune cells monocytes, macrophages, lymphocytes, and plasma cells (Serhan, 2005).

Tubal blockage was found to be significantly increased in the group of women with evidence of chronic inflammation most likely caused by intracellular parasite; $X^2=0.04$ ($P<0.05$). Immunoflourecent microscopy was used to detect Chlamydia but no organisms were isolated. This might be explained by the fact that the sensitivity of immunoflourecent microscopy is 60% in asymptomatic and 90% in symptomatic patients and unlikely to isolate Chlamydia from the exudates (Stamm, 1988). This finding confirms the conclusion of Morris who stated that chronic Chlamydia infection gives rise to chronic salpingitis and tubal blockage and can cause severe immunological mediated chronic inflammation.

Typically there is an exaggerated inflammatory response but Chlamydia can be isolated only briefly and in very small number (Morrison, 1990).

Conclusion:

Our study showed that cytological studies of peritoneal fluid are of value when correlate to tubal pathology in infertile women, while sterile fluid in cul- de –sac does not exclude PID either in acute or chronic stage. Cytological and bacteriological studies at the time of laparoscopic procedures are of no diagnostic value.

The association between tubal pathology and Cytological studies of peritoneal fluid was confirmed but did not add clinically valuable information during the diagnostic work up of infertile patient, while sterile fluid in cul- de –sac does not exclude PID either in acute or chronic stage.

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