Study of Nonspecific Cellular Immune Function (Leucocytospermia) on Asthenozoospermic , Oligozoospermic & Azoospermic Infertile Patients

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Abstract

Many studies had been established to detect the affects presence of leucocytes on semen quality and sperm function tests . Than after their effects on men fecundity.

This study had been occure on infertile (48) asthenozoospermic , (18) oligozoospermic & (13) azoospermic patients as well as to (10) control subjects .

Results study of this work obvious that presence of leucocytes in seminal fluid can be explained as :

1-Inflammatory response .

2- Nonspecific cellular immune response .

3- Cooperation and / or inverse cooperation of natural and adaptive

Immune responses to foreign antigenic epitopes.

الخلاصة

١- تمت الكثير من الدراسات بقصد الكشف عن مدى التأثير الحاصل بحالة ظهور كريات الدم البيضاء (Leucocytes) على كل من نوعية السائل المنوي وكذلك التأثير الحاصل في أختبارات كفاءة وظائف الحيمن (sperm function tests) وبالتالي مدى التأثير الحاصل في خصوية الرجل .

٢- هذه الدراسة تناولت (٤٨) شخصاً من غير الخصبين والذين كانوا يعانون من الضعف الواضح في حركة الحيامن (astherozoospermia) وكذلك (١٨) شخصاً من غير الخصبين والذين كانوا يعانون من قلة عدد الحيامن عن الحد الطبيعي (oligozoospermia) ، أضافة الى دراسة (١٣) من العقيمين المصابين باللانطفية (azoospermia) حيث تم المقارنة فيما بينهم وبين (١٠) من الاشخاص الخصبين كمجموعة سيطرة .

٣- أوضحت نتائج الدراسة بأن ظهور كريات الدم البيضاء في السائل المنوي بنسب تفوق الحد الطبيعي يمكن أن يعزى للاسباب التالية :-

٤- كاستجابة التهابية نتيجة لعدد من الأمراض الخاصة بالمسلك التناسلي .

٥- استجابة مناعية غير متخصصة على المستوى الخلوي .

٦- استجابة مناعية تعاونية مع / أو استجابة مناعية معكوسة لكل من المناعية الطبيعية والتكييفية للأجسام الغريبة المتخصصة بأحداث وتوليد الاستجابة المناعية (foreign antigenic epitopes).

Introduction

This study were estiblished according many attempts that had been made to answer the question of whether and to what extent WBCs – in particular polymorphonuclear (PMNs) can affect the quality of semen sample (Comhair et al., 1980; Gonzales et al., 1992; Anderson, 1995).

Therefore, this work were studied three different categories of infertile male factors which are includes ; asthenozoospermic, oligozoospermic and azoospermic patients as well as to the control subjects.

As the male reproductive system (Figure-I) includes the testis, a series of ducts or channels, accessory sex glands and the penis. The testis also produce the spermatocytes, the sperms in addition to the male sex hormones. The male reproductive mucosal compartment responds as an autoimmune response to sperm antigens (Meshan, 1984; Gilbert, 1988; Comhair, 1999; Wira et al., 2002).

Older paper showed a decrease in number and motility of spermatozoa in infertile patients with leucocytospermia (Talbert et al., 1987; Eggert et al., 1992).

Concerning the morphology of spermatozoa a reduction of normal forms was observed along with the decrease of their number and motility (Wolff et al., 1990; Gonzales et al., 1992).

These older findings have not been confirmed by other studies (Aitken and West, 1990; Tomlinson et al., 1993; Aitken and West, 1994), which involve large numbers of fertile men with leucocytospermia.

On the contrary, most reports seem to agree that leucocytospermia has a negative effect on sperm function tests in a different stages of the sperm – ova interaction processing due to WBCs impartment molecules secretions (Maruyama et al., 1985; Vogelpoel et al., 1991; Garvella and Lipovac, 1993; Chan et al., 1994).

an increased number of WBCs in combination with other criteria may be indicative of genital tract infection (Potitch et al., 1993 ; WHO, 1999). When assessing a semen analysis ; two groups of WBCs are present in semen , those with granular cytoplasm and those with agranular cytoplasm .The former includes polymorphonuclear granulocytes such as, neutrophils,eosinophils and basophils , while the later includes monocytes and lymphocytes .

Despite the presence of all above mentioned cells in semen samples, the most abundant are granulocytes – in particular polymorphonuclear (WHO, 1999).

The granulocytes were found as the predominant cell type (50 – 60%), macrophages follows (20 – 30%), and finally lymphocytes B and T lymphocyte account for very small proportion (2–5%) (John et al., 1999). Other cell types, however, are rare in semen samples (Wolff and Anderson, 1988; Eggert et al., 1992; Tomlinson et al., 1993; Aitken et al., 1994).

On the other hand, an increased number of immature germ cells may be a sign of abnormal spermatogenesis (Nieshlag et

al., 1997). Many investigations say that, Reactive oxygen species (ROS) are produced by leucocytes and spermatozoa in both fertile and infertile men as a result of lipid peroxidation. (Rao et al., 1989; Aitken et al., 1993). Excessive ROS causes oxidative stress, resulting in decreased sperm motility, viability and increased midpiece defects that impair sperm capacitation and acrosome reaction (Aitken, 1997; De Lamirande et al., 1995).

- Patients and methods :

The study consists of seventy nine infertile male patients . Those patients were subgrouped according to infertility type which includes; (48) of asthenozoospermic, (18) of oligozoospermic and (13) of azoospermic infertile patients who were involved in this study during their attendance of Babylon maternity and child care teaching hospital of Babylon university.

All those patients were evaluated by consultant of urologist surgeons assessing for the presence or absence of varicocele, hydrocele, cryptochidism, inguinal hernia operation, present or absent of the congenital abnormalities, complete history regarding sexual habits, venereal diseases, chronic diseases such as diabetes, and febrile disease at least throughtly during last one year were undertaken

In addition to the questioner for: age, alcohol consumption, smoking habits, ABO blood group, exposure for ionizing radiation and the type of infertility.

Seminal fluid analysis were done according to the WHO, (1990) .which is regaded that the normal concentration leucocytes value is < 1x 10 6 / ml.

Semen collection :

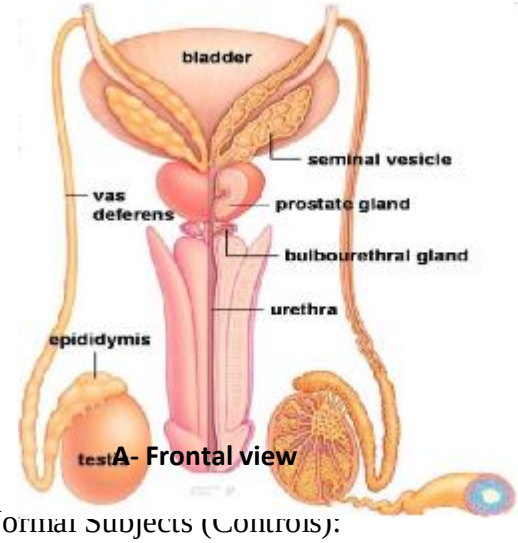
Semen or the samples of seminal fluid was collected (after 3–4 day of abstinence) directly into a clean, dry and sterile disposable Petri–dish by masturbation in room near the laboratory.

The container was labeled with the following information: name, age, abstinence period and time of sample collection.

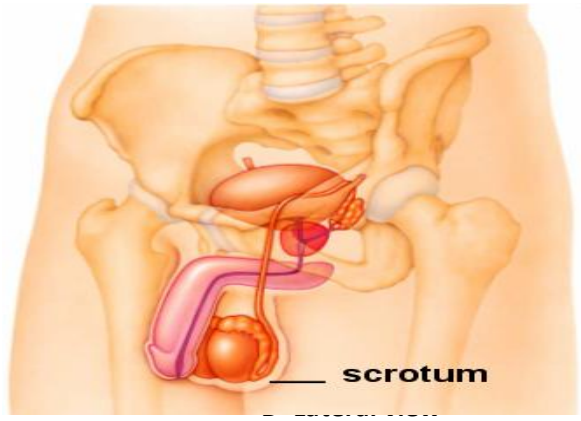
The specimens were incubated at 37°C for 30 minutes to allow liquefaction. The specimen was examined in details by macroscopic and microscopic examinations and the standard form of the WHO, (1999) was used to record the results of seminal fluid analysis.

Figure: (I)

The male reproductive system



A- Frontal view



Normal Subjects (Controls):

Ten healthy fertile subjects were used in this study as a control. groups . Physical and clinical examinations were showed by urologist clinician specialists.

Results

Semen of infertile men has shown maximum leucocytes concentration among Oligospermic then asthenozoospermic and azoospermic patients.

Leucocytospermia was ranged among infertile groups from 2.384×106 + 0.92 leucocytes /ml to 6.166×106 + 1.106 leucocytes /ml.

The Inter-groups ranges of leucocyte concentrations were; 2×106 – 25×106, 4×106–20×106 and 2×106–14×106 leucocytes /ml; for asthenozoospermic, Oligozoospermic and azoospermic patients, respectively (table-1)

Table (1)

Leukocytes Concentration Among Infertile Groups

Infertility Patient Group	Leukocytes Mean ± SE	Rate of Leukocytes positive: total patient	Leukocytes Positive range
(48)Asthenozoospermic patient	5.6383×106 ± 0.5628	45:48 (93.75%)	2×106 – 25×106
(18)Oligozoospermic Patient	6.1667×106 ± 1.1063	18:18 (100%)	4×106 – 20×106
(13)Azoospermic Patient	2.3846×106 ± 0.9236	8:13 (61.53%)	2×106– 14×106

Discussion

The ejaculation invariably contains other than spermatozoa. These include polygonal epithelial cells from the urethral tract, spermatogenic cells and leucocytes, which have been collectively referred to as (round cells). Leukocytes are present in most human ejaculations (Wolff and Anderson, 1988; Aitken and West, 1990; Aitken and West, 1994). The predominant cell type being the neutorphils.

Accurate assessment of the number of leucocytes is important because the excessive presence of these cells may indicate the existence of reproductive tract infection. A threshold concentration of leucocytes beyond which fertility will be impaired is difficult to define. The impact of these cells depends upon the site at which the leucocyte enters the semen. Furthermore, leucocytospermia may be associated with defects in the semen profile including reductions in volume of ejaculate, sperm concentration, and sperm motility, as wells as the loss of sperm function as a result of oxidation stress (Aiteken and west, 1990) and /or the secretion of cytokines (Hill et al., 1987).as showen in (table - 2) . ROS generated by the WBCs especially polymorphonuclear leucocytes (PMNS) or granulocytes, can exert a deleterious effect on human spermatozoa as indicated by marked loss of sperm motility and a reduced capacity for oocyte penetration, that contaminate PMNs is a major source of reactive oxygen species activity recorded in human sperm is in controvertible (Baker et al., 1996).

As a general guide, a normal ejaculate should not contain more than

1×10 6 leucocyte /ml (WHO, 1999). Such concentration (1 ×106/ml) was closely different from those results of leucocytospermia of asthenozoospermic,

oligzoospermic and azoospermic patients, which are apparently with high concentration of leucocytes; 5.638 ×106 ±0.562 (93.75%), 6.166× 10 6± 1.106 (100%), and 2.384 ×106 ±0.923 (61.53 %) respectively as shown in table (1). Infections may include the testicular tissue, vas deference or the sexual accessory gland (e.g. prostate, seminal vesicles and bulbo-urethral gland). Besides, the relationship between the presence of genital tract infection is controversial (Comhair et al.,1980; WHO, 1992). Our results of the leucocytospermic patients agreed with those of Talbert et al., (1987); Eggert et al., (1992) which had been referred to decrease in number and motility of spermatozoa, Leucocytospermia of the asthenozoospermic, oligozoospermic and azoospermic patients study also agreed with those study of Wolff et al., 1990; Gonzales et al.,1992; Potitch et al., 1993; Yanushpolsky et al., 1996). Whom concerning the morphology of spermatozoa and the reduction of normal forms, as well as the decrease of concentration motility spermatozoa which are relatively evident. In view of susceptibility of human spermatozoa to oxidative stress, the presence of neutrophils is likely to be damaged, particularly if the infiltration occurs at the level of the rete testis or epididymis conversely, the entry of leucocytes, at the moment of ejaculation, via the prostate or seminal vesicles is probably less harmful because of the powerful antioxidant effects of seminal plasma (Jones et al., 1979) . Hence this may be attributed to the fluctuating effect of leucocytospermia. Thus, such leucocytospermia may be beard in mind as:

I. Indication of genital infection Indication of urogenital infection .
II. Influxes of tissue harbouring leucocytes.
III. Cellular adjunct to infertility .

A part of normal nonspecific cellular immune surviellence expressed by human immune system.

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