Serum Amyloid A level in women with unexplained infertility

Thesis

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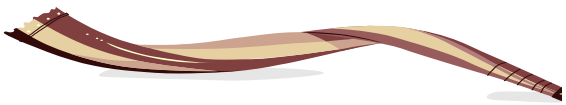

Zeinab Saad Abd El wahab

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List of Abbreviations

ACAT : Acyl-coenzyme A:cholesterol acyltransferase

APP : Acute phase proteins

A-SAA : Acute-phase serum amyloid A

CRP : C reactive protein

ESR : Erythrocyte Sedimentation Rate

FDA : Food and drugs administration

FF : Follicular fluid

FPRL1 : Formyl peptide receptor-like 1

FSH : Follicle stimulating hormone

GIFT : Gamete intrafallopian transfer

HCG : Human chorionic gonadotropin

HDL : high-density lipoprotein

ICI : Intracervical insemination of sperm

IL : Interleukin

IUI : Intrauterine insemination

IVF : In vitro fertilization

LH : Luteal hormone

NF-B : Nuclear factor-kappa B

NICE : National Institute for Clinical Excellence

OPU : Oocyte pick up

PRPs : Pattern recognition receptors

RPL : Recurrent pregnancy loss

SAA : Serum amyloid A

SD : Standard deviation

SPSS : Statistical package for social science

TGF : Transforming growth factor

TLR2 : Toll-like receptor 2

TNF : Tumor necrosis factor

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ABSTRACT

Background: By various molecular and cellular research efforts it was displayed that folliculogenesis, ovulation, and corpus luteum formation is considered as an inflammatory processes that hav raised the research interest to investigate that serum amyloid A as an inflammatory mediator to reveal its possible role in infertility.

Objective: This study aims to assess the association between SAA and unexplained infertility in women.

Methods: The current research study have been conducted at Ain Shams University Maternity Hospital during the period from September 2018 to April 2019. Women approached were recruited from outpatient clinic of Ain Shams University Maternity Hospital. The recruited 90 research study subjects have been divided into two research groups

Results: Serum amyloid A was statistically significantly higher among the unexplained research group versus control group (SAA had excellent discriminative value with an area under the ROC curve (AUC) of 0.992 (95% CI = 0.945 to 1.000, P-value <0.0001). A best cutoff criterion is SAA of 28.7 mg/l (sensitivity = 98%, specificity = 96%, J-index = 0.93)) denoting that amyloid A as an acute phase inflammatory modulator is higher among the unexplained research group reflecting a form of chronic inflammatory process in those category of cases. Analytical research results of multivariable binary logistic regression analysis as regards the correlation between SAA and unexplained infertility after adjustment for age and BMI, it was revealed that serum amyloid A an independent predictor for unexplained infertility.

Conclusions: Serum amyloid A as a biomarker for could be implemented as a predictability tool for unexplained infertility since it is a reflector of an chronic inflammatory process that is ongoing that could affect the process of implantation and ovulation however the clear molecular and cellular processes linked to the pathophysiological process that reflects the fertility hindering process should be implemented in future research efforts taking in consideration to have multicentric fashion of research with larger sample sizes to verify the value of serum amyloid A as biomarker that reflects the fertility potential besides the routine infertility work up.

Keywords: Amyloid A; Unexplained Infertility

PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRICS AND GYNECOLOGY

Title of the Protocol:

Serum Amyloid A level in women with unexplained infertility

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What is already known on this subject? AND What does this study add?
High follicular fluid amyloid A Level associated with reduced pregnancy rate. The aim of this study is to find out association between serum level of amyloid A protein and unexplained infertility .

1. INTRODUCTION/ REVIEW

Unexplained infertility is defined as failure to conceive after a period of 2 years of normal, regular, unprotected intercourse **(NICE, 2013).** A large variation in suggested definitions is seen in the literature including a failure to conceive over a period ranging from 1 to 3 years of unprotected intercourse in the face of normal baseline investigations **(Homburg, 2017).**

Possible etiologies of unexplained infertility: several possibilities have been proposed to explain why some couples fail to conceive in the absence of an identifiable cause:

1-Couples with unexplained infertility who are treated with IVF demonstrate reduced oocyte fertilization and embryo cleavage rates compared to couples in whom tubal factor is the cause of the infertility, although the rates of live birth per transfer are equivalent for both groups. Couples with unexplained infertility also had a higher rate of complete fertilization failure when treated with IVF than couples with tubal factor infertility (6 versus 3 percent). These results suggest that couples with unexplained infertility probably have subtle functional abnormalities in oocyte and/or sperm function. In this sense, IVF is also a diagnostic procedure **(Hornstein et al., 2014).**

2-Defective endometrial receptivity is thought to prevent normal attachment, invasion, and implantation of the blastocyst, and may account for some cases of unexplained infertility and recurrent pregnancy loss .No biomarkers have been validated for clinical diagnosis of these patients **(Lessey , 2011).**

Serum amyloid A (SAA) proteins are a family of apo lipoproteins associated with high-density lipoprotein (HDL) in plasma. It is also associated with inflammatory response highly similar to erythrocyte sedimentation rate and C reactive protein (CRP) **(Zhu et al.,2016)**.

Stimulation of hepatic production of acute phase proteins is by proinflammatory cytokines. The functions of positive acute phase proteins (APP) are regarded as important in optimization and trapping of microorganism and their products, in activating the complement system, in binding cellular remnants like nuclear fractions, in neutralizing

enzymes, scavenging free hemoglobin and radicals, and in modulating the host's immune response **(Jain et al., 2011).**

SAA is a highly conserved acute-phase protein, released in response to inflammation or infection. Production of acute-phase SAA (A-SAA) is stimulated by proinflammatory cytokines, such as (IL-6,IL-1, tumor necrosis factor(TNF) ,interferon and transforming growth factor (TGF)). The concentration of SAA increases dramatically during acute Inflammation and injury reaching withing 5-6 Hours levels that are 1000 fold greater than normal (<10 mg /l) **(Migita et al., 2011).**

Ovarian reproduction functions ie, folliculogenesis, ovulation, and corpus luteum formation are viewed as inflammatory processes .Follicular development involves cell recruitment and proliferation,angiogenesis, tissue remodling, and steroid production,regulated by pituitary-derived gonadotropins and by locally produced growth factors and cytokines**. (Urieli-shoval et al.,2013).**

2. AIM/ OBJECTIVES

Aim of the study: this study aims to assess the association between SAA and unexplained infertility in women.

Research question: In women with unexplained infertility does SAA level elevated?

Research Hypothesis: In women with unexplained infertility SAA level may be elevated.

3. METHODOLOGY: Patients and Methods

Place: Ain Shams University Maternity Hospital**.**

Study design: Case Control study.

Study population: Infertile women attending Ain Shams Universit y infertility outpatient clinic seeking fertility and fulfilling the inclusion criteria.

Inclusion criteria:

1-women with unexplained infertility (unprotected, regular intercourse for at least 2 years)**. (NICE,2013).**

2-Nulligravida ,Regular mensturation.

3-Age less than 40 years .

4- Normal Hormonal profile (day 2 (FSH,LH) , midluteal phase progesterone value, Prolactin, TSH), ,Transvaginal ultrasound, hysterosalpingography and detailed semen analysis are normal in the study group.

Exclusion criteria:

1-Age above 40 **2-** Smoking females **(Aneeela Jamil,et al 2015).**

3-Obese person (BMI>30) **(Yang ,et al.2006).**

4-Women with any systemic or medical diseases leads to elevation of SAA as:

a-Systemic lupus erythematosis **(Jin Kyun Park,2016).**

b-Rheumatoid arthritis **(Migita ,et al.2011).**

c-Sarcoidosis **(Bargagli ,et al.2014).**

d- Diabetes Mellitus **(Griffiths ,et al.2017).**

5-use hormonal contraception (for fertile group) **(Afshin ,et al .2015)**

Statistical Methods: up to our knowledge up till now no previous researches were published on a similar topic: it is case control study, so we will include 45 women in each group.

This study conducted on two groups of women:

Group 1 (study group): Women with unexplained infertility.

Group 2 (control group): Fertile women who had normal physical and pelvic examination, regular menstrual cycles, don't use hormonal contraceptive**,** had one child at least.

Study Procedure:

Women eligible for the study will be subjected to:

1-Detailed history taking: (Age, duration of marriage, medical, surgical, family and drug history, menstrual history (last menstrual period, regularity of mensturation) or urinary tract symptoms.

2- Complete general examination including: (Blood pressure , temperature, pulse, breast).

3- Laboratory investigation in the form of :

mid-luteal progesterone level , day2 (F.S.H, L.H), prolactin, TSH , Semen analysis of husband

For determination of SAA level, a 5-ml sample of venous blood will be taken from each participant under aseptic conditions, and centrifuged at 2500 g for 15 minutes at 4°C c. separated into serum aliquots,and stored frozen at −80°C until analysis of SAA ,which will be done by a double-antibody sandwich Enzyme Linked Immunosorbent Assay (ElISA) kits supplied by cusabio,USA according to manufactorers instruction .Each sample was tested in duplicate and the average will be taken. A cut off level of 30 mg/L SAA level will be chosen to reflect a real increase in its level (Griffith et al ,2017).

Outcome: Primary outcome:

- Difference between SAA level between cases and control.

Secondary outcome:

- correlation between SAA level with other parameters in the hormonal profile (e.g., FSH, LH, E2).

Patients' Consent:

The investigator will inform all participants about importance of the study and their role and rights, verbal informed consent will be obtained prior to drawing blood samples.

Ethics: The study will be approved from the ethical committee of the department of obstetrics and Gynecology, faculty of medicine, Ain-Shams University.