بسم الله الرحمن الرحيم

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تم عمل المسح الضوئي لهذة الرسالة بواسطة / سامية زكى يوسف

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات:

- بالرسالة صفحات لم ترد بالأصل
- بعض الصفحات الأصلية تالفة
- بالرسالة صفحات قد تكون مكررة
- بالرسالة صفحات قد يكون بها خطأ ترقيم

Faculty of Medicine
Menoufiya University
Clinical Pathology Department

ANTIPHOSPHOLIPID ANTIBODIES IN REPEATED FETAL LOSS

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF M.Sc. DEGREE IN CLINICAL , CHEMICAL PATHOLOGY

By

Amal Abd Al-Kader Abd Alaziz
M.B., B.Ch.

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Amal

CONTENTS

List of ABBREVATION

ACL - Ab:	Anticardiolipin - Antibody
AMA:	Antimitochondrial - antibody.
APL antibody :	Antiphospholipid antibody .
APTT :	activated partial thromboplastin time .
BFP. STS :	Biological false positive - serological test of syphilis
Ca^{++} :	Calcium .
chol :	cholesterol .
CL :	cardiolipin .
CNS :	central nervous system .
DIC :	Disseminated intravascular coagulopathy .
dKCT :	delta kaolin clotting time .
dRVVT :	dilute russel viper venoum time .
ds DNA :	double stranded DNA .
DV.T :	Deep venous thrombosis .
ELISA :	Enzyme linked immuno sorbent, assay.
HC APTT :	High concentration activated partial thromboplastin time
HRP :	horseradish peroxidase .
HIV :	Human immune deficiency virus .
LA :	Lupus anticoagulant .
M Ca Cl :	Molar calcium chloride .
PA :	Phosphatidic acid .
PAPS :	Primary antiphospholipid syndrome .

Table IV:

IgG antibodies against H. pylori in duodenal ulcer group after surgical and medical treatment.

type of treatment	+ve No	+ve %	-ve No	-ve %	total before treatment No	+ve %	X^2	P value
Surgical	0	0%	7	100%	7	100%	=3.65	Sign <0.05
Medical	5	56%	4	44%	9	100%		
Total	5	31%	11	69%	16	100%		

+ve : Positive for IgG antibodies against H.pylori.

-ve : negative for IgG antibodies against H.pylori.

No: Number of patients.

X^2 : Fisher extraction of Chi square.

P : Probability.

Sign : Significant (P < 0.05).

PBS :	Phosphate buffer saline .
Pc :	Phosphatidyl choline.
PE :	Phosphatidyl cthanolamine .
PG :	Phosphatidyl glycerol .
PI :	Phosphatidyl Inositol .
PNT :	Platelet neutralization test.
PS :	Phosphatidyl serine .
PT :	Prothrombin time .
RA :	Rheumatoid arthritis .
RIA :	Radio immuno assay .
SCAPTT :	Standard concentration activated partial thromboplastin tim
SLE :	Systemic lupus erythematosus .
SM :	Sphingo myelin.
TMB :	tetra methyl benzidine .
TTIT :	Tissue thromboplastin inhibition time .
VDRL :	Venereal Disease research laporatory antigen .

Table III:

IgG antibodies against H. pylori in gastric ulcer group after medical and surgical treatment.

type of treatment	+ve No	+ve %	-ve No	-ve %	total +ve before treatment No	total +ve before treatment %	X^2	P value
Surgical	2	33%	4	67%	6	100	=0.81	>0.05 N.S
Medical	13	54%	11	46%	24	100		
Total	15	50%	15	50%	30	100%		

+ve : Positive for IgG antibodies against H.pylori.

-ve : negative for IgG antibodies against H.pylori.

No: Number of patients.

X^2 : Fisher extraction of Chi square.

P : Probability.

N.S : Non significant (P>0.05).

INTRODUCTION :-

Antiphospholipid antibodies are not normally distributed. Their Presence in some patients lead to increased risk of vascular thrombosis, thromboembolic complications, thrombocytopenia and recurrent fetal.. loss. ***(Fields et al., 1989)*** .

IgG isotype was always associated with these complications .

These antiphospholipid antibodies (APL - Abs) occur commonly in auto immune diseases such as systemic lupus erythematosus (SLE) ***(Mc - Neil et al., 1991)*** . They are usually of IgG and IgM isotypes clinically diagnosed SLE have been thought to result in an increased risk of pregnancy loss .

Accordingly interest in autoimmune causes of level of APL - Ab were developed in women with unexplained repeated fetal loss ***(Dudly and Branch 1989)*** .

These APL - Ab include lupus anticoagulant (LA) and anticardiolipin (ACL) antibodies .

LA antibodies can be detected by prolongation of activated partial thromboplastin time (APTT) ***(Branch et al., 1987),*** but these tests used in detection of LA have low sensitivity for the prediction of adverse pregnancy out comes who had APL - Abs ***(Lockwood et al., 1989)*** .

ACL-IgG isotype considered as specific predictor of risk in fetal loss. ELISA is the most common method for detection of ACL associated with repeated fetal loss ***(Harris et al., 1983)*** .

AIM OF THE WORK :-

The aim of this study is to detect the level of anticardiolipin antibodies in patients with recurrent unexplained pregnancy loss to find out wheather ACL-Ab play arole in this condition or not .

"Chemistry and physical Properties of phospholipids"

Phospholipids are lipids containing in addition to fatty acid and an alcohol, a phosphoric acid residue . They are composed of a glycerol backbone with phosphodiester group at C3 linked to apolar head group alcohol, and two esterified Fatty acid chains at Cl and C2 (***Harper et al., 1977)*** .

Naturally occurring phospholipids contain saturated Fatty acids at Cl locus, but those at C2 position are usually unsaturated ***(Merill & Nichols 1985).*** Phospholipids derive their names from the head group alcohol. In human cells, these groups consist of either a nitrogenous base (Choline, ethanolamine or Serine), glycerol, inositol or sphingosine. the resulting phospholipids are :-

1 - phosphatidic acid :- "PA"

2 - phosphatidyl choline "PC"

3 - phosphatidyl ethanolamine "PE"

4 - phosphatidylserine "PS

5- phosphoglyceride "PG" (Cardiolipin)

6 - plasmologens.

7 - Sphingomyelins (SM) and

8 - phosphatidy Inositol "PI". ***(Harper et al., 1977)***

1) phosphatidic acid :- "PA"

PA is the simplest phospholipid which the alcohol is absent. It's molecule is referred to as "phosphatidyl" component in more complex structures. It is important in the synthesis of triglycerols and phospholipids. It is not found in great quantity in tissues.

2) phosphatidyl choline :- (Lecithin) "PC"

The lecithins contain glycerol and fatty acids as do the simple fats, but they also contain phosphoric acid and choline. The lecithins are widely distributed in the cells of the body. Dipalmityl lecithin is a very effective surface active agent preventing adherence due to surface tension, of the inner surfaces of the lung *(Harper et al., 1977)*

3)phosphatidyl ethanolamine (Cephalin) "PE"

Cephalin differs from lecithins only in that ethanolamine replaces choline., trimethylation of cephalin produceslecithin.*(James & Otto 1982)*

4) Phosphatidyl serine "PS"

It contains amino acid serine, It is found in tissues *(Harper et al., 1977)* e.g white matter (or myelin) of the nervous system has higher amounts *(Advan 1970)*

5) Phosphoglyceride (P.G)

This phosphoglyceride has phosphatidyl glycerol linked to the basic phosphatidyl till making it diphosphatidyl (Cardiolipin "CL") which is

unique in containing two diester phosphates linked by glycerol .

Pangborn (1947) who first discovered this phospholipid, reported his observation on partial hydrolysis of cardiolipin to give mixed fatty acids composed mainly of unsaturated fatty acids in the form of linoleic acid 72%, oleic acid 11%, linolenic acid 8% and palmitoleic acid 5.2%.

Cardiolipin constitutes a bout 11% of the mitochondrial outer membrane lipid and about 76% of inner membrane lipid ***(Guernieri & Stechmiller 1971);*** CL is high in the heart, little in brain, and not existed in red blood cells, it has an important role in cytochrome oxidase ***(Meod et al., 1986)***.

(Green & Castell 1987) suggested that one to two molecules of CL are deeply buried among the polypeptides of the enzyme complex, So,CL concentration in various tissues reflects the activity of that tissue.

6) Plasmologens:-

These compounds constitute as much as 10% of the phospholipids of brain and muscles ***(Harper et al., 1977)***

7) Sphingomyelins :- "SM"

Sphingomyelins are found in large quantities in brain and nerve tissues. On hydrolysis the sphingomyelins yield a fatty acid, phosphoric acid. choline and a complex amino alcohol,(Sphingosine)***(Harper et al., 1977).***

8) Phosphatidyl Inositol "PI" (lipositol)

"It was found to occur in phospholipids of brain tissues and of soyabeans as well as in other plant phospholipids *(Harper et al., 1977)*

The electrical charge of phospholipids :-

The chemical structure of the polar head group alcohol of phospholipids determines the net electrical charge or ionic state of the phospholipid. Pc, PE and SM are electrically neutral having both a negatively charged phosphate group and positively charged amino acid (i.e zwitter - ionic). PS,PG,PI and CL are all negatively charged or anionic *(MC–Neil et al., 1991).*

The physical properties of phospholipids:-

These can be studied in model membrane systems, simplest of which consists of a dispersion of the lipid in an aqueous buffer. In these dispersion, phospholipids will adopt one of three structures indicated in fig1. These are the micellar phase, the bilayer or lamellar phase typical of biological membranes and the hexagonal phase *(Cullis et al., 1985).*

The phase that phospholipid will a dopted depends on factors intrinsic to the molecule, such as nature of head group, length and degree of unsaturation of the fatty acid chains and extrinsic factors such as hydration, temperature, PH, ionic strength and presence of divalent cations (ca+), other lipids, and proteins *(Tilcock 1986).*