Apolipoprotein E polymorphisms in cases of recurrent pregnancy loss.

THESIS

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بسم الله الرحمن الرحيم

" وعلمك ما لم تكن تعلم

وكان فضل الله عليك عظيما"

صدق الله العظيم

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ABSTRACT

Recurrent pregnancy loss is one of the serious complications of pregnancy. Apolipoprotein E isoforms have been implicated in the development of this complication.The aim of this study is to evaluate the role of Apo E isoforms in inducing recurrent pregnancy loss .This study was conducted on 30 women experiencing recurrent pregnancy loss after excluding other causes and 20 healthy fertile control women. The genotype analysis involved DNA isolation followed by PCR amplification, then reversed hybridization using test strips containing allele specific oligonucleotide probes.Our results revealed that Apo E4 allele was significantly decreased in cases of recurrent pregnancy loss compared to controls while Apo E2 and Apo E3 had no significant effect.

Keywords:Apolipoprotein E, recurrent pregnancy loss, Apo E polymorphisms, Apo E isoforms.

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LIST OF ABBREVIATIONS:

AD	Alzheimer Disease
APC	Activated Protein C
Apo	Apolipoprotein
ARMS	Amplification Refractory Mutation System
cAMP	Cyclic adenosine monophosphate
CETP	Cholesteryl ester transfer protein
DVT	Deep Venous Thrombosis
	Epsilon
EDTA	Ethylenediaminetetraacetic acid
FER	Fractional esterification rate
HDL	High Density Lipoprotein
HMG-Co A reductase	3-hydroxy-3-methyl-glutaryl-CoA reductase
IDL	Intemediate Density Lipoprotein
KDa	Kilodalton
LDL	Low Density Lipoprotein
LPL	Lipoprotein lipase
MALDI TOF MS	Matrix-assisted laser desorption/ionization time of flight mass spectrometry
MTHFR	Methylenetetrahydrofolate reductase
PAI	Plasminogen Activator Inhibitor
PCR	Polymerase chain reaction
PNA	Peptide nucleic acid
RFLP	Restriction Fragment Length Polymorphism
RPL	Recurrent pregnancy loss
SNPs	Single nucleotide polymorphism

SSCP	Single Strand Conformation Polymorphism
TG	Triglycerides
VLDL	Very Low Density Lipoprotein

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INTRODUCTION
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INTRODUCTION:

Apolipoprotein E (Apo E) is a protein containing 299 amino acids synthesized in the liver and other organs ***(Eichner et al, 2002).*** It plays a key role in the metabolism of cholesterol and triglycerides by being the primary ligand for two receptors, the LDL receptor found on the liver and other tissues and an Apo E-specific receptor found on the liver. By binding to receptors in the liver, Apo E mediates clearance of chylomicrons and VLDL from the bloodstream ***(Mahley, 1988).***

The Apo E gene has three alleles – epsilon 2 (ε2), epsilon 3 (ε3), and epsilon 4 (ε4) – on the long (q) arm of chromosome 19 at position 13.2 ***(Scott et al, 1985 and Eichner et al, 2002).*** These isoforms differ in amino acid sequence at positions 112 and 158 ***(Rall et al, 1982).***

Apo E4 plays an important role in Alzheimer's disease ***(Parker et al, 2005 and Van der Flier et al, 2006)*** and in cognitive function ***(Bunce et al, 2004 and Blair et al, 2005).***

Individuals carrying the E4 allele have a higher total cholesterol level than people with the most common E3/E3 genotype, whereas those harboring the E2 allele have a lower total cholesterol level than those with E3/E3 (***Dallongeville et al, 1992*** and ***Hagberg et al, 2000).***

The E4 isoform of Apo E is associated with an increased risk of atherosclerosis and narrowing of arteries and subsequently coronary heart disease ***(Song et al, 2004 and Bennet et al, 2007).*** The mechanism that leads to this increased risk is unclear. The E4 isoform binds more weakly to LDL receptor than E3, thus decreasing clearance of excess cholesterol chylomicrons from the bloodstream (***Bennet et al, 2007.)***

Apo E4 could act in a similar way to increase the thrombosis of placental vessels and decrease placental blood flow leading to pregnancy loss or compromise. As pregnancy is a hypercoagulable state, it is not surprising then that the additive effect of an Apo E4 genotype superimposed on this hypercoagulable state would be expected to increase the risk of clotting (***Greer, 2000).*** Pregnant women are at increased risk for thromboembolism compared with non-pregnant women, with an underlying rate of venous thrombosis of about one per thousand pregnancies (***Pabinger et al, 2002).***

Apo E polymorphisms have been shown to play an important role in the lipid metabolism during pregnancy ***(McGladdery and Frohlich , 2001)***. Apo E polymorphism and pre-eclampsia has been studied and it was found that Apo E does not play a major role in the development of pre-eclampsia (***Makkonen et al, 2001*** and ***Francoual et***

al , 2002). It was suggested that the differential glycosylation of Apo E3, rather than protein sequence variants, is the probable explanation for preeclampsia ***(Atkinson et al, 2009)***.

The impact of Apo E polymorphisms on reproductive efficiency has been previously published. One study found the Apo E2 allele to be associated with the lowest reproductive efficiency, the E3 allele the highest and the E4 intermediate **(*Corbo et al , 2004).*** Another study looked at the role of Apo E polymorphisms in embryonic development suggesting that E4 may have protective effects during embryogenesis (***Zetterberg et al , 2002)*.**

The relationship between Apo E polymorphisms and recurrent pregnancy loss has been recently recognized, but it is not yet thoroughly investigated and there is little literature discussing this aspect.

It was found that Apo E4 allele (E3/E4 and E4/E4 genotypes) is higher in patients experiencing recurrent pregnancy loss (***Goodman et al, 2009).*** Another more recent study concluded that there is no association between Apo E polymorphisms and recurrent pregnancy loss ***(Bianca et al, 2009).***

AIM OF WORK:

The aim of our study is to evaluate Apo E isoforms in inducing recurrent pregnancy loss. Therefore, Apo E polymorphisms will be studied in patients suffering from recurrent pregnancy loss compared to healthy controls after excluding other causes of pregnancy.

REVIEW OF LITERATURE