

Original Research Article**Preeclampsia Risk and AGT M235T Gene in North India**

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Email: 0522rakesh@gmail.com**ABSTRACT****Background:** *Pregnancy induced hypertension is considered to be a multifactorial and multisystemic disorder with a genetic predisposition.***Aim:** *The aim of this original research article is to investigate the frequency of Angiotensinogen gene (AGT) Polymorphism in hypertension associated pregnancies as preeclampsia & eclampsia with normotensive uncomplicated pregnancies.***Method:** *This study was done in Rama Medical College & Hospital Kanpur. Blood sample from 102 cases of PIH and 100 cases of normotensive pregnant women collected and genomic DNA from each individual was isolated and genotyped for the M235T polymorphism of the angiotensinogen gene (AGT).***Result:** *Genomic investigation revealed that genotyped for the M235T deletion polymorphism of the angiotensinogen gene (AGT) is present in hypertension associated pregnancies as preeclampsia & eclampsia and absent in normotensive uncomplicated pregnancies.***Conclusion:** *Hypertension during the pregnancy is associated with M235T polymorphism of the angiotensinogen gene (AGT).***Keywords:** *PIH, Preeclampsia, Eclampsia, M235T, Angiotensinogen Gene, AGT, Pregnancy, Hypertension.***Introduction**

Preeclampsia is a progressive, multisystem disorder unique to pregnant women and is a leading cause of maternal death and contributes significantly to premature delivery.

It is characterized by hypertension & Proteinuria and its incidence is influenced by various factors such as parity, race & environmental factors ^[1].

The aetiology of preeclampsia is still unknown but genetic factor have been implicated since the syndrome show familial tendency ^[2].

Published report of pedigree analysis suggests that development of preeclampsia may be based on a single recessive gene or dominant gene with incomplete penetrance ^[3].

However more recent studies have suggested that the pattern of inheritance in multifactorial and depends on several genetic loci with greater or smaller contribution from environmental factor. Maternal pre-eclampsia was associated with 70% excess risk of pre-eclampsia in daughters in a geographical area of Sweden [4].

In addition not only maternal gene but also foetal gene may be implicated and maternal foetal interaction could not be ignored. The role of the placenta in the primary pathogenesis of the disorder indisputably indicates a fetal contribution to susceptibility to the disorder [5]. Reports of severe, very early-onset pre-eclampsia in cases of fetal chromosomal abnormalities such as diandric hydatidiform moles of entirely paternal genetic origin [6] are consistent with a role for paternally inherited fetal genes in the determination of clinical phenotype. This is supported by epidemiological studies reporting a higher rate of pre-eclampsia in pregnancies fathered by men who were themselves born of pre-eclamptic pregnancies [7].

Deciphering the genetic involvement in pre-eclampsia is challenging, not least because the phenotype is expressed only in parous women. Furthermore, in complex disorders of pregnancy, it is necessary to consider two genotypes, that of the mother and that of the fetus, which includes genes inherited from both mother and father. Maternal and fetal genes may have independent or interactive effects on the risk of pre-eclampsia [8].

Aim and Objective

The aim of this original research article is to investigate the frequency of Angiotensinogen gene (AGT) Polymorphism in hypertension associated pregnancies as preeclampsia & eclampsia with normotensive uncomplicated pregnancies.

Material Method

This study was done in Central Research Lab, Rama Medical College, Kanpur. Two Hundred two blood samples, each with 2ml Whole blood in EDTA vial collected from the Rama Hospital,

Rama Medical College, Kanpur. One Hundred two blood samples of this were from the women diagnosed for PIH and remaining one hundred from normotensive uncomplicated pregnancy.

Inclusion Criteria

- Gestational hypertension Without proteinuria or pathological oedema
- Pre-eclampsia-Hypertension and proteinuria with or without pathological oedema.
- Eclampsia – Pre-eclampsia complicated with convulsions and / or coma.
- Pre-eclampsia or eclampsia superimposed on chronic hypertension

Exclusion Criteria

- Chronic hypertension
- Essential hypertension
- Chronic renal disease (reno vascular)
- Coarctation of aorta
- Pheochromocytoma
- Thyrotoxicosis
- Connective tissue disease-systemic lupus erythematosus
- Pre-existing Diabetes mellitus (IDDM-Type 1)
- Pre-existing Diabetes mellitus (NIDDM-Type 2)
- Gestational Diabetes Mellitus (GDM)
- Twins Pregnancy

The blood samples collected from the Rama Hospital, Rama Medical College Kanpur, soon stored at -20°C in Central Research Lab, Rama Medical College, Kanpur.

Primer for Angiotensinogen polymorphism (AGT) in PIH was designed by integrated DNA technology 5'-GAT GCG CAC AAG GTC CTG TC-3' as forward primer

And 5'-GCG CGC GCC AGC AGA GAG GTT TGC CT-3' as reverse primer.

Primer designed with specification

S.No	Name	Sequence (5'-3')	Length	Vol. for 100 micro M	O.D	nMoles	MicroG	%GC	Tm °C	MW	Purification	Modification
1	Forward Primer	GAT GCG CAC AAG GTC CTG TC	20	296.4	6.3	29.6	181.3	60	56	6118	Desalted	None
2	Reverse Primer	GCG CGC GCC AGC AGA GAG GTT TGC CT	26	284.6	7.8	28.5	228.1	69.2	67	8013.2	Desalted	None

Qiagen Kit for whole blood used for extraction of DNA and standard protocol followed as provided in the Qiagen kit for DNA extraction.

Other materials used in the procedure are Agarose powder, PCR master mix kit (2x), PCR tube 0.2 micro L, 50X TAE buffer 200ml, 6X cresol-blue DNA loading dye, Eppendorf tube 1.5ml, micro tips 0.2 – 10 µl, micro tip 1ml, micro tips 100 – 200 µl, 1Kb DNA Ladder, Ethidium di bromide, double distilled water and Ethanol 99%.

Prepare Casting Tray: Swab casting tray by sprite>Seal the side by adhesive tape>insert the comb.

Prepare Agarose Gel: 1gm agarose powder add 98ml Double distilled water and 2ml TAE buffer>Boil 5min>Get cool as warm add 2 µl Etbr>Pour in casting tray>Get it cool to solidify>Take out comb, well ready.

Run Electrophoresis Gel: Add 600ml double distilled water and 10ml TAE buffer in electrophoresis chamber and place agarose gel>fill each well by all together 2µl 6xDNA loading dye, 2µl Double distilled water, 3µl DNA sample>Run electrophoresis @ 90 Mile Volt for 30 min>Place gel in BioRad>Run Image Lab> Save Photo for DNA identification.

Prepare Primer for action: - Follow standard protocol as per primer sheet provided and inject TE buffer and mix well. Now take 90 µl primer as storage solution and 10 µl in eppendoff tube as working solution and add 90 µl water which is protease, DNA, RNA free in working solution. Store both at -20°C.

Prepare PCR Solution for Test: - PCR tube>add 5µl water>1 µl Forward Primer>1 micro L Reverse Primer>3 µl DNA>10 µl Master Mix>20 µl PCR Solution Ready.

Prepare PCR Solution for Control: PCR tube>add 8µl water>1 µl Forward Primer>1 µl Reverse Primer>3 µl DNA>10 µl Master Mix>20 µl PCR Solution Ready.

Run PCR Machine: Set the PCR program as per standard protocol at melting temperature of 56°C and number of cycles are 35. Set the step 6th at 72°C and run.

It takes about 2 hrs to complete the amplification and finally store the PCR product at -20°C.

Electrophoresis for PCR product: Add 2 µl 6X DNA loading dye in each PCR product test & control>mix>Inject each in well>Inject 2 µl DNA ladder for reference>Run @90mv for 40 min> Place gel in BioRad>Run Image Lab> Save Photo for gene expression.

DNA Sequencing: The obtained DNA was sequenced by Chromous Biotech Limited at Bangaluru. The DNA band was cut and purified with the use of Quaigen kit and submitted for the sequencing.

Observation

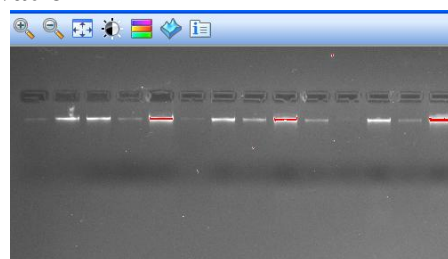


Figure 1: DNA identified in Control Sample using BioRad

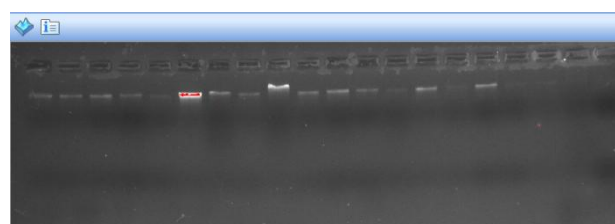


Figure 2: DNA identified in Test Sample using BioRad

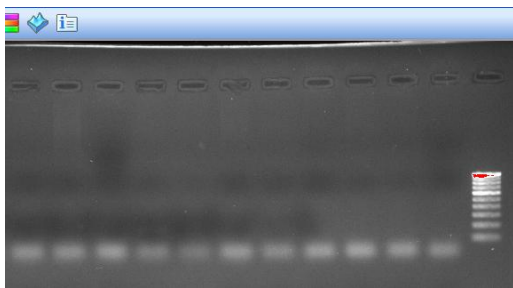


Figure 3: None of the AGT gene expressed in Control Sample after PCR in BioRad

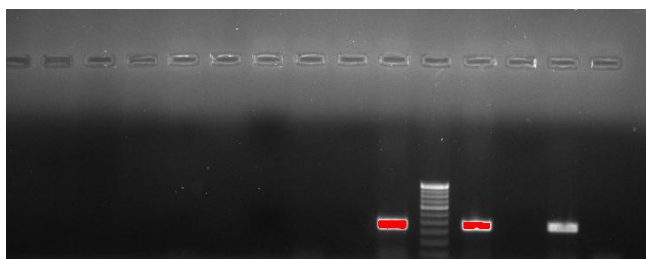


Figure 4: AGT gene expressed in Test sample after PCR in BioRad

By sequencing the gene the gene was found 659 bp long and with the use of NCBI and CLUSTALw the homology of our gene shows upto 294 bp. The fragment of DNA are showing the 99 % of homology with subject.

Homo sapiens angiotensinogen (AGT), mRNA Sequence ID: NM_000029.3 Length: 2587 Number of Matches: 2, Related Information Gene-associated gene details, GEO Profiles-microarray expression data Map Viewer-aligned genomic context Range 1: 1076 to 1369 GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
520 bits(281)	1e-144	290/294(99%)	1/294(0%)	Plus/Plus

```

Query                                     252
CTGCTAGTGG-
CCAGGGCAGGGCTGATAGCCAGGCCAGC
TGCTGCTGTCCATGGTGGTG 310
|||||
Sbjct                                     1076
CTGCTAGTGGCCAGGGCAGGGCTGATAG
CCAGGCCAGCTGCTGCTGTCCACGGTGG
TG 1135
    
```

```

Query                                     311
GGCGTGTTCACAGCCCCAGGCCTGCACCT
GAAGCAGCCGTTTGTGCAGGGCCTGGCTC
TC 370
    
```

```

Sbjct                                     1136
GGCGTGTTCACAGCCCCAGGCCTGCACCT
GAAGCAGCCGTTTGTGCAGGGCCTGGCTC
TC 1195
    
```

```

Query                                     371
TATACCCTGTGGTCTCCACGCTCTCTG
GACTTCACAGAACTGGATGTTGCTGCTGA
G 430
    
```

```

Sbjct                                     1196
TATACCCTGTGGTCTCCACGCTCTCTG
GACTTCACAGAACTGGATGTTGCTGCTGA
G 1255
    
```

```

Query                                     431
AAGATTGACAGGTTTCATGCAGGCTGTGAC
AGGATGGAAGACTGGCTGCTCCCTGACGG
GA 490
    
```

```

Sbjct                                     1256
AAGATTGACAGGTTTCATGCAGGCTGTGAC
AGGATGGAAGACTGGCTGCTCCCTGATGG
GA 1315
    
```

```

Query                                     491
GCCAGTGTGGACAGCACCCCTGGCTTTCAA
CACCTACGTCCACTTCCAAGGTAAG 544
    
```

```

Sbjct                                     1316
GCCAGTGTGGACAGCACCCCTGGCTTTCAA
CACCTACGTCCACTTCCAAGGGAAG 1369
    
```

Result

S. No	Sample	AGT Gene Expressed
1	100 Control	Zero
2	102 Test	26

It has found that zero AGT genes expressed in Normotensive pregnancy but 26 cases in PIH

found for M235T Angiotensinogen gene polymorphism (AGT).

Discussion

Hypertension and preeclampsia are similarly complex and multifactorial disorders with genetic and environmental elements. Numerous and extensive analysis of polymorphisms of the angiotensinogen gene reveal a relationship to severe hypertension and cardiovascular morbidity and mortality^[9].

Human studies have demonstrated an association of increased copies of the AGT gene and increased plasma AGT concentrations with elevated blood pressure^[10]. Animal studies using transgenic mouse and rat models which overexpress AGT and renin genes similarly show elevated blood pressure, endothelial dysfunction and renal abnormalities^[10,11].

The results of our exploratory study are consistent with some but not all prior studies of M235T and preeclampsia; Ward reported an association in a population of Caucasian women in Utah, and Kobashi reported a positive association in a Japanese population^[12,13]. Levesque using a large Caucasian French Canadian population did not find an association with M235T, however reported an association with T174M^[14].

In our study it has found that the AGT gene expressed in women who develop Preeclampsia and eclampsia, where as it remain unexpressed in women who do not develop hypertension during pregnancy.

AGT M235T polymorphism is associated with Pre-eclampsia in Chinese women. Furthermore, the gene polymorphism of the components of the renin-angiotensin system may contribute to the concentration alterations of sFlt1, VEGF, and PlGF in maternal serum, which causes disordered vasculogenesis contributing to Pre-eclampsia^[15].

Conclusion

Studies with large sample sizes adjusting for various confounding factors should be designed to confirm that

- 1) AGT M235T is associated with hypertension
- 2) AGT M235T is associated with PIH (Preeclampsia and Eclampsia)
- 3) AGT M235T is not associated with Normotensive Pregnancy
- 4) AGT M235T is associated with North Indian Population

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