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Association of *Adiponectin* (*ADIPOQ*) Gene Polymorphism with Gestational Diabetes Mellitus

Abdullah F. Yousif [1*], Raid M. H. Al-Salih[2] and Alaa H. Al-Naser[3]

[1]Directorate of Education Thi Qar, Ministry of Education.

[2]College of Science, University of Thi-Qar, Iraq

[3]College of Medicine, University of Thi-Qar, Iraq

*E-mail : Abd.fak_ch@utq.edu.iq

ABSTRACT

Gestational diabetes is defined as a carbohydrate intolerance that is first diagnosed during pregnancy and affects the health of the mother and the fetus. Having a specific genetic polymorphism can be a predisposing factor to developing some diseases. This study aimed to investigate the linked between *ADIPOQ* gene SNP (rs 2241766) with the development of GDM. Genomic DNA from blood samples were extracted by using DNA extraction kit (Geneaid, Uk), then *ADIPOQ* gene was amplified through conventional PCR and SNP was discover using restriction enzyme *Sma*I. The results showed that there was a marked association between genotypes and alleles for SNP 45 T / G and GDM. GG genotype (O.R. = 1.94, C.I. 95% = 0.82-4.58), TG genotype (O.R. = 1.43 , C.I. 95% = 0.58-3.52) and G allele (O.R. = 2.27 , C.I. 95% = 1.24-4.13) it was more common in the GDM group compared to the control group. The results show genotypes GG, TG and allele G frequencies increased in GDM group (46.7%, 35.5% and 64.4%, respectively) than in control group (31.1%, 26.7% and 44.4%, respectively). In other hand, the genotype TT and allele T showed decreased frequencies in GDM group (17.8 and 35.6%, respectively) than in control group (42.2% and 55.6%, respectively).

Keywords: *ADIPOQ* gene, SNP (rs 2241766), Gestational diabetes mellitus, RFLP.

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INTRODUCTION

The most modern definition of GDM is by the American Diabetes Association(ADA), as GDM has been defined as diabetes diagnosed in the second or third trimester (24-28 weeks) of pregnancy that was unclear of diabetes before pregnancy (ADA, 2020). GDM is a pregnancy complication characterized by impaired glucose tolerance, and it develops when the pancreatic cell reserve is not enough to compensate for the increased infrared radiation during pregnancy. As a result, there is an increase in liver glucose formation (gluconeogenesis), severe insulin resistance and consequently hyperglycemia (Mohammad, 2015).

GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. In fact, women with GDM are at a higher risk of developing or developing diabetes when they are not pregnant. As a result, GDM offers a unparalleled opportunity to study diabetes' early pathogenesis and to develop interventions to prevent the disease (Eades *et al*., 2017 and Lee *et al*., 2018). GDM has been linked with several adverse effects affecting the health status of mothers and newborns in the short and long term (Shriraam *et al*., 2013 and Sajani *et al*., 2014). These negative effects create an overburden on the health-care system's budget for antenatal care, intranasal care, and infant care, as well as the extended postpartum recovery (Danyliv and O'Neill, 2015).

The *ADIPOQ* gene is located on chromosome 3 at 3q27 and consists of five exons with a total length of 15,789 base pairs (bp). It is mainly expressed in adipose tissue, encodes proteins consisting of 244 amino acid residues, and is the main component of adiponectin that has the ability to influence metabolism and hormone synthesis in the organism (Xu *et al*., 2014). This study aimed to investigate the linked between *ADIPOQ* gene SNP (rs 2241766) with the development of GDM.

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MATERIALS AND METHODS

The Primers Used in the Interaction

The primers were lyophilized, dissolved in free ddH_2O to give a final concentration of 100 pmol/L as stock solution, and stored at -20 °C to prepare a 10 pmol/L concentration as work primer suspended, 10 µL of the stock solution in 90 µL of free ddH_2O water to reach a final volume of 100 µL. The molecular weight markers used in this study for DNA and PCR products were from Korea. The range of the markers were (100-2000 bp).

The Specific Primers of *ADIPOQ* Gene

Primers	Sequence	Tm (°C)	Product size	Reference
Forward	5'- GAAGTAGACTCTGCTGAGATGG -3'	57.06	372 bp	Takhshid *et al.*, 2015
Reverse	5'- TATCAGTGTAGGAGGTCTGTGATG -3'	57.24		

After several experiments to determine the optimal condition for (Initial denaturation and annealing), the temperature has changed through the work of (Gradient PCR) for all samples to determine the optimal condition, and the concentration of DNA template was changed between (1.5-3 µL), where these two factors were considered important factors in primer annealing with complement (Calkavur *et al*., 2015).

Extraction of Genomic DNA:

Genomic DNA was extracted from blood samples using the gSYAN DNA (frozen blood) Geneaid / UK extraction kit, and this was done according to the company's instructions.

Polymerase Chain Reaction:

Polymerase Chain Reaction (PCR) is a technique for amplifying a particular DNA sequence selectively. A pair of 18-24 bp oligonucleotide primers that are complementary to the unique sequences on each DNA strand flanking the target region are required for PCR. The stranded double-strand DNA was initially separated by high temperatures, and the subsequent cooling allowed the primers to solidify into complementary sequences. These primers were extended by a thermostable DNA polymerase to form the opposite strand. After that, the new double-stranded DNA (dsDNA) was denatured, and the

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procedure was repeated, resulting in exponential amplification of the specific DNA fragment (Mullis *et al*., 1986).

Agarose Gel Electrophoresis of DNA

Electrophoresis was performed to identify pieces of DNA after extraction or to detect the PCR result while the standard DNA was present to differentiate the beam size of the PCR result on the agarose gel.

Polymerase Chain Reaction Assay for Detection of the *ADIPOQ* Gene

The Maxime PCR PreMix Kit from Intron includes not only a variety of PreMix Kits based on experience, but also a 2X Master mix solution. Maxime PCR PreMix Kit (i-Taq) is the product what is mixed all component: i-Taq DNA Polymerase, DNTP admixture, reaction buffer, and so on-in one tube for 1 rxn PCR. This is the output that can obtain the best result with the most suitability systems. The first explanation is that it contains all of the required components for PCR, so we can do PCR just add a template DNA, primer set, and D.W . The second explanation is that it has a gel loading buffer to do electrophoresis, so we can do gel loading without any treating. It is favorable for different sample's experience by rapid and easy using method.

Table (1): Mixture of the specific interaction for determine *ADIPOQ* gene

Components	Volume
DNA	3 µL
Forward primer	10 picomols / µL (1 µL)
Nuclease free water	15 µL
Reverse primer	10 picomols / µL(1µL)
i-Taq PCR Pre Mix	5 µL
Final volume	25 µL

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Table (2): Reaction condition of restriction enzyme *Sma*I

Reagent	Volume
10x Buffer tango	3 µL
Nuclease free water	1 µL
PCR Product	5 µL
Restriction Enzyme *Sma*I	1 µL
Final volume	10 µL

Table (3): The optimum condition of detection *ADIPOQ* gene

No.	Step	T (°C)	Time	No. of cycle
1.	Initial Denaturation	95 °C	5 min.	1
2.	Denaturation-2	95 °C	30 sec	35
3.	Annealing	58 °C	30 sec	
4.	Extension-1	72 °C	30 sec	
5.	Extension -2	72 °C	10 min.	1

Statistical analysis

All statistical analysis was done using statistical package for the social sciences version 22. Odds ratio were used to quantifies the strength of the association between genotypes, alleles of *ADIPOQ* gene.

RESULTS AND DISCUSSION

Extraction of Genomic DNA

In order to study the genetic polymorphism of women with GDM and compared them to control women so the genomic DNA was extracted from blood samples of those subjects under study by using Genomic DNA extraction kit (Geneaid, UK) and in order to check the extraction procedure the eluted DNA were electrophoresed on an agarose gel (0.8%) and visualized by UV light as seen in Figure (2) a high molecular weight DNA bands.

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Figure (2): Gel electrophoresis of genomic DNA extracted from blood samples and ran through 0.8% agarose gel at 5 v/cm^2 for 1:20 hour.

Assessment of the Purity and DNA Concentration

It was determined (concentration and purity) by using Nanodrop spectrophotometer at 260/280 nm. Results were clarified in Table (4).

Table (4): purity and DNA concentration

	Mean ± SD	**Range**
DNA Concentration (ng/µL)	176.5±16.39	75.4-340.7
DNA Purity	1.92±0.11	1.6-2.1

Amplification of *ADIPOQ* Gene

The results obtained from polymerase Chain Reaction (PCR) was done for the detection of adiponectin gene in samples DNA extracted from pregnant women with and without GDM. The amplification of the *ADIPOQ* gene gave positive results for all the subjected samples (Figure 3). After the PCR technique have been done the products were gel electrophoresed as illustrated in Figure (3) a single band of amplified products with a molecular size of 372 bp.

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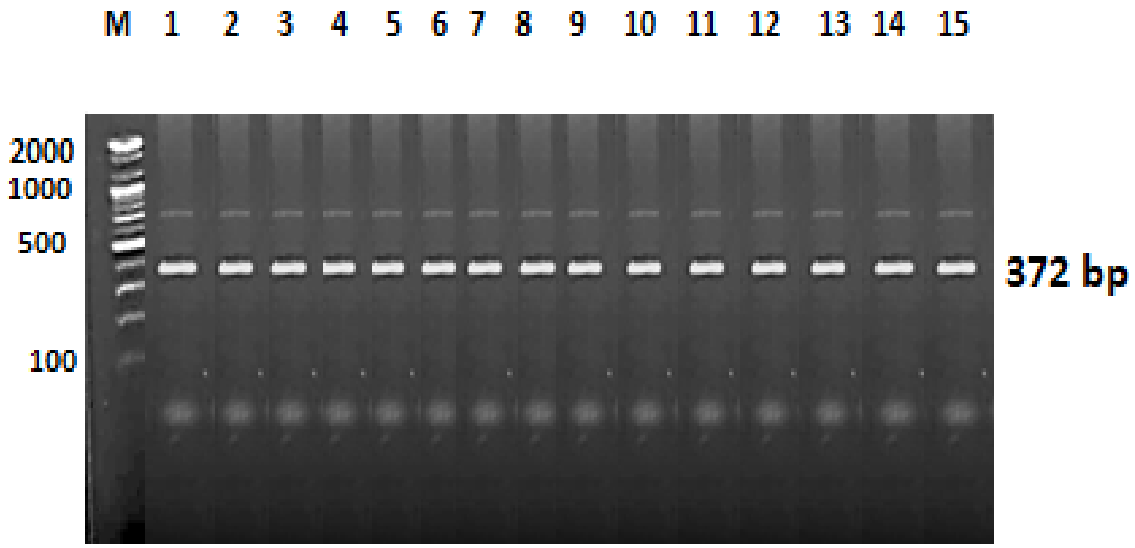

Figure (3): Gel electrophoresis for PCR products run on an agarose gel (1.5%) for 1:20 hour at 5 v/cm^2 in the presence of DNA Ladder marker (100-2000 bp).

Restriction Fragments Length Polymorphism (RFLP)

The polymorphism rs 2241766 have been detected by many researcher by using PCR-RFLP analysis technique. The results will create digestion fragments visible on agarose gel as the polymorphism result in the creation of a DNA sequence recognized by the restriction enzyme *sam*I. Patients have a wild types (TT) gene exhibited 372 bp fragment only. Those of heterozygote (TG) type show a three bands with a sizes of 163 bp, 209 bp and 372 bp; while, those of the homozygote (GG) type yielded 2 fragments of 163 bp and 209 bp sizes, as seen in (Table 5 and Figure 4).

Table (5): Digestion products and band size of *ADIPOQ* gene SNP (rs 2241766)

Genotype		No. Bands	Bands size (bp)
Wild type	TT	1	372
Homozygous	GG	2	163 and 209
Heterozygous	TG	3	163, 209 and 372

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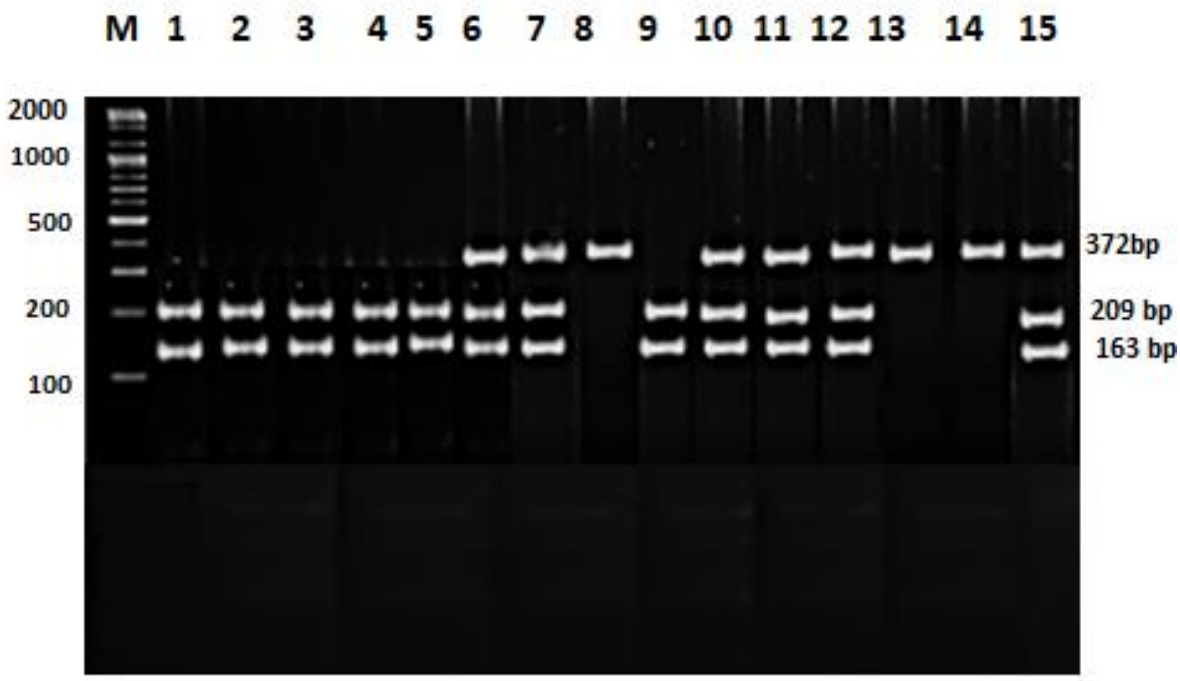

Figure (4): Gel electrophoresis of RFLP-PCR products. The RFLP-PCR product of SNP (rs 2241766) was digested by the restriction enzyme *Sma*I. The products of digestion were analyzed by 3% agarose gel electrophoresis visualized directly by ethidium bromide under ultra-violet illumination indicated the presence of the allele.

Line M: Marker of DNA

Lines 1, 2, 3, 4, 5 and 9: For individuals have the homozygote (GG) genotype exhibited two fragments of 163 bp and 209 bp sizes. Lines 6, 7, 10, 11, 12 and 15: For individuals have the heterozygote (TG) genotype that showed three fragments with sizes of 163 bp, 209 bp, and 372 bp. Lines 8, 13 and 14: For individuals have the wild type (TT) of 372 bp fragment.

The genotype and allele frequencies of *ADIPOQ* gene SNP (rs 2241766) in the GDM and Control groups in addition the statistical analysis is showing in Table (6). The results show that there was a marked association between genotypes and alleles of SNP (rs 2241766) and GDM. The GG genotype (O.R. = 1.94 , C.I. 95% = 0.82-4.58), TG genotype (O.R. = 1.43 , C.I. 95% = 0.58-3.52) and G allele (O.R. = 2.27 , C.I. 95% = 1.24-4.13) were more common in the GDM group compared to the control group. The

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results show genotypes GG, TG and allele G frequencies increased in GDM group (46.7%, 35.5% and 64.4%, respectively) than in control group (31.1%, 26.7% and 44.4%, respectively). In other hand, the genotype TT and allele T showed decreased frequencies in GDM group (17.8 and 35.6%, respectively) than in control group (42.2% and 55.6%, respectively). The relative risk for GDM : Genotypes GG, TG, TT , alleles G and T (1.500, 1.330, 0.421, 1.450 and 0.640 respectively).The relative risk for Control : Genotypes GG, TG, TT, alleles G and T (0.744, 0.879, 1.423, 0.640 and 1.450 respectively).

Table (6): Genotype and allele frequencies of *ADIPOQ* gene SNP (rs 2241766) in the GDM and control groups.

Genotype	Control No. (%)	GDM No. (%)	O.R.	C.I. (95%)	Relative risk for GDM	Relative risk for control
GG	14 (31.1%)	21 (46.7%)	1.94	0.82 - 4.58	1.500	0.744
TG	12 (26.7%)	16 (35.5%)	1.43	0.58 - 3.52	1.330	0.879
TT	19 (42.2%)	8.0 (17.8%)	0.30	0.11 - 0.78	0.421	1.423
Allele Frequency						
G	40 (44.4%)	58 (64.4%)	2.27	1.24-4.13	1.450	0.640
T	50 (55.6%)	32 (35.6%)	0.44	0.24-0.80	0.640	1.450

[O.R: Odds Ratio / **C.I. (95%):** Confidence Interval]

Bao *et al*. performed a systematic review and meta-analysis showing that adiponectin levels through the second trimester of pregnancy were decrease between pregnant women who later develop GDM than normal pregnant (Bao *et al*., 2015). Adiponectin may be involved in the pathogenesis of GDM. RS2241766 is synonymous with mutations in the exon 2 sub-area. Previous studies showed that *ADIPOQ* gene variation in two exons was closely concerning to expression of the same genes or adiponectin concentration. The genetic difference in *ADIPOQ* gene draw people's attentions as it might predispose humans to insulin resistance (Feng *et al*., 2019).

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Low *et al*. reveal that GDM patients who load the TG/GG genotype of adiponectin rs 2241766 had marked decrease plasma adiponectin levels than normal patients who load the TT genotype, denoting a possible role of the TG/GG genotype in plasma adiponectin levels in Malaysia (Low *et al*., 2011). Han *et al*. found that the GDM group diagnosed by the new International Association of Diabetes and Pregnancy Study Groups criteria had a higher distribution of TG/GG genotype and G allele frequency than the NGT group among Han women of the Nantong region in China. It was consider that adiponectin rs 2241766 might be linked with the propagation of GDM, and the G allele might be the final risk factor for GDM in Han women in this region (Han *et al*., 2015). RS 2241766 could possibly be linked with rising rate of weight gain in antenatal patients and decrease plasma adiponectin level thereafter reducing the risk of GDM. Beltcheva *et al*. found that T carriers shared no linked with T2DM and GDM in Bulgaria. Their results, however, could be essentially pronounced in individuals with the GTG haplotype, carriers of the main alleles of rs 2241766 (T), which have been found in various studies to be correlated with rise levels of adiponectin and maximal insulin sensitivity (Beltcheva *et al*., 2014).

Gueuvoghlanian-Silva *et al*.'s study specific that adiponectin rs 2241766 allelic variant was not linked with GDM, and no linked was found among adiponectin levels and this allelic variant (Gueuvoghlanian-Silva *et al*., 2012). In other study, Rizk *et al*. investigated the linked of two SNPs of *ADIPOQ* gene (+45 T/G and +276 G/T) and GDM. The results specific an linked among GG genotype of SNP +45 T>G (rs 2241766) and GDM among Arab residents in Qatar. They also found that there was no variation in the circulating adiponectin levels among GDM patients with various genotypes of SNP +45 T>G (rs 2241766) (Rizk *et al*., 2012). Beltcheva *et al*. examined the linked of three common SNPs of *ADIPOQ* gene (−11377 C/G, +45 T/ G and +276 G/T) and GDM. The results appear an linked among SNP +276 G/T and GDM, but not with anthers. Differences in demographic characteristics, genetic setting, environmental factors and diagnostic criteria for diagnosis of GDM could account for the discrepancy between the studies (Beltcheva *et al*., 2014). Given the reality that SNP +45 T>G (rs 2241766) is a synonymous mutation (Gly15Gly), the careful molecular mechanisms which would be accountable for linked of

SNP +45 T>G (rs 2241766) with GDM and T2D are yet obscure. A possible mechanism is the effects of this silent mutation on expression of *ADIPOQ* gene and circulating concentration of adiponectin. It has been reported that SNP +45 T>G (rs 2241766) in the *ADIPOQ* gene could affect the expression of adiponectin by influencing adiponectin mRNA processing or stability. It has also been recorded that SNP +45 T>G (rs 2241766) in the *ADIPOQ* gene has an linked with circulating levels of adiponectin (Takhshid *et al*., 2015).

CONCLUSION

The results appears that there was a marked correlated among genotypes and alleles of *ADIPOQ* gene SNP (rs 2241766) and GDM. The GG, TG genotypes and G allele of *ADIPOQ* gene SNP (rs 2241766) was an independent relative risk for GDM in inhabitance. Further studies are needed to explore the potential mechanisms by which SNP (rs 2241766) modulates predisposition to GDM.

REFERENCES

American Diabetes Association (ADA) (2020). "Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020 " *Diabetes Care* ,43(Supplement 1), S14-S31.

Bao, W. ; Baecker, A. ; Song, Y. ; Kiely, M. ; Liu, S. and Zhang, C. (2015). "Adipokine levels during the first or early second trimester of pregnancy and subsequent risk of gestational diabetes mellitus: a systematic review" *Metabolism*, 64(6), 756-764.

Beltcheva, O. ; Boyadzhieva, M. ; Angelova, O. ; Mitev, V. ; Kaneva, R. and Atanasova, I. (2014). "The rs266729 single-nucleotide polymorphism in the adiponectin gene shows association with gestational diabetes" *Archives of gynecology and obstetrics*, 289(4), 743-748.

Calkavur, S. ; Erdemir, G. ; Onay, H. ; Altun-Köroglu, Ö. ; Yalaz, M. ; Zekioglu, O. ; Aksu, G. ; Özkınay, F. ; Akercan, F. and Kültürsay, N. (2015). Mannose-binding lectin may affect pregnancy outcome. *The Turkish journal of pediatrics*, 57(1), 26-33.

Danyliv, A. ; and O'Neill, C. (2015). "Attitudes towards legalising physician provided euthanasia in Britain: The role of religion over time" *Social Science and Medicine*, 128, 52-56.

Eades, C. E. ; Cameron, D. M. and Evans, J. M. M. (2017). "Prevalence of gestational diabetes mellitus in Europe: a meta-analysis" *Diabetes Research and Clinical Practice*, 129, 173-181.

Feng, Y., Jiang, C. D., Chang, A. M., Shi, Y., Gao, J., Zhu, L., & Zhang, Z. (2019). "Interactions among insulin resistance, inflammation factors, obesity-related gene polymorphisms, environmental risk factors, and diet in the development of gestational diabetes mellitus" *The Journal of Maternal-Fetal & Neonatal Medicine*, 32(2), 339-347.

Gueuvoghlanian-Silva, B. Y. ; Torloni, M. R. ; Mattar, R. ; de Oliveira, L. S. ; Scomparini, F. B. ; Nakamura, M. U. and Daher, S. (2012). "Profile of inflammatory mediators in gestational diabetes mellitus: phenotype and genotype" *American Journal of Reproductive Immunology*, 67(3), 241-250.

Han, Y. ; Zheng, Y. L. ; Fan, Y. P. ; Liu, M. H. ; Lu, X. Y. and Tao, Q. (2015). "Association of adiponectin gene polymorphism 45TG with gestational diabetes mellitus diagnosed on the new IADPSG criteria, plasma adiponectin levels and adverse pregnancy outcomes" *Clinical and experimental medicine*, 15(1), 47-53.

Lee, K. W. ; Ching, S. M. ; Ramachandran, V. ; Yee, A. ; Hoo, F. K. ; Chia, Y. C. ; Sulaiman, W. A. W. ; Suppiah, S. ; Mohamed, M. H. and Veettil, S. K. (2018). "Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis" *BMC pregnancy and childbirth*, 18(1), 494, 1-20.

Low, C. F. ; Tohit, E. R. M. ; Chong, P. P. and Idris, F. (2011). "Adiponectin SNP45TG is associated with gestational diabetes mellitus" *Archives of gynecology and obstetrics*, 283(6), 1255-1260.

Mohammad, A. (2015). "Correlation between serum levels of adiponectin and tumour necrosis factor-alpha with insulin resistance index in gestational diabetes mellitus" Being a dissertation submitted to the national postgraduate medical college of Nigeria in partial

fulfillment of the requirement for the part II fmcpath (Chemical pathology) Examination.1-84.

Mullis, K. ; Faloona, F. ; Scharf, S. ; Saiki, R. ; Horn, G. and Erlich, H. (1986). "Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction" *In Cold Spring Harbor symposia on quantitative biology* , 51, 263-273.

Rizk, N. ; Sharif, E. A. L. M. ; Baloochi, S. M. and Pour, A. R. (2012). "The association of adiponectin gene polymorphism with gestational diabetes mellitus: The role of rs1501299 and rs2241766 variants" *Qatar Foundation Annual Research Forum*, 2012(1).

Sajani, T.T. ; Rahman, M. T. and Karim, M. R. (2014). "Maternal and fetal outcome of mothers with gestational diabetes mellitus attending BIRDEM Hospital" *Mymensingh medical journal*, 23(2), 290-298.

Sambrook, J. ; Fritschi, E. F. and Maniatis, T. (1989). "Molecular cloning: a laboratory manual" Cold Spring Harbor Laboratory Press New York.

Shriraam, V. ; Rani, M. A. ; Sathiyasekaran, B. W. C. and Mahadevan, S. (2013). "Awareness of gestational diabetes mellitus among antenatal women in a primary health center in South India" *Indian journal of endocrinology and metabolism*, 17(1), 146-148.

Takhshid, M. A. ; Haem, Z. and Aboualizadeh, F. (2015). "The association of circulating adiponectin and +45 T/G polymorphism of adiponectin gene with gestational diabetes mellitus in Iranian population" *Journal of Diabetes & Metabolic Disorders*, 14(1), 1-7.

Xu, J. ; Zhao, Y. H. ; Chen, Y. P. ; Yuan, X. L. ; Wang, J. ; Zhu, H. and Lu, C. M. (2014). "Maternal circulating concentrations of tumor necrosis factor-alpha, leptin, and adiponectin in gestational diabetes mellitus: a systematic review and meta-analysis" *The Scientific World Journal*, 926932.