

Association Serum Lipid Profile with Glutathione S- Transferas M and T genes Polymorphisms in Hypertension of Post-Menopausal Women

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ABSTRACT

Hypertension has been the most health problem in the world; it caused morbidity and mortality by its complications, some factors contributed in incidence this disease. The present study as cried out to investigate lipid profile types levels in post menopause women suffered from hypertension. Samples and data were collected from patinas and control, then lipid profile cholesterol, High Density Lipoprotein (HDL) , Triglycerides (TG) , Low Density Lipoprotein (LDL) and Very Low Density Lipoproteins (VLDL). The results show that mean of age was 58.135 ± 9.595 and 50.4 ± 5.834 for patient and control respectively, body mass index was 31.61 ± 6.86 , 29.99 ± 3.81 for patients and control respectively. More patients were overweight 48.27% and 40.54% of them have age less than 50 years. About 53.57% of patients had duration rang 5-10 years. The lipid profile levels were significant elevation in patients than control except TG , it were 447.83, 193, 414.43, 64.61 mg/dl for CHO, HDL, TG, LDL and VLDL respectively, The lipid profile levels positive associated with BMI in significant differences but didn't association with age and duration of disease. This results concluded that there was strong association between blood pressure and lipid profile level in post- menopausal women and this related with BMI in Iraq the genotyping of GST genes GSTM and GSTT show significant differences between GSTT deletion and normal in patients and control , 83.73% of patients GSTT gene was deleted. the deletion in GSTM was high in patients than control (32.43%). Pattern of two genes which represented by two genes normal or two genes deleted show significant differences between study groups about 24.32% of patients had two genes deleted and low percentage of it (10.81%) had normal genes. When analysis normal gene with deleted gene for GSTM and GSTT , there was no significant differences between groups.

Keywords: post- menopausal hypertension, lipid profile, women GSTT , GSTM.

INTRODUCTION

The prevalence of Hypertension disease has been increased in last decades and it's become important health problems in the world, it consider as risk factor of several disease such as cardiovascular disease (CVD, stroke, myocardial infarction, vision losing, heart and renal failure^{1,2} also it may lead to death duo to it complication³. Some factors contributed in incidence of blood pressure, also it consider as risk factors like age, life style, metabolic disorders, obesity and gender. Sever and chronic Stress, tension, smoking habitat, liquors, insufficient rest emotional disturbance and heredity. Some previous studies improved the association between hyperlipidemia and hypertension^{4,5}.

hypertriglyceridemia and hypercholesterolemia resulted from intake of saturated fats, different sources of calories and cholesterol with disturbance in lipid profile. also its associated with obesity and, consequently, hypertension^{6,7}. The decline in insulin function⁸, peripheral resistance increments, cardiac output, sympathetic tone and salt congestion⁶ are responsible mechanisms of hypertension. Brown (2008) found the

correlation among several factors with blood pressure, such as Basal Metabolic Index (BMI), serum cholesterol, HDL⁹.

The metabolism disorders of lipoproteins, both overproduction or deficiency known Dyslipidemias¹⁰. Dyslipidemia and hypertension association with some CVDs also its increased risk of Atherosclerosis, this relation is diverse among populations and this depending on the several factors like lifestyle, nutrition, genetic predisposition and environmental factors¹¹. Studies found that dyslipidemia in hypertension patients changed ratio of high total cholesterol and triglycerides or low-density lipoproteins. So there was a relation between the concentration of LDL and CVDs¹².

Role of the Oxidative stress have been studied in some disease it found strong association between hypertension and some biomarkers of oxidative stress. In Genetic studies there was variation in enzymes of ROS-generating²³ Furthermore, the antioxidant bioactivity is reduced in arteries which isolated from hypertensive humans and animals, also the signaling of redox-dependent was amplified and enhanced ROS

Table 1: study subject distribution according to BMI and age.

Categories	Patients	Control	Statics
BMI	31.618±6.8	29.996±3.813	P=0.3333 t = 0.9760
Age	58.135±9.5	50.4±5.834	P= 0.0018 t = 3.2841
Duration			
<5	28.57%		
5-10	53.57%		
>10	17.85%		
BMI			
Normal	10.34%	15%	X=1.419,
Overweig ht	48.27	50%	
Obese	41.37	35%	0.4920
Age			
<50	40.54%	75%	$\chi^2 = 34.5$
50-60	37.83%	25%	28
>60	21.63	0%	p= 0.0000

Table 2: lipid profiles levels in study subjects.

Categories	Patient	Control	Statics
Cholester ol	447.83±111	136.115±25.0	P=0.0001 T=12.3422
mg\dl			
HDL	138.6±7.43	32.496±18.98	P=0.0001 T= 6.2531
mg\dl			
TG	193± 87.43	152.765±	P=0.0563 t = 1.9502
mg\dl		38.89	
LDL	414.43±	73.066±	P=0.0001
mg\dl	194.38	35.94	t = 7.7516
VLDL	46.61±	30.553± 7.77	P= 0.0403
mg\dl	33.58		t = 2.1001

production²⁴. The Imbalances between oxidant/antioxidant system, can be increased level of lipid per-oxidation, and the altered lipid profile, the present study deal with GST gene polymorphisms with lipid profile in hypertensive patients Because GST.

The reviewers of GST data base showed 22 putatively functional genes and five pseudo genes. These genes encoded to proteins have biological process as well as detoxication and toxification mechanisms, by conjugation of reduced glutathione (GSH) with several substrates such as pharmaceuticals and environmental pollutants, also its function in oxidative index balance in biological tissues and cells²⁵.

METHODOLOGY

Study Design

A Case-control study implemented at the DNA lab / Babylon unv. / Iraq. Study included 55 women patients suffer from hypertension diagnostic in medical clinic during attended Mrjan teaching hospital. Sample collected according to ethical approval of Iraqi ministry of health. Questionnaire taken from patients included : BMI, age, occupation ,past medical history.

Exclusion criteria: pregnant women, smokers were excluded from present study. Lipid profile assay; the lipid profile including; total cholesterol, high density lipoprotein and triglyceride) were estimated by full automated reflotron plus system (Germany), a 32µl of serum were loading in specific strip in system which reading the results automatically¹³. Blood samples were collected for DNA extraction, its extracted from whole blood using (Genaid extraction kit) with modification according to²⁶, consternation and purity of DNA were estimated using nanodrp.

Primers, multiplex PCR was used in present study to detected GSTM and GSTT genes. The primers are GSTM1: forward: 5'-GAACTCCCTGAAAAGCTAAAGC-3', reverse: 5'-GTTGGGCTCAAATATACGGTGG -3'. GSTT1: forward: 5'-TTCCCTACTGGTCCCTCACATCTC-3', reverse: 5'-TCCCAGGTCACCGGATCAT-3'²⁷.

Amplification conditions and products size, experiments implemented using Multiplex PCR as a following; pre-denaturation for 5 min at 94C, then 35 cycles (1 min at 94C, 1 min at 58C, one min at 72C, at last 10 minutes at 72C). Genotypes were detected by the electrophoresis patterns of PCR products in agarose gel (1% agarose, 70 V, 20mA for 45 mints) the PCR size product was 215 bp for GSTM1 and 312 bp for GSTT1.

Statics, the results were statically analysis using Qi Square, t test and ANOVA one way analysis at p value <0.05).

RESULTS AND DISCUSSION

Hypertension is one of the important health problems in Iraq at last decade the number of disease incidence have been increased in different ages categories, in present study post-menopause women were choosing for estimation the association of lipid profile with hypertension diseases which become modern disease in this category of age, according to hormonal changes in this age and low activity of women and related hypertension with other chronic disease and its drugs.

Present results show that BMI of patients was obese 31.61% while BMI of healthy women was overweight 29.99, there was no significant differences between study groups. the distribution of study groups according to BMI classification which dependent by WHO show that high percentage of patients were overweight and obese (48.27 and 41.37)% respectively also control group show high percentage of participation individuals were overweight while obese and normal low percentage (35, 15)% respectively, other study deal with present result Mansour et al., found that overweight and obesity were more than half of the Basrah population¹⁴, Roka et al.,¹⁵ improved that BMI influenced hypertension in male and female, BMI correlated with life style, habitats , and individuals health. in comparison between patients and control, all of them show high percentage in overweight in non-significant differences, this may be because life style of Iraqi individuals such as nutrition, exercise, health awareness and hormonal changes in post-

Table 3: mean differences of lipid profile types according to BMI.

Categories	Patient mg\dl	Control mg\dl	P value	T test
Cholesterol Normal	410± 84.85	124.06± 15.93	0.0085	t = 6.1799
Overweight	463.57± 98.73	135.08±26.140	0.0001	t = 10.2089
Obese	472.5± 143.28	137.477± 26.74	0.0001	t = 6.8824
HDL Normal	100±28.28	17.85± 18.75	0.0276	t = 4.0206
Overweight	144± 81.50	32.582± 22.28	0.0004	t = 4.1883
Obese	162.5± 92.35	34.86± 15.13	0.0006	t = 4.0798
TG Normal	145± 134.35	145.4± 29.58	0.9960	t = 0.0054
Overweight	201.42± 61.75	149.26± 36.10	0.0363	t = 2.2531
Obese	283.33± 253.89	160.18± 43.86	0.1689	t = 1.4302
LDL Normal	466± 107.4	77.13± 21.27	0.0070	t = 6.6153
Overweight	417.57± 163.32	72.646±35.08	0.0001	t = 6.1916
Obese	520.33± 232.43	70.573± 39.85	0.0001	t = 5.7066
VLDL Normal	29±26.8	29.08±5.916	0.9960	t = 0.0054
Overweight	41.14± 121.4	29.852± 7.221	0.7849	t = 0.2768
Obese	66.90± 52.811	32.03± 8.77	0.0663	t = 1.9485

Table 4: mean differences of lipid profile types according to age categories.

Categories	Patients mg\dl	Control mg\dl	p-value	T test
Cholesterol				
<55	394±129.2	137.80±25.65	0.0001	t = 7.2785
55-65	465±82.25	132.166±25.36	0.0001	t = 9.5848
>65	518.75±69.5	0	0	0
HDL				
<55	142.4±87.05	29.6728±20.462	0.0001	t = 4.7198
55-65	119.285±26.15	39.083±14.3746	0.0001	t = 7.0005
>65	166.25±103.91	0	0	0
TG				
<55	202±72.03	156.55±37.53	0.2701	t = 1.1260
55-65	267.85±234.29	143.916±44.180	0.2213	t = 1.2670
>65	158.75±91.71	0	0	0
LDL				
<55	320.13±188.96	76.822±36.87	0.0001	t = 4.7290
55-65	403.71±131.68	64.3±35.26	0.0001	t = 6.1318
>65	610±166.47	0	0	0
VLDL				
<55	42.714±13.46	31.311±7.507	0.0096	t = 2.7887
55-65	53.57±46.85	28.78±8.836	0.2212	t = 1.2674
>65	41.25±32.61	0	0	0

Table 5: lipid profile types level according disease duration.

Categories	Cholesterol mg\dl	HDL mg\dl	TG mg\dl	LDL mg\dl	VLDL mg\dl
<5	441.11±72.7	114.44±29.62	303.33±287.09	418.22±119.8	60.66±57.41
5-10	421.3±131.9	146.4±83.64	182±83.7	377.46±207.8	38.42±16.73
>10	428±88.71	108±23.87	200±60.4	312.4±78.23	40±12.083
f test	0.082	0.986	1.436	0.590	1.240
P	0.921	0.387	0.257	0.562	0.306

menopausal of women which cause gain weight¹⁶, (table 1)

There was significant differences in age mean between patient and control although of each individuals age in present study was more than 45 years because study suggested for post menopause of females. Several physiological changes were developed During menopause, lead to incidence some diseases like cardiovascular disease, osteoporosis, vagina and uterus cancer also increase in body weight^{17,18}, resulted from

endogenous estrogen decline by accelerated weight gain¹⁹. In patients, disease was more frequent in age less than 50 while low percentage was found in age more than 60 years (table1).

According to disease duration, present study show that high percentage (53.57%) of patients were had (5-10) years, while 17.85% was had more than 10 years, in Iraq accelerated prevalence of hypertension was recorded in last decade, this may be because big changes which

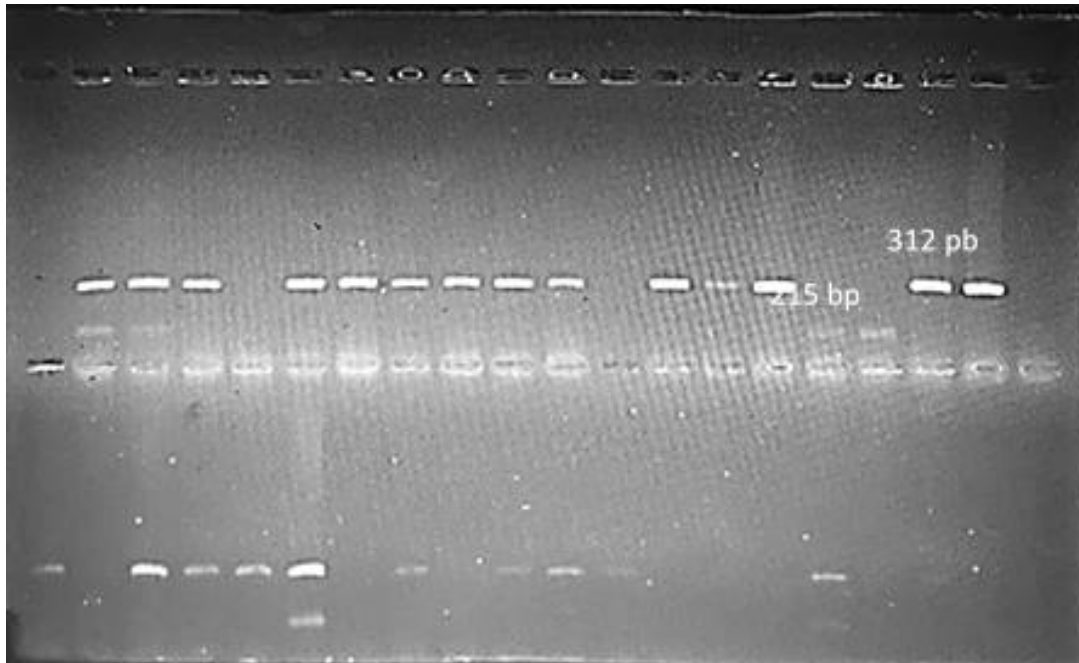


Figure 1: Electrophoresis patterns of GST genes polymorphisms using PCR-ARMS for patients and control the PCR size product was 215 bp for GSTM1 and 312 bp for GSTT1.

Table 6: statically analysis of genotyping of GSTM and GSTT genes in patients and control.

Categories	Contro 1 %	Patients %	χ^2	P value
GSTM+	88	64.86	3.621	0.057
GSTM-	12	32.43	,	1
GSTT1+	48	16.21	7.315	0.006
GSTT1-	52	83.73		8
GSTT+GSTM	48	10.81	6.892	0.008
GSTM-	12	24.32		7
GSTT-				
GSTM+GSTT	4	51.35	1.018	0.313
-				0
GSTM-	0	5.40		
GSTT+				

happened in Iraq like tension, increment of oxidative stress and environment pollution.

Lipid profile was detected in present study for both patients and control show in table (2), there was elevation in all types of lipid profile cholesterol, high density lipoprotein, triglyceride, low density lipoprotein and very low density lipoprotein, all of these parameters recorded significant differences at P value 0.05 except triglycerides the elevation was non-significant, investigators found in cohort study that the increment in lipid profile associated with development of hypertension in healthy women²⁰ also dyslipidemia was found in hypertension patients by (Charles et al.,²¹).

Table 3 show mean differences of lipid profile according to BMI, there was positive association between lipid profiles types and increment of weight in women suffered from hypertension, also in healthy women the elevation of lipid profile types relation with increment in BMI but the increment in patients was more than control in

significant differences except in triglyceride the elevation was non-significant. According to age categories there was disparity of lipid profiles types in patients, cho and LDL levels increased with age, while HDL was decreased in age category 55-65. TG and VLDL was increased in 55-65 category, some of differences were significance while other non-significant differences like TG in all age categories and VLDL in 55-65 Category (table 4).

The duration of diseases has been studied, there were three categories less than five years m 5-10 years and more than 10 years, the increment didn't dependent on increased duration of diseases, there were diversity in lipid profile types levels, all differences were non-significant at p value 0.05 using ANOVA one way test (table 5).

The elevation in the levels of CHO, LDL, TG, HDL and VLDL were found in several studies deal with hypertension patients however diverted of these elevation were differences according to some factors like lifestyle, nutrition, tension and stress, genetic factors, smoker and related with other disease like diabetes mellitus and metabolic disorder²². This study needs more investigations about heredity of dyslipidemia and genetic factor association with hypertension and lipid metabolism.

The genotype of GST genes were show in figure (1) and table (6), there was two bands 312 bp and 215 bp for GSTM and GSTT respectively, the results of present study using multiplex -PCR show that there were four types of genotyping GSTM deletion and normal, GSTT deletion and normal, GSTM normal and GSTT normal, GSTM deletion and GSTT deletion, GSTM normal and GSTT deletion, the last genotyping is GSSTM deletion and GSTT normal.

The statically analysis of genotyping show significant differences between GSTT deletion and normal in

Table 7: Mean differences of lipid profile types according to genotyping of GST genes for patients.

Categories	CHO	TG	HDL	LDL	VLDL
GSTM+	437.08± 109.08	246.25± 185.11	148.166± 88.557	392.33± 203.73	40.391± 20.729
GSTM-	443.33±169.99	160.83±77.04	116.66±33.120	445.83±180.53	38.5±26.766
GSTT1+	383.33±85.47	181.66±97.45	148.33±133.47	375.33±283.06	36.33±19.49
GSTT1-	460.32±112.14	224.58±168.84	136.96±61.20	422±177.83	48.66±35.62
GSTT+GSTM	410±93.452	207.5±113.54	180±160.20	453.5±328.76	41.5±22.70
GSTM+	444.73±115.96	243.157±45.1641	143.47±73.534	378.10±184.207	50.88±39.655
GSTT-					
GSTM-	330±42.43	130±28.28	85±7.07	219±55.15	26±5.66
GSTT+					
GSTM-	508.88±107.610	180±76.620	127.77±30.720	502.44±166.050	44.44±28.290
GSTT-					
F	1.041	0.816	0.558	0.781	0.491
P Value	0.407	0.576	0.788	0.605	0.839

patients and control, about 83.73% of patients GSTT gene was deleted. Also deletion in GSTM was high in patients than control (32.43) but in non-significant differences. Pattern of two genes which represented by two genes normal or two genes deleted, it show significant differences between study groups about 24.32% of patients suffer from deleted two genes and low percentage of it (10.81%) had normal genes. When analysis normal gene with deleted gene for GSTM and GSTT, there was no significant differences between groups (table 6).

The present results show there was strong association between GSTT and hypertension and low association between GSTM and hypertension disease in post-menopause females it deal with several studies that hypothesized that oxidative stress have major role in the hypertension pathogenesis, based on the changes in antioxidant enzymes which were reduction like superoxide dismutase and glutathione peroxidase in newly hypertensive patients; and higher production of Hydrogen peroxidase, Furthermore, hypertensive patients have higher lipid hydroperoxide production²⁸. In Indian population GSTM1 was a potential genetic factor and it may be an independent risk factor for hypertension development while GSTT1 may be contributed in protection against hypertension²⁹, the association between GST genes and hypertension explained by reduction in antioxidant activity which lead to defect in cells detoxification, and accumulation of these substances that causes mutations in hypertension related genes.

The association between lipid profile types and GST genes polymorphisms was show in table (7) there was no-significant variations among groups, the deletion in GSTM reduction in TG, HDL and VLDL levels and increased in CHO and LDL levels. While GSTT deletion reduced levels of HDL only while increased in CHO, TG, LDL and VLDL. The deletion in both genes caused decreased in TG and HDL while other types were increased. Other studies show there was no association among oxidative stress, lipid profile types and different disease like coronary artery disease it was no significant relationship between increased oxidative stress and abnormal lipid profile types³⁰ while it causes lipid profile disturbance in vitiligo patents³¹. From present results can

be concluded that lipid profile effected by GST polymorphisms but it need more investigations in male and pre-menopause female, because of the effective of sex hormones in lipid distribution and other cellular process in females.

REFERENCES

1. He J, Whelton PK. Epidemiology and prevention of hypertension. *Med Clin North Am* 1997;81:1077–97.
2. Whelton PK. Epidemiology of hypertension. *Lancet* 1994;344: 202–6.
3. IdemudiaJ,Ugwuja E. Plasma lipid profiles in hypertensive 1. Nigerians. *Internet Journal of cardiovascular research* 2009; 6(2)ht://www.ipub.com:80 at march 2011.
4. Hansen HS, Larsen ML. Hypertension and hyperlipidemia. *Ugeskr Laeger*. 2009; 171(24): 2028-30.
5. Feldstein CA. Statins in hypertension: are they a new class of antihypertensive agents? *Am. J. Ther.* 2010; 17(3): 255-62.
6. Hall JE, Brands MW, Henegar JR. Mechanisms of hypertension and kidney disease in obesity. *Ann. NY Acad. Sci.* 1999; 18(892): 91-107.
7. Kotsis V, Stabouli S, Papakatsika S, Rizos Z, Parati G. Mechanisms of obesity-induced hypertension. *Hypertens Res.* 2010; 33(5): 386-93.
8. Pastucha D, Talafa V, Malincikova J, Cihalik C, Hyjanek J, Horakova D. Obesity, hypertension and insulin resistance in childhood--a pilot study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2010; 154(1):77-81.
9. Brown CD, Higgins M, DonatoKA, Rohde FC, Garrison R, Obarzanek E. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res.* 2000; 8(9):605-19.
10. Syedm MA, Mark EC, and John ED. Management of 3. dyslipidemia in Adults, *American family physician*, May 1, 1998Wright state university school of medicine Day ton , ohio WWW aafp.org\afp\98050\ap\ahmed.htm\
11. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. For the DASH Collaborative research group *Acclinical trail of the*

- effects of dietary patterns on blood pressure. *N Engl J Med* 1997;336:1117–24.
12. Nagababu Pyadala¹, Ravindra Reddy Bobbiti¹, Rajaneesh Borugadda², Srilatha Bitinti², Soumendra Nath Maity³, Prudhvi Chand Mallepaddi⁴, Rathnagiri Polavarapu Assessment of lipid profile among hypertensive patients attending to a rural teaching hospital, Sangareddy *International Journal of Medical Science and Public Health* | 2017 | Vol 6 | Issue 1 71-76.
 13. Hameed N, Zaidan H, Jebor M, Al-Terehi M, (2017) Relationship between Lipid Profile and Some Physiological Markers in Pre and Post-Menopausal Type II Diabetic Females. *Journal of Global Pharma Technology*. 2017; 09(9):222-233.
 14. Mansour AA, Al-Maliky AA, Salih M (2012) Population Overweight and Obesity Trends of Eight Years in Basrah, Iraq. *Epidemiol* 2:110. doi:10.4172/2161-1165.1000110
 15. Roka R¹, Michimi A, Macy G. (2015) Associations Between Hypertension and Body Mass Index and Waist Circumference in U.S. Adults: A Comparative Analysis by Gender, High Blood Press *Cardiovasc Prev*. 2015 Sep;22(3):265-73. doi: 10.1007/s40292-015-0106-3. Epub 2015 May 27.
 16. Fadheel Q Prevalence and Determination of Obesity and Overweight among Employees Women in Iraq *J. Pharm. Sci. & Res*. Vol. 10(3), 2018, 540-543.
 17. Kwak, E.K.; Park, H.S. and Kang, N.M. (2014). Menopause knowledge, attitude, symptom and management among midlife employed women. *J Menopausal Med.*, 20: 118-1125.
 18. Al-Safi, Z.A. and Polotsky, A.J. (2015). Obesity and menopause. *Best Pract. Res. Clin. Obstet. Gynaecol.*, 29: 548–553.
 19. Polotsky, H.N. and Polotsky, A.J. (2010). Metabolic implications of menopause. *Semin. Reprod. Med.*, 28: 426–434
 20. Sesso HD, Buring JE, Chown MJ, Ridker PM, Gaziano JM. A Prospective Study of Plasma Lipid Levels and Hypertension in Women. *Arch Intern Med*. 2005;165(20):2420–2427. doi:10.1001/archinte.165.20.2420
 21. Charles U. Osuji, Emeka G. Omejua, Emmanuel I. Onwubuya, and Gladys I. Ahaneku, “Serum Lipid Profile of Newly Diagnosed Hypertensive Patients in Nnewi, South-East Nigeria,” *International Journal of Hypertension*, vol. 2012, Article ID 710486, 7 pages, 2012. <https://doi.org/10.1155/2012/710486>.
 22. Srivastava A and Binawara P ,Comparative Study on Lipid Profile of Hypertensive Patients and Non hypertensive Individuals *UK Journal of Pharmaceutical and Biosciences* Vol. 4(46-62 , 2016.
 23. Yildirim M, Baysal V, Inaloz HS, Can M. The role of oxidants and antioxidants in generalized vitiligo at tissue level. *J Eur Acad Dermatol Venereol* 2004;18(6):683–86.
 24. Nebert, D. W., & Vasiliou, V. (2004). Analysis of the glutathione S-transferase (GST) gene family. *Human genomics*, 1(6), 460-4.
 25. Nebert, D. W., & Vasiliou, V. (2004). Analysis of the glutathione S-transferase (GST) gene family. *Human genomics*, 1(6), 460-4.
 26. Al-Terehi M., Ghaleb R, Al-Oubaidy SH., Al-Saadi A., Zaidan H. (2016) Study TNF- α gene polymorphism in Type 1 Diabetic Patients Using ARMS technique *Journal of Chemical and Pharmaceutical Science CPS* Volume 9 Issue 3,1107-1111.
 27. Al-Terehi, M. Hasan, A. Muhammed, H. Mohsen I. Al-Saadi A., Zaidan H. (2016) Polymorphisms of Glutathione-S-Transferase M1 and T1 Genes in Breast Cancer Tissue *Journal of Chemical and Pharmaceutical Sciences*, 9,4,2011-14.
 28. Baradaran, A., Nasri, H., & Rafieian-Kopaei, M. (2014). Oxidative stress and hypertension: Possibility of hypertension therapy with antioxidants. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*, 19(4), 358-67.
 29. Ritambhara, Anup Kumar, Daya Shankar Lal Srivastava, Sivakumar Vijayaraghavalu and Munish Kumar, GSTM1/GSTT1 Gene Polymorphism in North Indian Population and their Association to Hypertension. *BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA*, December 2017. Vol. 14(4), p. 1269-1275.
 30. Rao, V., & Kiran, R. (2011). Evaluation of correlation between oxidative stress and abnormal lipid profile in coronary artery disease. *Journal of cardiovascular disease research*, 2(1), 57-60.
 31. Arun Kumar Metta, Sandhya Metta, Imran Kazmi, Govind Raj (2016) Evaluation of oxidative stress and lipid profile in patients of generalized vitiligo *International Journal of Medical Science and Public Health* | 2016 | Vol 5 | Issue 03