

# **IDENTIFICATION OF GSTT1 NULL GENOTYPE AND GLUTATHIONE LEVELS IN CHRONIC KIDNEY DISEASES**

Submitted in partial fulfillment of the requirements for the award of  
Bachelor of Technology degree in Biotechnology

by

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**SATHYABAMA**  
INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)  
Accredited with Grade "A" by NAAC  
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# SATHYABAMA

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### Certification of Project Work

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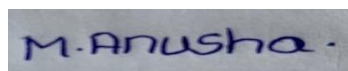
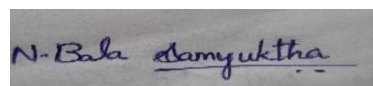
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## DECLARATION

We NARRA BALA SAMYUKTHA (37230049) and MANDALA ANUSHA (37230042) hereby declare that the Project Report entitled “**IDENTIFICATION OF GSTT1 NULL GENOTYPE AND GLUTATHIONE LEVELS IN CHRONIC KIDNEY DISEASES**” done by us under the guidance of Dr. SUDHA (Internal) and Dr. SABITHA.Y (External) managing director at Ciencia Life Sciences, Hyderabad is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

**DATE: 15/ 4 / 21**

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A rectangular box containing the handwritten signature "M. Anusha." in blue ink.A rectangular box containing the handwritten signature "N. Bala samyuktha" in blue ink, with a horizontal line underneath the name.

**SIGNATURE OF THE CANDIDATE**

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## ABSTRACT

Glutathione s-transferases (GSTs) belong to a family of ubiquitous and multifunctional enzymes that work as one of the endogenous antioxidants in our body (GSTs) belong to a family of ubiquitous and multifunctional enzymes that work as one of the endogenous antioxidants in our body. Chronic kidney disease (CKD) is a condition associated with a poor prognosis and high impact on the healthcare system. Achieving timely and appropriate diagnosis and therapeutic prescription in these patient populations has a major impact on morbidity and mortality indices. Lack of specific biomarkers of the efficiency of the kidney as well as of dialysis therapy in depurating the organism from different types of circulating toxins is a major clinical limitation. The glutathione S-transferase (GST) quality family encodes qualities that are basic for certain life forms, as well as for detoxification instrument, by means of conjugation of diminished glutathione (GSH) with various substrates such as pharmaceuticals and natural pollutants. The GST gene family comprises 16 genes in 6 subfamilies – alpha (GSTA), mu(GSTM), omega(GSTO), pi (GSTP), theta (GSTT), and zeta (GSTZ), etc. So, this study is about the association of GSTT1 null genotype and glutathione levels in chronic kidney disease to develop as biomarkers. This study was designed to look into the association of GST polymorphism with oxidative stress in chronic kidney disease (CKD). Two groups of patients (30 in each): people without CK, people with CK were considered for study. Genotyping was done on STT1 genes using a multiplex polymerase chain reaction. Serum GST as a marker of oxidative stress was measured spectrophotometrically. Based on genotyping, subjects were categorized. GST levels were lower among subjects with cancellation in one/both GSTt1 genes. A negative correlation GST level was observed among genotypes with GSTT1 gene deletions. The presence of GSTT1 null genotype is significant for the disease. But due to low size, it's not confirmed. GSTT1 erasures separately or together were related to lower GST levels and higher oxidative push CKD. Interestingly, GSTT1 deletion appears to be associated with C.

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## ABBREVIATIONS

|                   |                                    |
|-------------------|------------------------------------|
| UV                | Ultra Violet                       |
| CKD               | Chronic Kidney Disease             |
| GST               | Glutathione S Transferase          |
| MgCl <sub>2</sub> | Magnesium Chloride                 |
| Tris HCL          | (Hydroxymethyl) Hydrochloric Acid  |
| Rcc               | Renal cell Carcinoma               |
| AKI               | Acute Kidney injury                |
| ESRD              | End Stage Renal Infection          |
| HD                | Hemodialysis                       |
| TE Buffer         | Tris EDTA Buffer                   |
| EDTA              | Ethylene Diamine Tetra Acetic Acid |
| KCl               | Potassium Chloride                 |
| PCR               | Polymerase Chain Reaction          |

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## CHAPTER - 1

### INTRODUCTION

#### 1.1 - Chronic Kidney Disease (CKD):

Chronic kidney disease (CKD) is a type of kidney disease in which there will be gradual loss in kidney function over a period of time (months to years). At the start there were generally no symptoms. Later the symptoms may consider leg swelling, feeling tired, vomiting, loss of appetite, and confusion. Obey involves an increased risk of heart disease, high blood pressure, bone disease, and anemia. Chronic kidney disease (CKD) is condition linked with poor prognosis and high effect on the healthcare system. Performing timely and proper diagnosis and therapeutic prescription in these patient populations has a major effect on morbidity and mortality indices. Causes of chronic kidney disease involve diabetes, high blood pressure, glomerulonephritis, and polycystic kidney disease (Lindner A et al., 1974). Threat factors involve a family history of chronic kidney disease. Chronic kidney disease influenced 753 million individuals around the world in 2016, 417 million females and 336 million guys. In 2015 it caused 1.2 million passing's, up from 409,000 in 1990. The causes that contribute to the noteworthy number of passing are tall blood weight at 550,000, taken after by diabetes at 418,000, and glomerulonephritis at 238,000. CKD is originally without symptoms and is usually detected on routine screening blood work by either an increase in serum creatinine, or protein in the urine. (Kazancioğlu. R et al., 2013) As the kidney work diminishes: Blood weight is expanded due to liquid over-burden and generation of vasoactive hormones made by the kidney by means of the renin–angiotensin framework, expanding the chance of creating hypertension and heart disappointment. Urea accumulates, leading to azotemia and eventually uremia (symptoms ranging from lethargy to pericarditis and encephalopathy). Due to its tall systemic concentration, urea is excreted in eccrine sweat at tall concentrations and crystallizes on skin as the sweat vanishes ("uremic ice"). Potassium gathers within the blood (hyperkalemia with an extent of indications counting discomfort and possibly lethal cardiac arrhythmias). Hyperkalemia ordinarily does not create until the glomerular filtration rate falls to less than 20–25 ml/min/1.73

m2, at which point the kidneys have diminished capacity to discharge potassium). Hyperkalemia in CKD can be exacerbated by acidemia (which leads to extracellular movement of potassium) and from need of affront. Liquid abundance side effects may extend from mellow edema to life-threatening respiratory edema. Hyperphosphatemia comes about from destitute phosphate disposal within the kidney. Hyperphosphatemia contributes to expanded cardiovascular hazard by causing vascular calcification. Circulating concentrations of fibroblast development factor-23 (FGF-23) increment continuously as the kidney capacity for phosphate excretion decreases which may contribute to cleared out ventricular hypertrophy and expanded mortality in individuals with CKD. e function of enzymes and increases excitability of cardiac and neuronal membranes by the promotion of hyper Hypocalcemia results from 1,25 dihydroxy vitamin D3 deficiency (caused by high FGF-23 and reduced kidney mass) and resistance to the activity of parathyroid hormone. Osteocytes are mindful for the expanded generation of FGF-23, which may be a strong inhibitor of the protein 1-alpha-hydroxylase (capable for the change of 25-hydroxycholecalciferol into 1, 25 dihydroxy vitamin D3). Afterward, these advances to auxiliary hyperparathyroidism, kidney osteodystrophy, and vascular calcification that helps hinder cardiac work. An uncommon result is the occasion of the exceptional condition named calciphylaxis. Changes in mineral and bone digestion system that will cause (Sparks M. A. *et al.*, 2014).

1) Abnormalities of calcium, phosphorus (phosphate), parathyroid hormone, or vitamin D metabolism

2) Abnormalities in bone turnover, mineralization, volume, straight improvement, or quality (kidney osteodystrophy)

3) Vascular or another soft-tissue calcification. CKD-mineral and bone clutters have been related with destitute results. Metabolic acidosis may result from diminished capacity to produce sufficient alkali from the cells of the proximal tubule. Acidemia affects thalami (Jordi Bover and Mario Cozzolino 2011)

Anemia is common and is particularly prevalent in those needing hemodialysis. It is multi factorial in cause, but incorporates expanded irritation, lessening in erythropoietin, and hyperuricemia driving to bone marrow concealment. In afterward stages, cachexia may create, driving to inadvertent weight misfortune, muscle squandering, shortcoming and anorexia. Sexual brokenness is exceptionally common in both men and ladies with CKD. A larger part of men have a decreased sex drive, trouble getting an erection, and coming to orgasm, and the issues get more awful with age. A majority of ladies have issues with sexuality. excitement, and painful monthly cycle and issues with performing and enjoying sex are common . People with CKD are more likely than the common populace to create atherosclerosis with ensuing cardiovascular infection, an impact that will be at slightest somewhat interceded by uremic poisons. Individuals with both CKD and cardiovascular malady have an importantly more awful forecast than those with as it were a cardiovascular illness. The three most common causes of CKD in arrange of outright recurrence as of 2015 are diabetes mellitus, hypertension, and glomerulonephritis. Around one of five grown-ups with hypertension and one of three grown-ups with diabetes have CKD. In case the cause is obscure, it is called idiopathic.

#### **1.1.1 By anatomical location:**

Vascular disease includes large vessel disease such as bilateral kidney artery stenosis and small vessel disease such as ischemic nephropathy, hemolytic-uremic syndrome, and vasculitis Glomerular

#### **1.2 Disease comprises a diverse group and is classified into:**

Primary glomerular malady such as central segmental glomerulosclerosis and IgA nephropathy (or nephritis) auxiliary glomerular infection such as diabetic nephropathy and lupus nephritis. Tubulointerstitial malady incorporates medicate- and toxin-induced unremitting tubulointerstitial nephritis, and reflux nephropathy. Obstructive nephropathy, as exemplified by reciprocal kidney stones and kind prostatic hyperplasia of the prostate organ. Once in a while, stick worms contaminating the

kidney can cause obstructive nephropathy. Intrinsic malady such as poly-cystic kidney disease.

Mesoamerican nephropathy may "be a new form of kidney malady that may well be called agrarian nephropathy". A tall and so-far unexplained number of modern cases of CKD, alluded to as the Mesoamerican nephropathy, has been famous among male specialists in Central America, basically in sugar cane areas within the marshes and Nicaragua. Warm push from long hours of piece-rate work at tall normal temperatures of around 36 °C (96 °F) is suspected, as are agricultural chemicals.

**1.2.1 Glutathione S-transferases: (GSTs)**, already known as ligands, contain a family of eukaryotic and prokaryotic stage II metabolic isozymes best known for their capacity to catalyze the conjugation of the decreased frame of glutathione (GSH) to xenobiotic substrates for the reason of detoxification. The GST family comprises three super families: the cytosolic, mitochondrial, and microsomal moreover known as MAPEG proteins. Individuals of the GST superfamily are greatly assorted in amino corrosive arrangement, and an expansive division of the groupings stored in open databases are of obscure work. The Protein Work Activity (EFI) is utilizing GSTs as a show superfamily to recognize modern GST capacities.

GSTs can constitute up to 10% of cytosolic protein in a few mammalian organs. GSTs catalyze the conjugation of GSH by means of a sulfhydryl gathering to electrophilic centers on a wide assortment of substrates in order to create the compounds more water-soluble. This movement detoxifies endogenous compounds such as peroxidase and empowers the breakdown of xenobiotics. GSTs may too tie poisons and work as transport proteins, which gave rise to the early term for GSTs, ligandin (Aguilera I et al., 2004)

**1.2.2 Glutathione S-transferase theta-1:** GST Theta-1 is an enzyme that in humans is encoded by the GSTT1 gene.

Glutathione S-transferase (GST) theta 1 (GSTT1) may be a part of a superfamily of proteins that catalyze the conjugation of diminished glutathione to an assortment of electrophilic and hydrophobic compounds. Human GSTs can be isolated into five

primary classes: alpha, mu, pi, theta, and zeta. The theta class includes GSTT1 and GSTT2. The GSTT1 and GSTT2 share 55% amino acid sequence identity and both were claimed to have an important role in human carcinogenesis. The GSTT1 gene is located approximately 50kb away from the GSTT2 gene. The GSTT1 and GSTT2 genes have a similar structure, being composed of five exons with identical exon/intron boundaries. GSTT1 null genotype and glutathione levels in chronic kidney disease to develop as biomarkers.

Human GSTs are isolated into different classes and subclasses. Among the distinctive subclasses, in human kidney GST  $\mu$  lesson is primarily localized within the tubules though GST  $\theta$  lesson is exceedingly communicated in liver and kidney. Both these hereditary loci are known to be exceedingly polymorphic and a few of these polymorphisms lead to alter within the expression of the protein, either subjectively or quantitatively and consequently can render people helpless to different illnesses counting CKD. Among the different known hereditary polymorphisms of GST, the GSTM1 and GSTT1 qualities are the foremost broadly examined, basically since the invalid variations of these qualities might result in decreased GST expression driving to decrease antioxidant defense.

Furthermore, they have noteworthy frequencies in a few later reports, counting one from our research facility. Within the past few a long time, a few have appeared to be displayed in the healthy Indian populace. Think about the affiliation of hereditary polymorphisms of GST with the improvement of CKD, generally of the diabetic root, but without any conclusive results. In spite of the fact that Fujita et al. found no affiliation of GSTM1 erasure with diabetic nephropathy in Japanese sort 2 DM patients, Yang et al (2013). appeared that GSTT1 invalid genotype was a hazard calculate for the development of diabetic nephropathy within the Chinese populace. In 2005, Kim et al. detailed that GSTM1 invalid genotype is related with sort 2 diabetic nephropathy within the Korean populace. We have come over as it were one past report considering the GST polymorphisms in connection to CKD where the creators concluded that the invalid polymorphism of the detoxifying proteins, GSTT1 and GSTM1, were related with the chance of creating end-stage renal disease (ESRD).

The mechanism by which GST polymorphism leads to CKD isn't well-understood. In a past consider, Hayek et al. have appeared that GSTT1 invalid genotype is related with increment in markers of lipid peroxidation among diabetics. A later consideration from our research facility has moreover illustrated the part of GST polymorphism within the advancement of oxidative push in preterm labor cases. This ponder was outlined to examine the part of the GST polymorphism in deciding the variety in susceptibility of people to CKD and to pinpoint the plausible underlying mechanism.

Two supergene families encode proteins with glutathione S-transferase (GST) movement: the family of solvent chemicals comprises at slightest 16 qualities; the partitioned family of microsomal proteins comprises at slightest 6 qualities. These two GST families are accepted to apply a basic part in cellular assurance against oxidative stretch and poisonous outside chemicals. They detoxify an assortment of electrophilic compounds, counting oxidized lipid, DNA and catechol items produced by responsive oxygen species-induced harm to intracellular particles. An expanding number of GST qualities are being recognized as polymorphic. Certain alleles, especially those that bestow disabled catalytic movement (Eg GSTM1 (\*)0, GSTT1(\*)0), may be related with expanded affectability to poisonous compounds. GST polymorphisms may be infection altering; for illustration, in subgroups of patients with basal cell carcinoma or bronchial hyper-responsiveness, certain GST show up to apply a measurably noteworthy and naturally important effect on malady vulnerability.

Glutathione S-Transferase (GST) may be a stage II protein and catalysis response between glutathione and an assortment of electrophilic compounds, counting a few natural carcinogens. In man, at slightest 20 isoenzyme shapes of GST have been distinguished and numerous of them appear hereditarily based on changeability of chemical movement. The GSTT1 isoenzymes show a few polymorphisms, counting a monozygotic cancellation, which have been related with an expanded chance for creating neoplastic diseases. There's geological and ethnic variety in genotype frequencies for both qualities. The accessible information recommends that cancer frequency changes among Italian districts, being higher in Northern than in Southern zones, in spite of the fact that it is obscure whether this wonder is to be credited to hereditary and/or natural variables. We performed a case-control ponder to assess

the GSTT1 polymorphisms in an arrangement of cancer patients in Basilicata, a Southern Italian locale, and in corresponding controls.

The results obtained illustrate that the event of GST polymorphisms within the Basilicata populace isn't distinctive from other Italian districts and propose that the populace inferable hazard related with these genotypes may be very tall. GSTM1 homozygous invalid genotype was associated with an expanding chance of cancer, particularly in females. The most grounded affiliation was with colon and breast cancers. For the GSTT1 quality, the comes about were suggestive of a diminished hazard of cancer-related with the invalid genotype. Thus, similar considerations on these and other vulnerability qualities are justified since they can offer assistance to distinguish helpless subgroups of individuals who can be focused on for cancer prevention.

Chemical substances shown in tobacco are metabolized by proteins from the P450 cytochrome family and glutathione-S-transferases (GSTT1 and GSTM1) (Weak et al., 2010). Glutathione is an antioxidant tripeptide, composed of glycine, cysteine and glutamic corrosive. It may be a cofactor in numerous proteins and detoxification processes.

### **1.3 POLYMORPHISM:**

Polymorphism is the occurrence of two or more clearly diverse morphs or shapes, moreover alluded to as elective phenotypes, within the populace of a species. To be classified as such, morphs must involve the same living space at the same time and have a place to a panmictic populace (one with irregular mating). Put basically, polymorphism is when there are two or more conceivable outcomes of a characteristic on a gene. For example, there is more than one possible trait in terms of a jaguar's skin coloring; they can be light morph or dark morph. Due to having more than one conceivable variety for this quality, it is named 'polymorphism'. In any case, in the event that the puma has one conceivable characteristic for that quality, it would

be named "monomorphic". For illustration, in case there was as it were one conceivable skin color that a puma may have, it would be named monomorphic.

The term polyphenism can be utilized to clarify that the distinctive shapes emerge from the same genotype. Hereditary polymorphism may be a term utilized to some degree in an unexpected way by geneticists and molecular scientists to portray certain changes within the genotype, such as single nucleotide polymorphisms which will not continuously compare to a phenotype, but continuously compares to a department within the genetic tree. See below.

Polymorphism is common in nature; it is related to biodiversity, hereditary variety, and adjustment. Polymorphism as a rule has the capacity to hold an assortment of shapes in a populace living in a shifted environment. The foremost common case is sexual dimorphism, which happens in numerous life forms. Other cases are mimetic shapes of butterflies (see mimicry), and human hemoglobin and blood sorts. Agreeing to the hypothesis of advancement, polymorphism comes about from evolutionary forms, as does any aspect of a species. It is heritable and is modified by natural determination. In polytheism, an individual's hereditary cosmetics permits for diverse morphs, and the switch component that decides which transform appears is natural. In hereditary polymorphism, the hereditary cosmetics decides the transform. The term polymorphism moreover alludes to the event of basically and practically more than two diverse sorts of people, called zooids, inside the same organism. It could be a characteristic include of cnidarians. For case, Obelia has nourishing people, the gastrozooids; the people competent of agamic propagation as it were, the gonozooids, impact styles; and free-living or sexually replicating people, the medusa haryana. Adjusted polymorphism alludes to the upkeep of distinctive phenotypes in populations (Baburaj, V.P., 2007).

#### **1.4 GST POLYMORPHISM:**

GSTT1 are polymorphic and their deleted variants (null genotypes) result in a complete loss of functional activity. Various studies have shown a positive association between the GSTM1 and GSTT1 null genotypes and an increased risk of bladder, skin, lung, and oral cancer.

Glutathione-S-transferases are chemicals included in tobacco carcinogen digestion system, and qualities encoding these chemicals are highly polymorphic. We compared the frequencies of GSTT1 null polymorphisms in women with cervical cancer and in a control group, as well as to determine possible associations between such polymorphisms, cigarette smoking and the prognosis of cervical cancer. The series comprised 135 cervical cancer patients and 100 ladies without cancer. Genotypes were explored by PCR. The comes about were compared utilizing the Chi-square test or Fisher's correct test and survival investigation by Kaplan-Meier test and Log-rank. Among the cases, the recurrence of GSTM1 quality invalid polymorphism was 22.2%, and for the GSTT1 quality it was 48.5%. Among the controls, the recurrence of the GSTM1 quality invalid polymorphism was 45.0%, whereas for GSTT1 it was 56.0%. A significant association was found between smoking and cervical cancer ( $P = 0.0062$ ;  $OR = 2.16$ ). In an unexpected way from GSTM1, the GSTT1 invalid polymorphism was not related with cervical cancer hazard in this ponder. The GSTT1 invalid genotype was altogether related with worse prognosis. The overall survival rate for the cervical cancer group was 78.5%, and when stratified by genotypes, survival was higher in patients presenting at least one of the alleles, GSTT1 or GSTM1, indicating a higher risk of death for those presenting dual nullity ( $P = 0.031$ ;  $RR = 2.458$ ) (Abdel-Rahman et al., 1996)

A possible association between glutathione S-transferase theta 1 quality (GSTT1) polymorphism and the risk of developing prostate cancer is as of now fervently talked about, but proof from different epidemiologic ponders remains hazy. This examination was performed to survey whether an affiliation between GSTT1 polymorphism and prostate cancer chance exists by utilizing meta-analysis to combine comparable considerations, subsequently expanding test estimate and factual centrality, as well as to recognize designs in different ponders. The affiliation reports were recognized from the PubMed database and the Cochrane Library on Walk 1, 2013, and information from qualified considers (from 1999-2012) were synthesized. Thirty-eight reports were included in this meta-analysis on the association of the null genotype of GSTT1 with prostate cancer risk. No solid association between the GSTT1 invalid genotype and prostate cancer hazard may be set up for the overall population(odds

ratio = 1.11, 95% confidence interval: 0.97, 1.27;  $P = 0.13$ ). However, the GSTT1 null genotype was distinctly associated with prostate cancer risk in Caucasians (odds ratio = 1.24, 95% confidence interval: 1.03, 1.48,  $P = 0.02$ ). In conclusion, the GSTT1 null genotype is related with prostate cancer chance in Caucasians, but not within the overall population.

### **1.5 ROLE OF ANTIOXIDANTS IN CHRONIC KIDNEY DISEASES:**

Antioxidants have an effective role in sustaining the metabolism of biomolecules. They crucially avoid the outbreaks of oxidative stress in their host cells. The concentration of a particular antioxidant is a necessary factor which determines its fate in that particular metabolism. This nutrient absorption of antioxidants balances their pool or fraction in our system, so it becomes a major investigative approach to assess their role in certain diseases.

Constant kidney disease (CKD) could be a significant risk factor for untimely cardiovascular malady and passing. Expanded oxidative push in individuals with CKD has been embroiled as a potential causative calculation for a few cardiovascular maladies. Antioxidant treatment may decrease cardiovascular mortality and dreariness in individuals with CKD (Jun M and Venkataraman 2012)

People with chronic kidney disease (CKD) have a tall hazard of creating heart infection and passing on rashly. In spite of the fact that heart infection has numerous causes, harm caused by destitute oxygen trade within the body's cells (oxidative stretch) is thought to be a major issue. Individuals with CKD frequently have proof of oxidative stress and this can be emphatically related with the rate of kidney malady movement. We surveyed current prove to assess how antioxidant treatment impacted results for patients with CKD. By and large, we found that antioxidant treatment did not decrease the chance of heart illness or passing in people with CKD, but that this seems to shift depending on CKD arrangement. There were a few proofs to propose that individuals on dialysis may benefit from antioxidant treatment, which these treatments may diminish the hazard of kidney infection getting to be more awful. In any case, these come about are based on exceptionally restricted evidence and

encourage ponders are required to affirm if antioxidant treatment can be of advantage for individuals with CKD.

Majority of studies indicated that antioxidants are helpful in hemodialysis patients, reducing the risk of cardiovascular events and increasing the quality of dialysis (Kalantar .k - Zadeh and Balakrishnan V. S. 2006)

## CHAPTER - 2

### REVIEW OF LITERATURE

Abbou Kaddous, and Mallika ,Tbahriti, H. F. *et al.*, (2013) have studied the Effect of different stages of chronic kidney Disease and Renal replacement therapies on Oxidant-Antioxidant Balance in uremic patients. By Lipids and proteins peroxidation, Antioxidant, Measurements and statistical analysis. The outcome showed that HD (Hemodialysis) accentuates lipids, while PD aggravates protein oxidation. The activity of antioxidant enzymes was changed by impaired renal work and by both dialysis medicines.

Blaithin. A and Murray, P. T. *et al.*, (2010) have studied based on the Urinary glutathione S-transferases in pathogenesis and demonstrative harm taking after cardiac surgery: a basic survey. Outcome showed that GSTs in minimizing oxygen free radicals exposure in the renal tubules during CPB may shed further light into their role as promising biomarkers of cardiac surgery-associated AKI (acute kidney injury).

Bras.J and Nefrol *et al.*, (2015) have studied based on the GSTM1 and GSTT1 genes null polymorphisms in kidney cancer susceptibility: prove based on a meta-analysis. Outcome showed that No associations were found between polymorphisms of GSTM1 null (OR = 1.015, 95%CI =0.897 to 1.147) and GSTT1-null (OR = 1.081, 95%CI = 0.791 to 1.479) and renal cancer.

Gutierrez-Amavizca B.E and Orozco-Castellanos.R *et al.*, (2013) have studied based on the GSTM1, GSTT1, and MTHFR polymorphisms to end-stage renal disease of unknown etiology in Mexicans. By Deoxyribonucleic acid (DNA) extraction and genotyping and statistical analysis. Results appeared that the GSTM1 invalid polymorphism shows up to be related with the ESRD (End-stage renal infection) of obscure etiology in patients in Mexico.

Hamid Nomani and Ali Aidy *et al.*, (2016) have studied based on the Association between GSTM1, GSTT1, and GSTP1 variants and the risk of end-stage renal disease (2016). The outcome showed that the GST-null allele (GSTT1-null/GSTM1-

null) is a risk factor ESRD and carriers of this allele have high levels of MDA (malondialdehyde).

Hui-Yuan Cheng and Tian-Biao Zhou *et al.*, (2012) have studied based on the Relationship between GSTM1/GSTT1 Null Genotypes and Renal Cell Carcinoma Risk: A Meta-Analysis. The outcome showed that GSTT1 null genotype is associated with the RCC (Renal cell carcinoma) susceptibility in Caucasians and Asians and the dual null genotype of GSTT1-GSM1 is associated with RCC (Renal cell carcinoma) risk in Asian.

Mimic-Oka. J and Tsimic *et al.*, (1992) have studied based on the Glutathione and its related chemicals that infringe blood cells in numerous stages of incessant renal inadequate. Outcomes showed that GR (glutathione reductase) and GST activities normal human lymphocytes are increased when incubated with plasma from uremic patients.

Sonja suvakov and Aleksandra Stefanovic *et al.*, (2012) have studied based on the Glutathione S-transferase A1, M1, P1, and T1 null or low genotype are associated with enhanced oxidative damage among oxidative damage among hemodialysis patients. The outcome showed that the level of oxidative and carbonyl stress was most pronounced in the subgroup of patients with all four null or low-activity GSTM1, GSTT1, GSTP1, and GSTA1 genotypes.

Sudip k. Datta and Vivek Kumar *et al.*, (2010) have studied based on the Association of glutathione S-transferase M1 and T1 gene polymorphism with oxidative stress in diabetic and nondiabetic chronic kidney diseases by DNA extraction and genotype, Biochemical analysis, and statistical analysis, results through Demographic data and routine biochemical investigations, Genotyping, Genotype-Phenotype Correlation. The outcome appeared that GSTT1 cancellation shows up to be related to both diabetic and nondiabetic CKD independent of the GSTM1 status.

Y Yang and MT Kao *et al.*, (2004) have studied based on the Glutathione S-transferase T1 cancellation may be a hazardous figure for creating end-stage renal illness in diabetic patients. The outcome showed that homozygous deletion of the GSTT1 gene is a risk factor for developing the end-stage renal disease in diabetic patients.

Merad-Boudia and Marie Chantal Jaudon *et al.*, (1996) have studied based on the Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. The outcome showed that resulting Oxidative stress could contribute to the development of accelerated atherosclerosis and other long-term complications in uremic patients.

Veronique witko-Sarsat and Tilman Drueke *et al.*, (2004) have studied based on the Restoring glutathione as a therapeutic strategy in chronic kidney disease. Outcomes showed that Disabled resistant reactions are related to a decrease within the glutathione concentration of immune tissue.

John L.E and R.S Houlston *et al.*, (2000) have studied based on the Glutathione S transferase  $\mu 1$  (GSTM1) status and bladder cancer risk: a meta-analysis by statistical analysis . Outcome showed that the interactions between the GSTM1 and other polymorphisms on the risk of bladder cancer and their interactions with environmental risk factors will only be addressed by well –designed studies based on samples sizes commensurate with the detection of small genotypic risks.

Chih-Hsien Wang, and Vint-Cent Wu *et al.*, (2000) have studied based on the Urinary  $\pi$ -glutathione S-transferase predicts Advanced Acute kidney injury Following Cardiovascular surgery by study population (conducted by biomarker investigators from NSARF (the national Taiwan university study group on acute renal) , Clinical Data Collection, Sample collection, Biomarker Measurements, Outcome Definitions, Statistics Analyses. The outcome showed that the performance of the best cut-off value of both  $\alpha$ -and  $\pi$  GST are thus evaluated prospectively in this study in order to elucidate their respective performance as critical AKI biomarkers, in terms of dialysis or mortality.

## **CHAPTER 3**

### **AIM AND SCOPE**

#### **3.1 AIM:**

To identify the GSTT1 null genotype and glutathione levels in chronic kidney diseases and To estimate the amount of Glutathione in plasma using Moron procedure by drawing a standard curve using known concentration of Glutathione by spectroscopic method.

#### **3.2 SCOPE OF THE PRESENT INVESTIGATION:**

The present study can be applied to predict Oxidative stress by estimating Glutathione levels in the CKD patients. This case-control study was analyzed through statistical analysis. Chronic kidney disease (CKD) could be around the world open wellbeing issue, with expanding rate and predominance, high costs, and destitute results

- There is even a substantially higher prevalence of the earlier stages of chronic kidney disease (CKD), with adverse outcomes, including loss of kidney function, cardiovascular disease (CVD), and premature death. Methodologies to progress results will require a worldwide effort coordinated at the prior stages of CKD.
- The rationale for a global initiative to address this problem is simple and self-evident. The epidemic of CKD is global. The adverse results of CKD are all-inclusive, as are the basic science and evidence-based methodologies for anticipation, location, assessment, and treatment. In spite of the fact that chance components and assists for care change locally, it is critical to extend the proficiency of utilizing accessible mastery and assists in moving forward the care and results of CKD around the world.
- To provide a clear understanding to both the nephrology and non-nephrology communities of the evidence base for the K/DOQ Identification and classification of seriousness of CKD.
- To develop global consensus for the adoption of a simple definition and classification system for CKD, clarifications and modifications to current guidelines to facilitate more widespread implementation of activities for patient care and physician and open instruction around the world.
- To identify a collaborative research agenda and plan that would improve the evidence base and facilitate the implementation of the definition and classification of CKD. (Mekki K. *et al.*, 2013)

## **CHAPTER 4**

### **MATERIALS AND METHODS**

#### **4.1 METHODOLOGY:**

The materials that are used for the process are:

##### **4.1.1 EQUIPMENTS:**

- ☐ Test tubes
- ☐ Eppendorf tubes
- ☐ Vacutainer
- ☐ Beakers
- ☐ Conical flasks
- ☐ Pipette
- ☐ Pipette tips
- ☐ Pipette tips stand
- ☐ Test tube racks

##### **4.1.2 CHEMICALS:**

- ☐ Agarose Powder
- ☐ TE buffer
- ☐ Forward Primer
- ☐ Reverse Primer
- ☐ Master mix solution
- ☐ Restriction endonuclease (MSP1) Solution,
- ☐ Spirit Solution
- ☐ 10X TBE buffer

- ☐ 1X TBE buffer
- ☐ Tris HCl
- ☐ KCl
- ☐ MgCl
- ☐ EDTA
- ☐ 50ul blood plasma
- ☐ Sodium phosphate buffer (pH 8)
- ☐ 0.6mM DTNB

#### **4.1.3 TECHNIQUES:**

- ☐ Gradient PCR,
- ☐ Agarose gel electrophoresis,
- ☐ Laminar airflow hood,
- ☐ Transilluminator.

#### **4.1.4 DEVICES**

- ☐ Reagent Bottles
- ☐ Electrophoresis tray
- ☐ Gel Comb
- ☐ Weighing scale
- ☐ Power Supply

#### **4.1.5 SOFTWARE:**

- ☐ EXCELSHEETS
- ☐ MS WORD
- ☐ EPIINFO

#### **4.2 - Sample Collection:**

Venous blood samples (5ml) were collected in EDTA vacutainers of farmers.

Immediately after the collection, they were transported and stored at -20 C. The subsequent day. Genomic DNA was extracted and used for polymerase chain reaction (PCR) to determine the genotype.

#### **4.2.1 DNA Isolation:**

The isolation of DNA is a fundamental first step in any assay of molecular techniques involving in genetic identity analysis. The initial isolation of DNA from a sample source can be tedious, involving steps that yield

the highest quantity of DNA at the expense of speed and convenience.

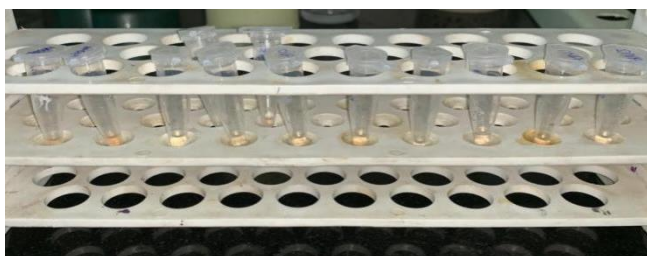


***Fig 4.1 Blood collection in Vacutainer***

Many techniques currently used for DNA lead to significant dilution of the sample, materials or require the precipitation of material especially difficult. This consideration is especially important in the field of forensic analysis and genetic identity where DNA is extracted from extremely small amounts of starting materials often recovered from suboptimal conditions.

### 4.3 DNA isolation from blood:

The technique presented in this report can be used to isolate DNA from a variety of sample media, including fresh or dried blood and as little as 10ml of blood. In addition, the sample and thought put of the technique enables a large number of the sample to proceed quickly (Datta, S.K., et al., 2010).



***Fig 4.2 DNA Isolation in Pipette tubes***

#### 4.3.1 Preparation of Reagents:

##### **TKM1 Solution:**

- ☐ Tris HCl (PH 7.6)
- ☐ KCl
- ☐  $MgCl_2$
- ☐ EDTA

### 4.4 Properties of agarose gel:

Agarose gel may be a three-dimensional lattice shaped of helical agarose particles in supercoiled bundles that are amassed into three-dimensional structures with channels and pores through which molecules can pass. The 3-D structure is held along with hydrogen bonds and can subsequently be disturbed by warming back to a fluid state. The dissolving temperature is diverse from the gelling temperature,

depending on the sources, agarose gel incorporates a gelling temperature of 35–42 °C and a softening temperature of 85–95 °C.

## **Procedure:**

### **4.4.1 Casting of gel:**

The gel was prepared by dissolving the agarose powder in an appropriate buffer, such as TAE or TBE, to be used in electrophoresis. The agarose is dispersed in the buffer before heating it to a near-boiling point, but avoids boiling. The melted agarose is permitted to cool adequately some time recently pouring the arrangement into a cast as the cast may twist or split in case the agarose arrangement is as well hot. A comb is set within the cast to form wells for stacking tests, and the gel ought to be totally set some time recently used. The concentration of gel influences the determination of DNA partition. The agarose gel is composed of microscopic pores through which the atoms travel, and there's an reverse relationship between the pore measure of the agarose gel and the concentration – pore estimate diminishes as the thickness of agarose filaments increments. Tall gel concentration moves forward partition of littler DNA atoms, whereas bringing down gel concentration licenses expansive DNA molecules to be separated. The process allows fragments extending from 50 base sets to a few megabases to be isolated depending on the gel concentration utilized. The concentration is measured in weight of agarose over the volume of the buffer utilized (g/ml). For a standard agarose gel electrophoresis, a 0.8% gel gives great partition or determination of expansive 5–10kb DNA parts, whereas 2% gel gives great determination for little 0.2–1kb parts. 1% gels is frequently utilized for a standard electrophoresis. Tall rate gels are habitually fragile and may not set evenly, though moo rate gels (0.1-0.2%) are fragile and not basic to handle. Low-melting-point (LMP) agarose gels are as well more sensitive than commonplace agarose gel.

Low-melting point agarose may be utilized on its possessor or at the same time with standard agarose for the partition and confinement of DNA.

#### **4.4.2 Loading of samples:**

Once the gel has set, the comb is evacuated, taking off wells where DNA tests can be stacked. Stacking buffer is blended with the DNA test some time recently the blend is stacked into the wells. The loading buffer contains a thick compound, which may be glycerol, sucrose, or Ficoll, that raises the thickness of the test so that the DNA test may sink to the foot of the well.[28] If the DNA sample contains residual ethanol after its preparation, it may float out of the well. The loading buffer too incorporates colored colors such as xylene cyanol and bromophenol blue utilized to screen the advance of the electrophoresis. The DNA samples are loaded using a pip (Kim, Y. H et al., 2012).



***Fig 4.3 Sample loading***

#### **4.4.3 Electrophoresis:**

Agarose gel slab in electrophoresis tank with groups of colors showing advance of the electrophoresis. The DNA moves towards anode. Agarose gel electrophoresis is most commonly done on a level plane in a submarine mode whereby the chunk gel is totally submerged in a buffer amid electrophoresis. It is additionally conceivable, but less common, to perform the electrophoresis vertically, as well as on a level plane

with the gel raised on agarose legs utilizing a suitable apparatus. The buffer utilized within the gel is the same as the running buffer within the electrophoresis tank, which is why electrophoresis within the submarine mode is conceivable with agarose gel.



**Fig 4.4 Electrophoresis**

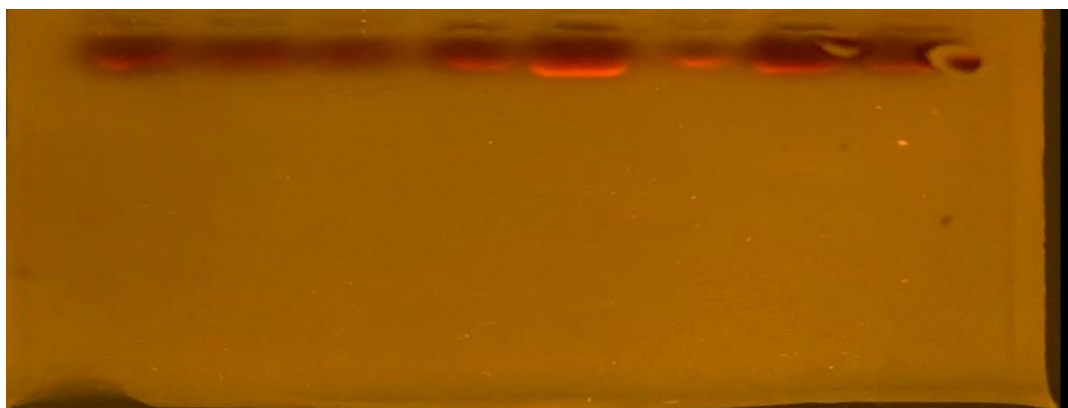
For optimal resolution of DNA more noteworthy than 2kb in measure in standard gel electrophoresis, 5 to 8 V/cm is suggested (the separate in cm alludes to the remove between terminals, hence this prescribed voltage would be 5 to 8 duplicated by the separate between the anodes in cm). Voltage may too be constrained by the reality that it warms the gel and may cause the gel to soften in case it is run at tall voltage for a delayed period, particularly in the event that the gel utilized is LMP agarose gel. As well tall a voltage may moreover decrease determination, as well as causing band streaking for expansive DNA particles. As well, moo a voltage may lead to broadening of the band for little DNA parts due to dispersion and diffusion.

Since DNA is not visible in characteristic light, the advance of the electrophoresis is observed utilizing colored colors. Xylene cyanol (light blue color) comigrates expansive DNA parts, whereas Bromophenol blue (dull blue) co moves with the littler parts. Less commonly utilized colors incorporate Cresol Ruddy and Orange G which

relocate ahead of bromophenol blue. A DNA marker is additionally run together for the estimation of the atomic weight of the DNA parts. Note that as it may be that the measure of circular DNA like plasmids cannot be precisely gaged utilizing standard markers unless it has been linearized by confinement process, on the other hand, a supercoiled DNA marker may be utilized.

#### **4.4.4 Staining and visualization:**

DNA as well as RNA are ordinarily visualized by recoloring with ethidium bromide, which intercalates into the major grooves of the DNA and fluoresces beneath UV light. The intercalation depends on the concentration of DNA and hence, a band with a tall concentration will show a better sum of DNA compared to a band of less intensity. The ethidium bromide may be added to the agarose solution before it gels, or the DNA gel may be stained later after electrophoresis. Distaining of the gel is not necessary but may produce better images. Other strategies of recoloring are accessible; illustrations are SYBR Green, GelRed, methylene blue, brilliant cresyl blue, Nile blue sulfate, and precious stone violet. SYBR Green, GelRed and other comparative commercial items are sold as more secure choices to ethidium bromide because it has been appeared to be mutagenic in Ames test, in spite of the fact that the carcinogenicity of ethidium bromide has not truly been set up. SYBR Green requires the utilization of a blue-light transilluminator. DNA stained with crystal violet can be viewed under natural light without the use of a UV transilluminator which however does not produce the strong bands (Kim, Y. H et al., 2012).



***Fig 4.5 DNA Visualization***

When stained with ethidium bromide, the gel is seen with an bright (UV) transilluminator. The UV light excites the electrons inside the fragrant ring of ethidium bromide, and once they return to the ground state, light is discharged, making the DNA and ethidium bromide complex fluoresce. Standard transilluminators use wavelengths of 302/312-nm (UV-B), however exposure of DNA to UV radiation for as little as 45 seconds can produce damage to DNA and affect subsequent procedures, for example reducing the efficiency of transformation, in vitro transcription, and PCR. Exposure of the DNA to UV radiation therefore should be limited. Using a higher wavelength of 365 nm (UV-A run) causes less harm to the DNA but too produces much weaker fluorescence with ethidium bromide. Where multiple wavelengths can be selected in the transilluminator, the shorter wavelength would be used to capture images, while the longer wavelength should be used if it is necessary to work on the gel for any extended period of time.

#### **4.5 Downstream procedures:**

The separated DNA bands are frequently utilized for assist methods, and a DNA band may be cut out of the gel as a cut, broken down, and decontaminated. Contaminants be that as it may affect a few downstream methods such as PCR, and too softening point agarose may be favored in a few cases because it contains less of the sulfates that can influence a few enzymatic responses. The gels may too be utilized for blotting techniques.

#### **4.6 Buffers:**

In general, the ideal buffer should have great conductivity, create less heat and have a long life. There are a number of buffers utilized for agarose electrophoresis; common ones for nucleic acids incorporate Tris/Acetate/EDTA (TAE) and Tris/Borate/EDTA (TBE). The buffers utilized contain EDTA to inactivate numerous nucleases which require divalent cation for their work. The borate in TBE buffer can be tricky as borate can polymerize, and/or connected with cis diols such as those

found in RNA. TAE has the least buffering capacity, but it gives the most excellent determination for bigger DNA. This implies a lower voltage and more time, but a distant better product.



***Fig 4.6 PCR (polymerase chain reaction)***

## **4.7 Primer:**

A primer may be a brief single-stranded nucleic acid utilized by all living life forms within the start of DNA synthesis. The enzymes responsible for DNA replication, DNA polymerases, are as it were able of including nucleotides to the 3'-end of an existing nucleic corrosive, requiring a groundwork be bound to the layout some time recently DNA polymerase can start a complementary strand. Living living beings utilize exclusively RNA preliminaries, whereas research facility procedures in organic chemistry and atomic science that require in vitro DNA amalgamation (such as DNA sequencing and polymerase chain response) as a rule utilize DNA groundworks, since they are more temperature steady.

#### **4.8 The cycling reactions:**

There are three major steps in a PCR, which are rehearsed for 30 or 40 cycles. This is often done on a computerized cycler, which can warm and cool the tubes with the response blend in a really short time.

##### **4.8.1 Denaturation at 94°C:**

During the denaturation, the twofold strand dissolves open to single stranded DNA, all enzymatic responses halt (for case: the expansion from a previous cycle).

##### **4.8.2 Annealing at 54°C:**

The primers are wiggling around, caused by the Brownian movement. Ionic bonds are continually shaped and broken between the single-stranded groundwork and the single-stranded template. The more steady bonds final a small bit longer (preliminaries that fit precisely) and on that small piece of twofold stranded DNA (template and primer), the polymerase can connect and begins replicating the layout. Once there are many bases built within, the ionic bond is so solid between the layout and the groundwork, that it does not break anymore.

##### **4.8.3 Extension at 72°C:**

This is the ideal working temperature for the polymerase. The preliminaries, where there are a couple of bases built in, as of now have a more grounded ionic fascination to the format than the strengths breaking these attractions. Primers that are in positions with no exact match, get loose again (because of the higher temperature) and don't give an extension of the fragment.

The bases (complementary to the layout) are coupled to the groundwork on the 3' side (the polymerase includes dNTP from 5' to 3', perusing the format from 3' to 5' side, bases are included complementary to the template).

#### **4.9 Procedure:**

Typically, PCR comprises an arrangement of 20–40 rehearsed temperature changes, called warm cycles, with each cycle commonly comprising two or three discrete temperature steps (see figure underneath). The cycling is frequently gone before by a single temperature step at a really tall temperature (>90 °C (194 °F)), and taken after by one hold at the conclusion for last item expansion or brief capacity. The temperatures utilized and the length of time they are connected in each cycle depend on assortment of parameters, counting the chemical utilized for DNA synthesis.

The concentration of bivalent particles and dNTPs within the response, and the dissolving temperature ( $T_m$ ) of the primers. The individual steps common to most PCR methods are as follows:

##### **GSTT1-**

5'-TTCCTTACTGGTCCTCACATCTC-3' and

5'- TCACCGGATCATGGCCACC-3'

##### **Globulin-**

5'-GCCCTCTGCTGCTAACAAGTCCTAC-3' and

5'-GCCCTAAAAAGAAAATCGCCCAATC-3'

#### **4.9.1 Initialization:**

This step is as it were required for DNA polymerases that require warm enactment by hot-start PCR.[12] It comprises of warming the response chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes.

#### **4.9.2 Denaturation:**

This step is the first regular cycling occasion and comprises warming the response chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules

#### **4.9.3 Annealing:**

In the following step, the response temperature is brought down to 50–65 °C (122–149 °F) for 20–40 seconds, permitting toughening of the preliminaries to each of the single-stranded DNA formats. Two diverse primers are ordinarily included within the response blend: one for each of the two single-stranded complements containing the target locale. The preliminaries are single-stranded groupings themselves, but are much shorter than the length of the target locale, complementing as it were exceptionally brief groupings at the 3' conclusion of each strand.

It is basic to decide an appropriate temperature for the tempering step since proficiency and specificity are unequivocally influenced by the strengthening temperature. This temperature must be moo sufficient to permit for hybridization of the preliminary to the strand, but tall sufficient for the hybridization to be particular, i.e., the primer ought to tie as it were to a superbly complementary portion of the strand, and no place else. On the off chance that the temperature is as well moo, the primer may tie imperfectly. On the off chance that it is as well tall, the groundwork may not tie at all. An ordinary toughening temperature is around 3–5 °C below the  $T_m$  of the groundworks utilized. Steady hydrogen bonds between complementary bases are shaped as it were when the groundwork arrangement exceptionally closely matches the layout sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.

#### **4.9.4 Extension/Elongation:**

The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA polymerase of Taq polymerase (*Thermus aquaticus*). Polymerase is approximately 75–80 °C (167–176 °F), though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by including free dNTPs from the response blend that are complementary to the format within the 5'-to-3' heading, condensing the 5'-phosphate gather of the dNTPs with the 3'-hydroxy gather at the conclusion of the early (prolonging) DNA strand. The exact time required for stretching depends both on the DNA polymerase utilized and on the length of the DNA target locale to amplify. As a rule of thumb, at their ideal temperature, most DNA polymerases polymerize a thousand bases per

diminutive. Beneath ideal conditions (i.e., in case there are no impediments due to restricting substrates or reagents), at each extension/elongation step, the number of DNA target groupings is doubled. With each successive cycle, the initial format strands additionally all recently created strands gotten to be layout strands for the following circular of elongation, driving to the exponential (geometric) enhancement of the particular DNA target region. The forms of denaturation, tempering and stretching constitute a single cycle. Different cycles are required to intensify the DNA target to millions of duplicates. The equation utilized to calculate the number of DNA duplicates shaped after a given number of cycles is  $2^n$ , where  $n$  is the number of cycles. In this way, a response set for 30 cycles comes about in 230, or 1073741824, copies of the first double-stranded DNA target region.

#### **4.9.5 Final elongation:**

This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature extend required for ideal movement of most polymerases utilized in PCR) for 5–15 minutes after the final PCR cycle to guarantee that any remaining single-stranded DNA is fully elongated.

#### **4.9.6 Final hold:**

The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products

| <b>Temperature</b> | <b>Time:</b> |
|--------------------|--------------|
| 940C               | 3min         |
| 940C               | 30 sec       |
| 600C               | 1min         |
| 720C               | 55 sec       |
| 720C               | 8 min        |
| 80C                | Infinite     |

**Total 30 cycles**

#### **4.10 Restriction Digestion:**

Restriction Digestion is the method of cutting DNA atoms into smaller pieces with extraordinary proteins called Confinement Endonucleases (now and then fair called Limitation Chemicals or RE's). These extraordinary enzymes recognize particular groupings within the DNA molecule (for case GAATTC) wherever that grouping happens within the DNA. Restriction Digests start by blending the DNA and the RE, but it's unfortunately not very as basic as that. Confinement Chemicals are sensitive and ought to be treated carefully. Since chemicals are proteins and proteins denature as the temperature is expanded, RE's are continuously put away in a cooler until they are used. In reality, all of the fixings in a Limitation Process are kept on ice until it's time for the response to begin. The actual response conditions change from one protein to another, and incorporate temperature, NaCl and/or MgCl<sub>2</sub> concentration, pH, etc. All of these factors but temperature are optimized by blending the chemical and DNA with a buffer particular for the protein of choice. [Water bath]Once all the fixings are blended within the response tube, the tube is hatched at the Limitation Enzyme's ideal temperature for 1 hour or longer. At that point at last when the process has run for the fitting sum of time, the response tube is put back on ice to anticipate nonspecific corruption of your DNA. Once the Confinement Process is completed, Agarose Gel Electrophoresis is performed to isolate the process parts by estimate and visualize.

#### **4.11 DNA Ladder:**

A molecular-weight estimate marker, too referred to as a protein step, DNA stepping stool, or RNA step, may be a set of benchmarks that are utilized to recognize the inexact estimate of a particle run on a gel amid electrophoresis, utilizing the rule that atomic weight is contrarily corresponding to movement rate through a gel framework. Hence, when utilized in gel electrophoresis, markers successfully give a logarithmic scale by which to appraise the estimate of the other parts (giving the part sizes of the marker are known). Protein, DNA, and RNA markers with pre-determined part sizes and concentrations are commercially accessible. These can be run in either agarose

or polyacrylamide gels. The markers are stacked in paths adjoining to test paths some time recently the graduation of the run. DNA ligation is the method by which straight DNA pieces are associated with each other by means of covalent bonds; more particularly, these bonds are phosphodiester bonds. Here, a 100bp duplex DNA piece is somewhat ligated. The consequence of this is often that dimers of 200bp, trimers of 300bp, tetramers of 400bp, pentamers of 500bp, etc. will shape. Also, a parcel of the 100bp dsDNA will remain. As a result, a DNA "ladder" composed of DNA pieces of known molecular mass is created on the gel (Loit, E. et al., 2015).

#### **4.12 Effects of gel conditions:**

As with experimental samples, the conditions of the gel can affect the molecular weight size marker that runs alongside them. Factors such as buffer, charge/voltage, and concentration of gel can influence the versatility and/or appearance of your marker/ladder/standard. These components have to be taken into consideration when selecting a marker and when analyzing the ultimate comes about on a gel. 1.2% agarose gel appears in two distinctive DNA steps colored with Gel Ruddy recolor.

#### **4.13 Buffers:**

Buffers act to

- 1) Establish pH,
- 2) Give particles back conductivity. In DNA electrophoresis, the TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA) are the typical buffers of choice. TBE buffer is favored for little DNA pieces, while TAE is way better suited for parts more prominent than 1500 base sets. In terms of buffering capacity, TAE is lower when compared to TBE; this for the most part comes about in slower portability of the DNA. TBE is additionally able to offer better resolution. It must be famous that water cannot act as a substitute for one of these buffers, as the DNA will not move along the gel. Furthermore, utilizing water rather than a buffer will result within the gel melting.

#### **4.14 Charge/Voltage:**

In terms of voltage, the suggested extent is between 4 and 10 V/cm (i.e., volts/cm). Agarose gels are ordinarily run at a voltage of 5 V/cm. The separate unit, cm, alludes to the removal between the cathodes (i.e., the anode and the cathode) and not the length of the gel itself. Voltages as well distant underneath or over this extent will influence the portability and the determination of the groups. Moo voltages will diminish the versatility and will cause the groups to broaden. On the other hand, tall voltages will diminish the determination of the groups. This can be to a great extent due to the truth that voltages that are as well tall can cause the gel to overheat, and even melts.

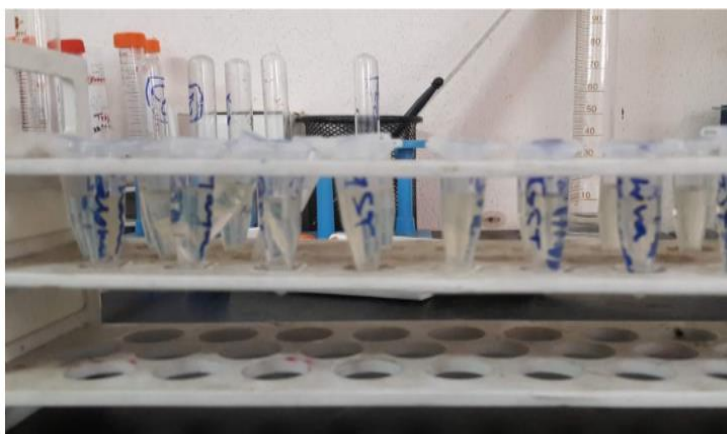
#### **4.15 Concentration:**

Agarose concentration must be taken under consideration when selecting a marker. The gel percentage affects the migration of the DNA. Generally, the higher the gel concentration, the slower the rate at which the DNA will move through the gel. This is in addition to the part atomic weight plays within the movement of a DNA marker or test, that's to say, that the higher the molecular weight, the slower the DNA will migrate. Gel concentration moreover influences the capacity to imagine the groups run out on the gel. Littler groups are superior settled on the next rate gel, while expanded molecular-weight groups are more effortlessly visualized on a lower percentage gel.

#### **4.16 DETERMINATION OF REDUCED GLUTATHIONE:**

- A serial range of control and cases plasma samples were analyzed for GSH with the respective serial count.
- Initially 25µl of plasma was taken in sterilized Eppendorf tubes. Then, 475µl of sodium phosphate buffer was added to make it up to a volume of 500µl. To this 1ml of 0.6mM DTNB was added.

➤ Yellow color was obtained after a 10 minutes incubation period and this was measured at 412nm against a blank containing 50μl of 5% TCA in place of supernatant. (Duan, X. *et al.*, 2015)



***Fig 4.7 Known Samples of Glutathione***

#### **4.17 Epi info:**

Epi Data is a factual program for the study of disease transmission created by the Centers for Malady Control and Avoidance (CDC) in Atlanta, Georgia (US). From a user's viewpoint, the foremost vital capacities of Epi Information are the capacity to quickly create a survey, customize the information passage handle, rapidly enter data into that survey, and after that analyze the information. For epidemiological employments, such as episode examinations, being able to rapidly make an electronic information section screen and after that do quick examination on the collected information can spare significant sums of time versus utilizing paper surveys.

Epi Data uses three particular modules to achieve these errands: Shape Creator, Enter, and Investigation. Other modules incorporate the Dashboard module, a mapping module, and different utilities such as StatCalc.

Electronic questionnaires are made within the Frame Originator module. Person questions can be put anyplace on a page and each shape may contain numerous

pages. The client is given a tall degree of control over the form's appearance and work. The client characterizes both the question's prompt and the arrangement of the information that's to be collected. Information sorts incorporate numbers, content strings, dates, times, and Boolean. Clients can too make drop-down records, code tables, and comment legal areas. One of the more powerful features of Form Designer is the capacity to program insights into a frame through a highlight called "check code". Check code permits for certain occasions to happen depending on what activity an information passage individual has taken. For illustration, in case the information section individual sorts "Male" into an address on sexual orientation, any questions relating to pregnancy might at that point be covered up or crippled. Skip designs, message boxes, and math operations are moreover accessible. Social database modeling is upheld, as clients may interface their frame to any number of other shapes in their database.

The "Classic Analysis" module is where clients analyze their information. Consequence and send out capacities exist that permit for information to be changed over between plain-text, CSV, Microsoft Excel expectations, Microsoft Access, MySQL, Microsoft SQL Server, and other groups. Numerous progressed factual schedules are given, such as t-tests, ANOVA, non-parametric measurements, cross organizations and stratification with gauges of chances proportions, hazard proportions, and chance contrasts, calculated relapse (conditional and unrestricted), survival examination (Kaplan Meier and Cox relative risk), and examination of complex overview data. "Visual Dashboard" module may be a lighter-weight Investigation component that's designed to be simple to utilize, but does not contain the total set of information administration highlights that the "Classic Examination" module does. Utilizing the Outline module, information can be shown either by geographic reference or by GPS coordinates.

Older versions of Epi Information contained a Report module and a Menu module. The Report module permitted the client to alter and organize the crude yield from other Epi Information modules into satisfactory reports. The menu module permitted for the altering and rearranging of the essential Epi Data menu structure. This module was effective enough that a few applications have been built off of it (in forms of Epi Information earlier to form 7), counting the National Electronic Broadcast

communications Framework for Observation (NETSS) for Epi Data 6. Not at all like the other modules, the menu module does not have a design-mode client interface, but instead dwells in a .mnu record whose scripts must be altered physically. In Epi Data 7, the Visual Dashboard accepts a few of the fundamental capacities of the report module.

Epi Data 7 includes a number of wholesome anthropometric capacities that can help in recording and assessing estimations of length, stature, weight, head circumference, and arm circumference for children and young people. They can be utilized to calculate percentiles and number of standard deviations from the cruel (Z-scores) utilizing the CDC/WHO 1978 development reference, CDC 2000 development reference, the WHO Child Development Reference, or the WHO Reference 2007.

It replaces the Nut Stat and Epi Nut modules found in earlier forms of Epi Information.

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StatCalc - 2x2 Tables

Strata 1 | Strata 2 | Strata 3 | Strata 4 | Strata 5 | Strata 6 | Strata 7 | Strata 8 | Strata 9

|          |       | Outcome |         |         |
|----------|-------|---------|---------|---------|
|          |       | Yes     | No      | Total   |
| Exposure | Yes   | 27      | 15      | 42      |
|          | Row % | 64.29%  | 35.71%  | 100.00% |
|          | Col % | 87.10%  | 42.86%  | 63.64%  |
|          |       |         |         |         |
| Exposure | No    | 4       | 20      | 24      |
|          | Row % | 16.67%  | 83.33%  | 100.00% |
|          | Col % | 12.90%  | 57.14%  | 36.36%  |
|          |       |         |         |         |
| Total    |       | 31      | 35      | 66      |
|          | Row % | 46.97%  | 53.03%  | 100.00% |
|          | Col % | 100.00% | 100.00% | 100.00% |

Odds-based Parameters

|                        | Estimate | Lower  | Upper   |
|------------------------|----------|--------|---------|
| Odds Ratio             | 9.0000   | 2.5906 | 31.2670 |
| MLE Odds Ratio (Mid-P) | 8.6770   | 2.6106 | 34.5525 |
| Fisher Exact           |          | 2.3271 | 41.5441 |

Risk-based Parameters

|                 | Estimate | Lower   | Upper   |
|-----------------|----------|---------|---------|
| Risk Ratio      | 3.8571   | 1.5332  | 9.7037  |
| Risk Difference | 47.6190  | 26.8269 | 68.4112 |

Statistical Tests

|                 | X <sup>2</sup> | 2 Tailed P |
|-----------------|----------------|------------|
| Uncorrected     | 13.9039        | 0.00019240 |
| Mantel-Haenszel | 13.6932        | 0.00021523 |
| Corrected       | 12.0578        | 0.00051576 |

|              | 1 Tailed P | 2 Tailed P |
|--------------|------------|------------|
| Mid-P Exact  | 0.00009967 |            |
| Fisher Exact | 0.00018150 | 0.00026245 |

**Fig 4.8: Epi info**

#### 4.18 Statistical analysis:

Allele frequencies were calculated by gene counting methods. Presence of the particular allele was designated as wild genotype and absence or deletion of the allele was designated as null genotype. The association between GST T1 genotypes,

null genotype and glutathione levels in chronic kidney patients. Patients were analyzed using odds ratio (OR) derived from logistic regression analysis using EPI6 software (Epiinfo6 CDC). Because GST T1 null genotypes and glutathione levels may interact in CDK further analysis combining the GST T1 genotypes was carried out and their significance was determined by calculating the p value.

*Table 4.1 Components used for PCR*

|                              |              |
|------------------------------|--------------|
| Master Mix                   | 25µl         |
| Globulin/ Forward<br>Reverse | 6 µl<br>6 µl |
| GST/ Forward<br>Reverse      | 6 µl<br>6 µl |
| DNA                          | 4 µl         |
| DH20                         | 7 µl         |
| Total                        | 60 µl        |

#### 4.19 PCR CONDITIONS:

The entire mixture was added and incubated at the following thermal conditions

*Table-4.2 Incubation temperature for PC*

|                   | TEMPERATURE( <sup>0</sup> C) | TIME   |
|-------------------|------------------------------|--------|
| Denature template | 95 <sup>0</sup> C            | 1 min  |
| Denature          | 95 <sup>0</sup> C            | 20 sec |
| Annealing         | 60 <sup>0</sup> C            | 1 min  |
| Elongation        | 77 <sup>0</sup> C            | 1 min  |
| Final Elongation  | 7 <sup>0</sup> C             | 1 min  |

| cases |       |                | controls |     |      | Sheet3       |            |        |           |          |              |
|-------|-------|----------------|----------|-----|------|--------------|------------|--------|-----------|----------|--------------|
|       | A     | B              | C        | D   | E    | F            | G          | H      | I         | J        | K            |
| 1     | S. No | Name           | Age      | Sex | Or   | Control/Test | Creatinine | lepoly | Urea      | BUN      | Gluthathione |
| 2     | 1     | Mrs. Akther E  | 59       | F   | Test | 2.4          | W          |        |           |          | 3.8839       |
| 3     | 2     | Mr. Jus Rao    | 76       | M   | Test | 4.4          | Null       |        | 131       | 65       | 0.7941       |
| 4     | 3     | Mrs. Rashee    | 72       | F   | Test | 2.2          | W          |        |           |          | 3.6761       |
| 5     | 4     | Mrs. Zabunis   | 61       | F   | Test | 3.4          | W          |        |           |          | 4.1377       |
| 6     | 5     | Mr. Yousuf K   | 45       | M   | Test | 4.2          | W          |        |           |          | 3.9123       |
| 7     | 6     | Mr. Yodagiri   | 78       | M   | Test | 2            | Null       |        | 63        | 31       | 0.4218       |
| 8     | 7     | Mr. Ravinder   | 61       | M   | Test | 2.4 mg/dl    | W          |        |           |          | 4.1675       |
| 9     | 8     | Mrs. Mubeer    | 32       | F   | Test | 2.1 mg/dl    | W          |        |           |          | 3.1231       |
| 10    | 9     | Mr. Srinivas I | 45       | M   | Test | 2.6 mg/dl    | Null       |        |           |          | 0.9231       |
| 11    | 10    | Mrs. Shahna    | 62       | F   | Test | 3.8 mg/dl    | W          |        | 53 mg/dl  | 26 mg/dl | 3.9828       |
| 12    | 11    | Mr. Shekar     | 63       | M   | Test | 2.4 mg/dl    | Null       |        |           |          | 0.9203       |
| 13    | 12    | Mr. Siddiq Al  | 62       | M   | Test | 8.4 mg/dl    | W          |        | 13 mg/dl  | 61 mg/dl | 4.4138       |
| 14    | 13    | Mrs. Shahna    | 52       | F   | Test | 2.5 mg/dl    | Null       |        | 71 mg/dl  |          | 0.9214       |
| 15    | 14    | Ms. Asra       | 26       | F   | Test | 7.4 mg/dl    | Null       |        | 266 mg/dl |          | 0.5241       |
| 16    | 15    | Mrs. Pushpa    | 64       | F   | Test | 4.5          | Null       |        |           |          | 1.7123       |
| 17    | 16    | Mrs. Shamek    | 40       | F   | Test | 0.7          | Null       |        |           |          | 0.4177       |
| 18    | 17    | Mr. Venkatar   | 42       | M   | Test | 2.1 mg/dl    | Null       |        | 47 mg/dl  | 22 mg/dl | 0.7069       |
| 19    | 18    | Dr. Uma Kulu   | 39       | F   | Test | 8.8 mg/dl    | Null       |        |           |          | 1.1853       |
| 20    | 19    | Mr. Ramudu.    | 43       | M   | Test | 5.9 mg/dl    | Null       |        |           |          | 0.9486       |
| 21    | 20    | Mr. Khairudd   | 72       | M   | Test | 2.5 mg/dl    | Null       |        | 63 mg/dl  | 31 mg/dl | 0.5241       |
| 22    | 21    | Mr. Mohd Me    | 45       | M   | Test | 4.3 mg/dl    | W          |        | 113 mg/dl | 56 mg/dl | 2.9123       |
| 23    | 22    | Mr. M. Abdul   | 83       | M   | Test | 2.1 mg/dl    | Null       |        |           |          | 0.8169       |
| 24    | 23    | Mr. Mohamn     | 60       | M   | Test | 14.5 mg/dl   | Null       |        | 137 mg/dl |          | 0.9216       |
| 25    | 24    | Mr. Anjaneyu   | 68       | M   | Test | 2.0 mg/dl    | Null       |        | 82 mg/dl  |          | 1            |
| 26    | 25    | Mr. Shyam K    | 51       | M   | Test | 7.5 mg/dl    | W          |        | 105 mg/dl |          | 4.0138       |
| 27    | 26    | Mrs. Shahna    | 62       | F   | Test | 3.8 mg/dl    | W          |        | 53 mg/dl  | 26 mg/dl | 0.9828       |
| 28    | 27    | Mr. Shekar     | 63       | M   | Test | 2.4 mg/dl    | Null       |        |           |          | 0.9214       |
| 29    | 28    | Mr. Siddiq Al  | 62       | M   | Test | 8.4 mg/dl    | W          |        | 13 mg/dl  | 61 mg/dl | 1.0138       |
| 30    | 29    | Mrs. Shahna    | 52       | F   | Test | 2.5 mg/dl    | Null       |        | 71 mg/dl  |          | 1.841        |
| 31    | 30    | Ms. Asra       | 26       | F   | Test | 7.4 mg/dl    | Null       |        | 266 mg/dl |          | 0.5241       |
| 32    | 31    | Mr. Ravi Mek   | 33       | M   | Test | 14.5 mg/dl   | W          |        |           |          | 1.854        |
| 33    | 32    | Mr. Sai Babu   | 62       | M   | Test | 2.0 mg/dl    | W          |        |           |          | 2.584        |
| 34    | 33    | kamaya         | 63       | M   | Test | 14.5 mg/dl   | null       |        |           |          | 1.7123       |
| 35    | 34    | akamarao       | 51       | M   | Test | 2.0 mg/dl    | w          |        |           |          | 3.851        |
| 36    | 35    | mahesh         | 48       | M   | Test | 7.5 mg/dl    | Null       |        |           |          | 0.4177       |

Fig 4.9 control list

| cases |       |                   | controls |     |              | Sheet3     |         |      |      |     |                    |
|-------|-------|-------------------|----------|-----|--------------|------------|---------|------|------|-----|--------------------|
|       | A     | B                 | C        | D   | E            | F          | G       | H    | I    | J   | K                  |
| 1     | S. No | Name              | Age      | Sex | Control/Test | Creatinine | lepoly  |      | Urea | BUN | glutathione umol/l |
| 2     |       | 1 Mahesh.N        | 50       | M   | Control      | 0.4        | W       |      |      |     | 3.9576             |
| 3     |       | 2 suraya.Y        | 64       | M   | Control      | 0.8        | W       |      |      |     | 3.9305             |
| 4     |       | 3 Swarna.Jaki     | 54       | F   | Control      | 0.2        | W       |      |      |     | 3.8056             |
| 5     |       | 4 Mr. Srinivas I  | 49       | M   | Control      | 0.7        | W       |      |      |     | 3.2138             |
| 6     |       | 5 Mr. Sridhar     | 25       | M   | Control      | 0.8        | W       |      |      |     | 4.8916             |
| 7     |       | 6 S.Hemant        | 31       | M   | Control      | 1          | W       |      |      |     | 3.8209             |
| 8     |       | 7 Mr. Ravi Mek    | 33       | M   | Control      | 0.6        | W       |      | 20   |     | 3.6551             |
| 9     |       | 8 Mr. Sai Babu    | 62       | M   | Control      | 1.1        | W       |      | 41   | 19  | 2.7528             |
| 10    |       | 9 Mr. T.R.R. Ra   | 63       | M   | Control      | 0.5        | null    |      |      |     | 3.8352             |
| 11    |       | 10 Mr. Abdul Ba   | 50       | M   | Control      | 0.4        | W       |      |      |     | 2.8943             |
| 12    |       | 11 Mr. Chandra    | 64       | M   | Control      | 0.8        | W       |      |      |     | 3.8682             |
| 13    |       | 12 Mrs. Surya V   | 38       | F   | Control      | NS         | W       |      |      |     | 4.4614             |
| 14    |       | 13 Mr. Durga Pr   | 24       | M   | Control      | 0.9        | W       |      | 19   |     | 2.5151             |
| 15    |       | 14 Mr. Abraham    | 40       | M   | Control      | 0.8        | W       |      | 23   |     | 4.7123             |
| 16    |       | 15 Mr. Srinu Kar  | 40       | M   | Control      | 0.8        | W       |      | 21   |     | 4.3162             |
| 17    |       | 16 Mrs. Batool I  | 70       | F   | Control      | NS         | W       |      |      |     | 4.218              |
| 18    |       | 17 Mrs. Ragini E  | 74       | F   | Control      | 0.7        | W       |      | 44   | 21  | 3.4138             |
| 19    |       | 18 Mrs. Swarna    | 54       | F   | Control      | 0.2        | W       |      |      |     | 2.726              |
| 20    |       | 19 Mrs. Archana   | 45       | F   | Control      | 0.1        | W       |      |      |     | 4.3148             |
| 21    |       | 20 Mrs. Krishna   | 30       | F   | Control      | 0.8        | W       |      |      |     | 4.4337             |
| 22    |       | 21 Mr. Khan MS    | 70       | M   | Control      | 0.5        | W       |      |      |     | 3.5148             |
| 23    |       | 22 Mr. S.A. Moh   | 36       | M   | Control      |            | W       |      |      |     | 3.6284             |
| 24    |       | 23 Mr. Khasim     | 60       | M   | Control      | 0.8        | Control | Null |      |     | 4.2133             |
| 25    |       | 24 Mrs. Rehana    | 63       | F   | Control      | 0.2        | W       |      |      |     | 3.7123             |
| 26    |       | 25 Mrs. Sarasw    | 41       | F   | Control      | NS         | W       |      |      |     | 4.3162             |
| 27    |       | 26 Mr. Rajeev     | 31       | M   | Control      | NS         | W       |      |      |     | 4.218              |
| 28    |       | 27 Mr. Srinivas I | 49       | M   | Control      | 0.7        | W       |      | 13   | 6   | 3.4138             |
| 29    |       | 28 Mr. Sridhar    | 25       | M   | Control      | 0.8        | W       |      | 19   |     | 4.726              |
| 30    |       | 29 Mr. Sai Hem    | 31       | M   | Control      | 1          | W       |      | 21   | 10  | 4.3148             |

Fig 4.10 cases list

## CHAPTER- 5

### RESULTS AND DISCUSSION

The category of the CKD patients with null genotype and reduced glutathione levels as per characteristics of the study and control groups are presented in Table-5.1. From this data it was observed that the patients were mostly males between the age group of 33 to 83 years and standard deviation for males is  $SD \pm = (58 \pm 13.07)$ . And females are between the age of 26 to 78(). But males are more than females in both patients and non-patients category of these case studies. The details of their personal creatinine tests are given in Table-5.1.

In Table-5.1 we present some of the characteristics of cases and controls. Besides age match, we also present creatinine tests for both controls and cases; however there was only a small percentage of female participation in this study. We have recorded the creatinine test for males in cases  $SD \pm (5.39 \pm 4.31)$  and in controls  $SD \pm (0.74 \pm 0.200)$ . Creatinine test for females in cases  $SD \pm (3.96 \pm 2.44)$  and in controls  $SD \pm (0.36 \pm 0.30)$ . Males are recorded high in both patients and outpatients category. This table is about case study of sample preparation. These data have some correlation with genotype; hence we have included the details.

*Table- 5.1 Case study of sample information*

| PARAMETERS   | CASES             | CONTROLS          |
|--------------|-------------------|-------------------|
| Sample Size  | 35                | 31                |
| Female       | 13                | 10                |
| Male         | 22                | 21                |
| Age (Female) | $45.23 \pm 20.40$ | $53.7 \pm 14.95$  |
| Age (Male)   | $58 \pm 13.07$    | $44.76 \pm 14.78$ |

|                  |             |              |
|------------------|-------------|--------------|
|                  |             |              |
| Creatinine(M)SD± | 5.39 ± 4.31 | 0.74 ± 0.200 |
| Creatinine(F)SD± | 3.96 ± 2.44 | 0.36 ± 0.30  |

Differences between the levels of antioxidants in the controls and case samples.

In table- 5.2 this case study is about some of the characteristics of cases and controls. From this data we observed that males are more in glutathione case study than females. Males between the age of 33 to 83 years. And the females are 26 to 78 years. However there was only a small percentage of female participation in this study. Here we recorded the reduced glutathione in cases and controls in this case study. In males the glutathione levels in cases are SD± (1.42±1.05) in controls are (3.99 ± 0.60) . In females the glutathione levels in cases are SD± (2.71 ± 1.61) in controls are SD ±(3.67± 0.611) .Glutathione levels are lower in cases and normal in controls (Banerjee, B.D., et al., 2010)

*Table 5.2 Category Study on Glutathione Level*

| PARAMETERS            | CASES       | CONTROLS    |
|-----------------------|-------------|-------------|
| Sample Size           | 35          | 31          |
| Female                | 13          | 10          |
| Male                  | 22          | 21          |
| Age (Female)          | 49.7±15.4   | 5.37±14.9   |
| Age (Male)            | 58±13.07    | 44.7±14.7   |
| Glutathione Levels(M) | 1.42±1.05   | 3.99 ± 0.60 |
| Glutathione Levels(F) | 2.71 ± 1.61 | 3.67± 0.611 |

## 5.1 Null Genotyping of the GSTT1

The Null genotype distribution as analyzed by PCR, is presented in table -5.3. We also analyzed the genotype frequencies with respect to the wild type and null type of the cases and controls. This table shows the comparative distribution of the Null genotype of GSTT1 between patients and controls. It seems that there is a marked significant difference between patients and controls in the GSTT1 null genotype s,

The frequencies of GSTT1 variants (table-5.3) are presented in Yates corrected Chi-square and p-values. The frequency of cases and controls in wild type and null genotype in the total population of GSTT1 were determined. In null type the frequencies of GSTT1 in patients are 20 and in controls are 4. And the wild type( normal) frequencies of GSTT1 in patients are 15 and in controls are 27.

In Table-5.1, the null genotype frequency of both patients and non-patients category is to compare cases with controls. It was found that Null genotypes of GSTT1 more in cases and Wild type of GSTT1 were found more in controls. It is seen that null type frequency in GSTT1 is higher in cases .When compared to healthy controls.

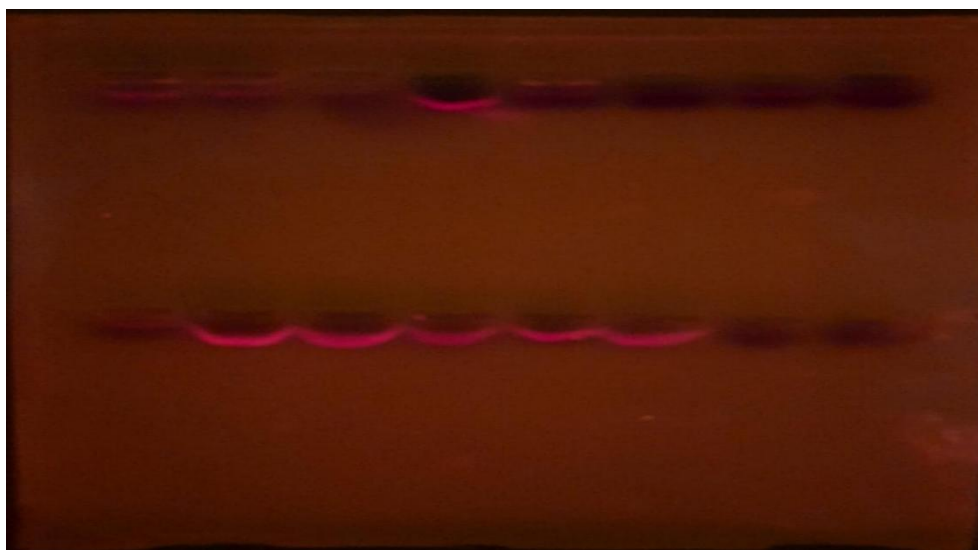
And it shows that OR (odd ratio) is associated with the null genotype of GSTT1, adjusted for age. In both controls and cases the deletion is 9.0. (Chi-square)  $X^2$  is 13.9039. The frequency of the cases and controls in null genotype in the total population in P-value (0.00019249) in patients and controls with deletions. Our results have a significant odd ratio (9.0) in patients with deletions. And p-value is 0.00019240. It is significant .That polymorphism is responsible for the disease. But due to low size it's not confirmed. (Banerjee, B.D., et al., 2010)

The p- value is  $<0.005$ = significant.

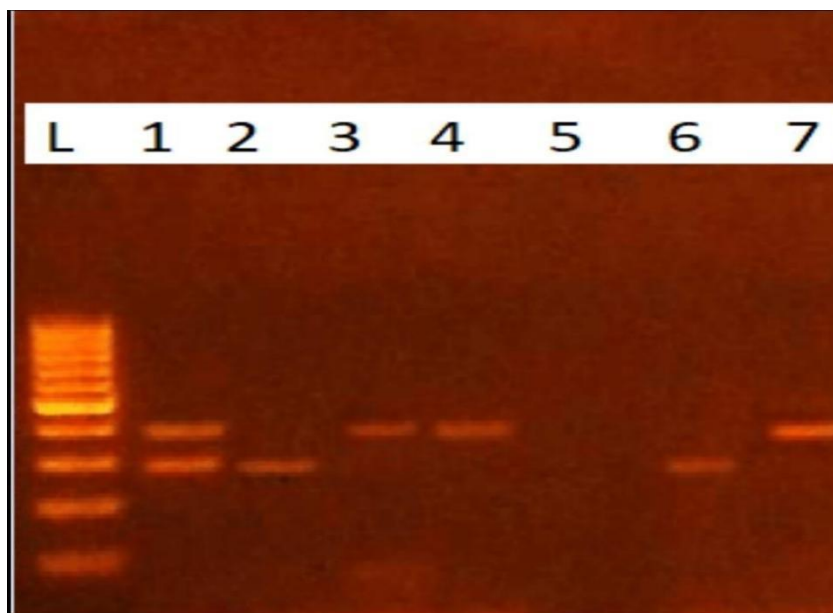
The p-value is  $>0.005$ = insignificant.

*Table 5.3 The association of GSTT1 gene polymorphism*

|          | Control (n = 31) | Case (n = 35) | Odd ratio              | $\chi^2$ | P - Value  |
|----------|------------------|---------------|------------------------|----------|------------|
| Normal   | 27               | 15            | 1                      | 1        | 1          |
| Deletion | 4                | 20            | 9.0 (2.5906 - 31.2670) | 13.9039  | 0.00019240 |



***Fig 5.1 DNA Visualization***



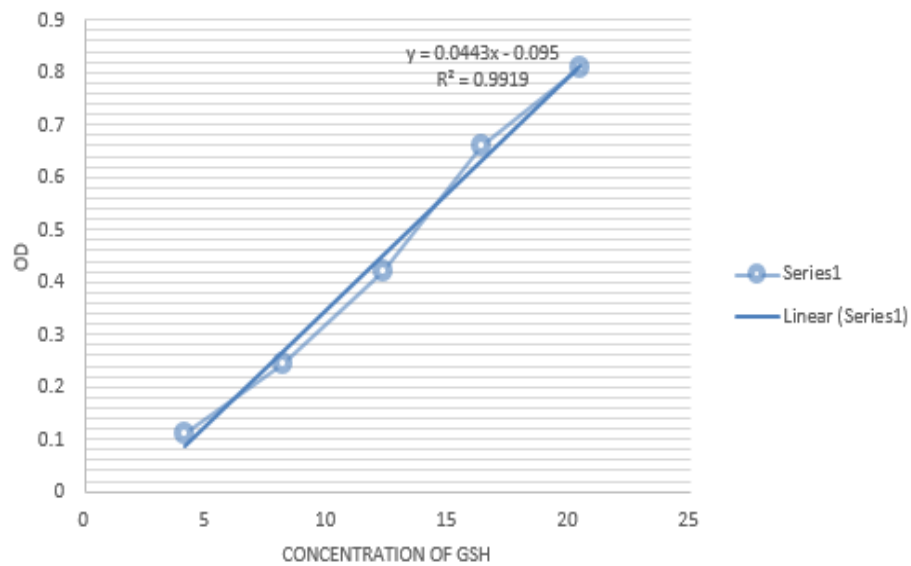
**Fig 5.2 DNA Ladder**

## **5.2 GRAPH:**

From the graph 5.1 .The intercept  $Y=0.0443x-0.095$  so acquired is applied to calculate the concentration of GSH in every plasma sample by substituting their OD values in the corresponding formula. (By subtracting the OD value with intercept and divided by slope = Concentration of glutathione) . The reduced levels of GSH were derived from the resultant GSH VALUES in the case samples. The relationship between the declined levels of Glutathione is reflected from the calculated GSH values from the graph. A standard graph was prepared by taking concentration of GSH on x-axis and OD on y-axis. The GSH content is calculated from the standard curve and expressed as ul/ml of blood (Duan, X. et al., 2015).

Intercept  $Y = 0.0443X$

Slope = 0.095



**Graph 5.2 Standard curve for GSH (Glutathione)**

**Table 5.4 GST ESTIMATING PLOT**

| X-AXIS | Y-AXIS |
|--------|--------|
| 4.092  | 0.11   |
| 8.185  | 0.245  |
| 12.28  | 0.422  |
| 16.372 | 0.66   |
| 20.468 | 0.81   |

## **CHAPTER - 6**

### **SUMMARY AND CONCLUSION**

GST polymorphism and its association with CKD are yet to be well established. Our study was designed to find out whether the GSTT1 Null genotype may be involved in chronic kidney diseases. Accordingly, controls and cases are two different groups of people were recruited: (1) people with CKD (2) people without CKD. The two groups of peoples (controls, cases) were included to exclude the confounding effects of glutathione levels and GSTT1 null genotype that may be known to be the largest cause of CKD. The blood samples collected from these patients were analyzed to study the genetic polymorphism and oxidative stress through statistical analysis.

GSTT1 null genotype appears to be more important in CKD patients. Genetic polymorphisms of the genes coding for the enzymes involved in the metabolism of genotoxins result in different phenotypes with respect to their ability to detoxify these agents.( Mittal, B., et al., 2006)

The  $\mu$  (GSTM1) and  $\theta$  (GSTT1) members of the GST multi-gene family, which are polymorphic in humans are involved in detoxifying mutagenic electrophilic compounds and an increased frequency of these GST gene deletions have been associated with several diseases. The polymorphism in the GSTT1 gene loci is caused by the gene deletion. This results in virtual absences of enzyme activity of this enzyme activity in individuals with the GSTT1 null phenotype. The polymorphisms affecting the activity of these enzymes may affect the processes contributing to the development of the diseases.

The major strength of the consideration could be a decently tall test measure and irregular determination of think about subjects without any inclination of ethnicity, occupation since the ponder carried out in urban set up having blended populace and are uncovered to OCPs through environment only. Be that as it may, there are quite a couple of disadvantages. Since this can be a hospital-based case-control consideration, it has potential restrictions due to need of populace control and in nonappearance of any early record of nearness or nonattendance of them. Since we reviewed OCP level in subjects having CKD, in this way, it is troublesome to set up

cause and effect. Assist follow-up considers are required to set up OCP as a go between of CKD.

This study raises critical questions around the immunization status of the GSTT1 antigen of sound benefactors in stem cell transplantation. Beneath certain GSTT1 donor/recipient hereditary combinations, invalid benefactors ought to be screened for the nearness of GSTT1-specific cells before the transplant since it may have imperative clinical suggestions.

This investigation has analyzed in depth the genotype frequencies with respect to several parameters as described in the series of tables presented here. The results indicated Chronic kidney patients had a significant disease but it is not confirmed. GSTT1 deletion mutations were found to be very high. This is the only study that examines the effect of the GSTT1 null genotype in chronic kidney diseases. The results showed a significant odds ratio (9.0) in patients with deletion of GSTT1 null genotype and the p-value is (0.00019240). Our study demonstrated that GSTT1 null genotype may be involved in the cause of chronic kidney disease. GSTT1 null genotype lowers glutathione activity in the body and causes oxidative stress leading to kidney damage, risk of chronic kidney disease. Therefore genotyping of GSTT1 polymorphisms may be a useful biomarker in determining the risk factor for chronic kidney diseases (Harrison, D.J., et al., 1989).

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