

Expression of Apolipoprotein A1 and Apolipoprotein B Genes in Iraqi Patients with Chronic Kidney Disease: A Clinical and Laboratory Correlation

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ABSTRACT

Background: Chronic kidney disease (CKD) is associated with dyslipidemia and cardiovascular complications, where apolipoproteins A1 (ApoA1) and B (ApoB) play critical roles in lipid metabolism and disease progression. Evaluating their gene expression can provide insights into the pathophysiological mechanisms linking lipid abnormalities to CKD. **Objective:** This study aims to analyze the expression levels of the ApoA1 and ApoB genes in CKD patients and correlate them with biochemical markers to assess their role in disease progression. **Methods:** A case-control study was conducted on 100 CKD patients and 50 healthy controls. Gene expression levels of ApoA1 and ApoB were measured using quantitative real-time polymerase chain reaction (qRT-PCR). Laboratory investigations included serum lipid profile (total cholesterol, LDL, HDL, triglycerides), kidney function tests (creatinine, eGFR, blood urea nitrogen), and inflammatory markers (CRP, IL-6). Statistical analysis assessed the correlation between gene expression and biochemical parameters. **Results:** ApoA1 expression was significantly downregulated in CKD patients compared to controls ($p < 0.01$), correlating with reduced HDL levels and increased inflammatory markers. In contrast, ApoB expression was significantly upregulated ($p < 0.05$), associated with elevated LDL and triglyceride levels, indicating a higher cardiovascular risk in CKD patients. A significant inverse correlation was observed between ApoA1 expression and serum creatinine levels ($r = -0.48$, $p < 0.001$), while ApoB expression positively correlated with disease severity ($r = 0.52$, $p < 0.001$). **Conclusion:** Altered expression of ApoA1 and ApoB genes in CKD patients suggests a key role in dyslipidemia and cardiovascular complications. The study underscores the potential of ApoA1 and ApoB gene expression as biomarkers for monitoring disease progression and guiding therapeutic interventions in CKD patients.

Keywords: Cystatin C, End Stage Renal Disease, ELISA, Real Time PCR.

INTRODUCTION

The chronic kidney diseases (CKD) has 9.1% prevalence rate worldwide [1], and becoming a significant global public health issue. By 2040, CKD is anticipated to overtake heart disease as the fifth biggest cause of deaths in the world. It is related to substantial morbidity and mortality [2]. Decreased kidney function may result in various issues, including imbalances in salt and water, anemia, weak bones, hypertension, cardiovascular disease, and waste product accumulation in the body [3]. On living quality and financial stability for families, CKD has a significant impact. Without taking into account societal expenditures and lost productivity, the average cost of care per patient as the condition develops to end stage renal diseases ranging between (20.110\$-100.593\$). Kidney disease is an undetectable killer because it is hard to diagnose and detect in the earliest stages (1-3a), when the condition is asymptomatic. Symptoms will become apparent and the patient begins to seek healthcare when they reach an end-stage renal disease (ESRD) where their kidney function is less than 50% [4]. The advancement of CKD is at risk due to metabolic abnormalities [5]. One of the most significant aspects of metabolic disorders is dyslipidemia (Fox CS, 2004), which can worsen glomerular and tubulointerstitial illness through the interaction of oxidative stress and inflammation (Ruan XZ, 2009). As a result, increases in lipid levels are crucial signs of declining renal function. In contrast to the conventional lipid parameters, apolipoprotein-B (Apo-B), a particular lipoprotein content in blood lipid, did not garnered great attentions in prior investigations on a relationship between dyslipidemia and CKD [6].

Serum ApoB level has been shown in recent articles to be superior biomarkers to diagnose the risk of CVD, prognosis and lipid reducing treatment compared to established lipid's variables. Few prior research have been conducted, and the findings are not conclusive to whether Apo-B has a role in CKD development [7]. Dyslipidemia, a buildup of atherogenic particles, and disturbance of lipoprotein metabolism are all linked to decreased renal function [8]. Serum lipid abnormalities could become a separate risk factor for CKD development [9]. Serum-bound apolipoprotein Apo B is made up of two sub-units, Apo-B 100 & Apo-B 48. It is necessary for the creation of different plasma lipoprotein particle and carries plasma lipids. All phases of CKD result in an increase in ApoB, and since VLDL Apo-B 100 catabolism is slower in CKD, buildup of Apo-B containing lipoproteins can be more of a result of decreased clearance than of increased synthesis [10]. In research on animals, it was discovered that hyperlipidemia increased glomerulosclerosis and tubule interstitial damages, which resulted in the onset and progressions of kidney impairment [11]. All nucleated cells consistently produce cystatin C, a 13-kDa cysteine proteinase inhibitor protein, which is freely filtered by the kidney with almost complete reabsorption and catabolism in the proximal tubule and minimal urine excretion [12]. Therefore, patient variables such gender, age, body size and composition, and nutritional status have a significantly less impact on serum cystatin C levels. It was first put forth as a glomerular filtration biomarkers in 1985; it has now been debated and tested for more than 30 years as to how much more clinically

useful it is than creatinine [13]. When assessing GFR in patients who are critically ill, Cystatin C is a sensitive indicator of even small variations in the parameter, so in clinical practice, it can be preferred over creatinine [14]. Although studies on the correlation between Apo B level and CKD and CysC levels and CKD have been conducted, it is not obvious if Apo-B and CysC levels play a substantial cooperative role in the various stages of CKD. The results of earlier study are equally contradictory. The present study aims to investigate the association between S. Apo-B & CysC levels and CKD using Iraq health check-up facilities. If confirmed, this theory will offer an alternative strategy for CKD prevention and therapy.

Methods

Study population: About 100 individuals who were screened for serum ApoB and creatinine have been included in the study from December 2022 to April 2023. It was carried out in Baghdad hospital / medical City-Iraq. Changes in nutritional status may occur along with the severe kidney disease, which could be a confusing factor that influences serum creatinine measurement and produces unreliable estimations of renal function. The cross-sectional study has 100 (n=100; male 50, female 50) eligible participants diagnosed with chronic kidney diseases. Finally, to determine the connection between blood ApoB level and the incidence of CKD, 100 individuals and 50 healthy volunteers (25 male; 25 female) were added. During the study, no information that may be used to identify a specific person was retained. Inclusion criteria: Patients with chronic kidney disease were involved in this study and history was obtained via a questionnaire verifying the medical history and basing on the renal function test. Exclusion criteria: Patients with diabetes mellitus, liver failure, Alcoholics, Smokers, patients suffering from HIV, HCV, Multiple Myeloma, Renal Carcinoma and who have undergone transplantation in the recent, patients under immunotherapy or Chemotherapy and patients under medication of statins, steroids, Insulin, contraceptive pills are all excluded from the study. Subjects demography details are collected through a questionnaire with their informed consent, during the sampling procedure. Sample collections: Blood samples were collected from anterior axillary veins on empty stomachs. The automatic biochemical analyzer was used to measure biochemical and blood routine parameters while patients were fasting, after two hours of blood collection. Procedures and methods were carried out according to laboratory protocols and instructions [15]. Blood sample were divided into two aliquots. The first aliquot was used as a serum to investigate biochemical parameters including renal function tests (urea, creatinine), detect uric acid and electrolytes level. The serum fraction was kept the deep freeze for Cystatin C and Apolipoprotein Assaying. Second aliquot was added into anticoagulant tube containing (EDTA) and used for gene sequencing. About 250µL was collected from the EDTA tube and added with Trizol reagent and used for gene expression studies. The concentrations of blood albumin, uric acid, urea, serum creatinine were estimated by automatic biochemical analyzer. Serum sodium, potassium, calcium and phosphorus was measured in all 130 subjects. The polyethylene glycol enhanced immunoturbidometry was used to detect the Apolipoprotein A-I (Apo-A-1) & ApoB. Biochemical assays: Urea (Normal Range: 16.6 - 48.5mg/dl), uric acid (normal range: Male 3.4-7.0mg/dl, Female 2.4-5.7mg/dl), albumin levels (Normal range: 3.5 - 5.5g/dl). were estimated by the Cobas-C-111® Clinical Chemistry Automated Systems. Processing of samples was done according to the guidelines

of the manufacturer. Serum creatinine was estimated using Jaffe method. (Normal Range: Male 0.7 – 1.2mg/dl; Woman 0. 5–0.9mg/dl). Serum electrolyte levels including Calcium (Ca; Normal range: 8.5-10.5mg/dL), Sodium (Na, normal range: 135-145 mmol/L), Potassium (K, normal range: 3.5-5.0 mmol/L), and Phosphate (PO₄, normal range: 2.5-4.5 mg/dL) were measured using automated clinical chemistry systems like the Cobas c 111®. Estimation of Human Cystatin C and Apolipoprotein B (APOB): Levels of serum Cystatin C and Apolipoprotein B were measured by using ELISA kits. Absorbance of the samples was recorded in microtitre plate reader at 450nm. Concentration of Test sample concentrations were measured by the use of a standard graph Cys-C ($y=1.0889x^2 + 1.5212x$; $R^2= 0.9988$) and standard Apolipoprotein B ($y=1.1775x^2 + 1.3773x$; $R^2= 0.9978$). Concentration was represented in ng/ml. In brief, a 96-well microtiter plate was coated by overnight incubations at 4°C with an anti apo-B 48 monoclonal antibody 4C8. Plate was washed with phosphate buffered saline and 50µL aliquots of 100 fold dilute serum was added in duplicate to wells and incubated at room temperature (20–25°C) for 2hr. About 50µL aliquot of apoB-48 standards (5ng/mL to 80ng/mL) was added to the respective wells and incubated at RT. After washing, 50µL of biotin-conjugated anti apoB-48/ B-100 was added to all the wells and put on a shaker for 1 hour. Approximately 50µL of HRP conjugated avidin solution was added and incubated at RT for 30 minutes. After washing, 50µL of chromogenic substrates were added and incubated on a shaker for 20 minutes until color development. The Spectra Fluor Plus (Tecan USA) plate reader was used to record the absorbance at 450 nm [16].

Gene Expression Studies

RNA extraction from the blood: The extraction of total RNA from the samples was carried out by using *Trans Zol/Up Plus* RNA Kits reagent (TransGene, biotechnology ER501-01) as prescribed by the manufacturer. About 1ml of blood was centrifuged at 12,000rpm for 1min and the pellet obtained was resuspended in 1000µl TransZol Up and incubated overnight at -23°C. To the contents, 200µl of chloroform was added then incubated at RT for 3min after vortexing. The contents were then centrifuged at 10,000 rpm for 15min at 2-8°C. The colorless upper aqueous phase containing the RNA was transferred to a fresh tube and added with chilled ethanol. The precipitate obtained was added to the spin columns and centrifuged at 12,000 rpm for 30sec at RT. About 500µl of CB9 was added to the spin columns and spun down at 12,000rpm for 30sec at RT. The flow through was discarded. About 500µl of WB9 was added into the spin columns and spun at 12,000rpm for 30sec at RT. Further centrifugation was done to eliminate any leftover ethanol, and then air-dried for several minutes. The spin column was washed with 50-200µl of RNase-free Water and the eluted RNA was collected after centrifuging at 12,000rpm for 1min. The pure extracted RNA was then stored at -20°C for further use. **miRNA extraction:** Total miRNA was extracted from the samples using the *EasyPure®* miRNA Kit Reagent (TransGen, biotech. ER601-01) as mentioned in manufacturer's manual. About 1ml of elute collected from previous chapter was centrifuged for 1min at 12,000rpm and the pellet obtained was resuspended in 1000µl LB10 and incubated overnight at -23°C. To the contents, 200µl of chloroform was added and incubated for 3min following vortexing. The contents were then centrifuged at 2-8 °C for 15 minutes at 10,000rpm and the colorless upper aqueous phase containing the RNA was transferred to a fresh tube and added with 1/3rd volume of 96% -100% ethanol. The lysate

formed was added to a miRNA spin column and centrifuged for 30sec at 12,000rpm. About 500µL of WB10 was added into the spin columns and spun at 12,000rpm for 30sec at RT. Further centrifugation was done to eliminate any leftover ethanol, and then air-dried for several minutes. The spin column was washed with 50-200µl of RNase-free Water and the eluted RNA was collected after centrifuging at 12,000rpm for 1min. The pure extracted RNA was then stored at -20°C for further use. RNA concentration and purity assessment: RNA obtained was checked for concentration and purity using Quantus Fluorometer (Thermo Fisher Scientific, USA) so as to use the sample as template for RT PCR. The samples ranged in RNA concentration from 73-147ng/µl, while the absorbance of the samples was measured at both 260 and 280nm to determine the purity. The presence of A260/A280 ratio of around 2 suggested that the sample of RNA is pure.

cDNA Synthesis from mRNA and miRNA:

First strand cDNA synthesis, reaction component: cDNA was synthesized using the EasyScript® One-Step gDNA removal and cDNA Synthesis SuperMix Kit. Total RNA was reverse-transcribed to complementary DNA (cDNA) as suggested by the manufacturer. The

reaction volume was maintained at 20µl and about 4µl of total RNA was used in this step. The anchored Oligo(dT)18 Primers (0.5µg/µL), Random Primers (0.1µg/µL) along with 1µL of *EasyScript*® RT/RI Enzyme Mix was used in the reaction. The content was mixed thoroughly and incubate for 10min at 25°C. In qPCR, the contents are incubated with an anchored oligo (dT) 18 primer for 15min at 42°C. The reaction temperature parameters are in the following order: 85 °C for 5sec, 42°C for 15min and finally 25°C for 10min.

Quantitative Real Time PCR (qRT-PCR):

Expression of *Apo B* and *Apo A*: The expression levels of the *Apo B* and *Apo A* genes were estimated by the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, a sensitive technique for the quantifying of steady-state mRNA levels. To confirm the expression of the target genes (*Apo B* and *Apo A*), a quantitative real-time qRT-PCR SYBR Green assay was used. Primers were designed and procured from Alpha DNA Ltd. (Canada). The endogenous control gene *GAPDH*'s mRNA was used as housekeeping gene and is used as normalizer. The expression was quantified using $2^{-\Delta\Delta Ct}$ method. Table 1 shows the primers used in the reactions.

Table 1: Table Showing the List of Primer Sequences for *Apo B* and *Apo A* Genes.

Gene		Sequence	GC%	Tm	Product length
ApoA	FW	CATTTCTGGCAGCAAGATGA	45	66.2	968bp
	RV	GCCTTCAAACCTGGGACACAT	50	68.25	
ApoB	FW	AGAGGACAGAGCCTTGGTGGAT	54.55	73.18	395bp
	RV	AGAGGACAGAGCCTTGGTGGAT	54.55	73.18	

Expression of *miRNA 27b*: The level of expression for *miRNA 27b* gene was determined by the use of qRT-PCR SYBR Green test. Endogenous control gene *U6* mRNA serves as housekeeping gene and used as normalize. The Quantitative Real Time PCR (qRT-PCR) was carried out using the QIAGEN Rotor gene Q Real-time PCR System (Germany). The expression levels and fold changes of the *Apo B*, *Apo A* and *GAPDH* genes were assessed using the *Trans Start*® Top Green qPCR Super Mix kit by measuring the threshold cycle (Ct). All the reactions are performed in duplicates. About 10µM of each of the primers were used along with 2µl of cDNA template.

The cycling protocol consisted of initial denaturation at 95°C for 5min, followed by 30 cycles of denaturation at 95°C for 30sec, annealing at 58°C for 30sec, extension at 72°C for 50sec followed by final extension at 72°C for 5-10min. PCR product sequencing: The Promega PCR Clean-Up Kit was used to purify the PCR products before they were forwarded to a sequencing company (Macrogen, Seoul, South Korea) for analysis. The sequence of nucleotides acquired for each primer was examined using the Basic Local Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to produce the most homologous alignment.

Statistical Analysis

For determining the correlation between S. Apo-B & Apo-A levels and prevalence of CKD, the widely used technique of logistic regression was chosen. Frequencies and percentages were used to represent categorical variables. Interquartile range (IQR) and the median were used to express continuous variables. Kruskal Wallis test is used to compare 2 groups with skewed distributions, and the Student's t-test was used to compare two groups with normal distributions. The entire study's data was entered in a master chart and tallied after that.

Calculations of the variables' frequency, percentage, means, Standard Deviation (SD), median, lowest, and maximum values were made. The Statistical Package for Social Sciences (SPSS) (Faculty version) for Windows was used to analyse the data. Statistical significance was determined as a p value < 0.01 were considered statistically highly significant and a p value < 0.05 was considered statistically significant.

Results

There was a total of 150 subjects with a median age of 45 years and there were 65 males (50%) and 65 females (50%) with no significant difference observed among groups with respect to age and gender (p > 0.05). The comparison of serum uric acid values of the subjects (3.891 ± 0.467) showed no significant rise than the normal range. All of the healthy volunteers also were found to show uric acid levels within the normal range (p < 0.001). All of the subjects showed an elevated level of albumin of 12.417 ± 2.151 which is abnormally higher when compared to normal range representing CKD (Normal range: 3.5 - 5.5g/dl). On the other hand, control groups showed a normal albumin level (p<0.01).

We found the urea values of all the control groups were found to be within the normal range [Table 2]. The urea value of the subjects (139.03 ± 41.100) was found to be significantly higher than the control (33.1 ± 7.227) representing CKD (p<0.01). All of the healthy volunteers' levels of creatinine was within normal ranges (p<0.05). The comparison of serum creatinine values of subjects with control showed that in all the groups serum creatinine value (8.984 ± 3.086) were significantly higher than the control (0.96 ± 0.1302) (p<0.001). On the other hand, as those are the most prevalent macro electrolytes and are associated with CKD, we chose sodium, potassium, calcium,

and chloride [Table 3]. The serum electrolytes level (Na⁺ and Ca²⁺) for subjects showed no significant rise than the normal range ($p < 0.01$). In contrast, serum K⁺ and PO₄³⁻ levels showed an abnormal rise

when compared to the normal range ($p < 0.01$). All of the healthy volunteers showed a normal range values of all the four electrolytes in the study ($p < 0.01$) [Table 3].

Table 2: Serum Markers of Subjects and Control Groups. (n=150; subjects 100, control 50). The Values were Averages of Triplicates and Represented as Values $\pm$ SD.

	Creatinine	Urea	Albumin	Uric Acid
Subjects	8.984 $\pm$ 3.086	139.03 $\pm$ 41.100	12.417 $\pm$ 2.151	3.891 $\pm$ 0.467
Control	0.96 $\pm$ 0.1302	33.1 $\pm$ 7.227	4.54 $\pm$ 0.548	4.53 $\pm$ 0.559

Table 3: Table showing the Serum Electrolytes of the Subjects and the Control Groups. (n=150; Subjects 100, Control 50). The Values were Averages of Triplicates and Represented as Values $\pm$ SD.

	Ca	Na	K	Po ₄
Subjects	8.524 $\pm$ 0.867	136.84 $\pm$ 3.243	5.403 $\pm$ 0.948	5.433 $\pm$ 1.434
Control	9.09 $\pm$ 0.3417	141.4 $\pm$ 2.1908	4.34 $\pm$ 0.4148	3.58 $\pm$ 0.5281

Serum ApoB, ApoA and CysC levels: The mean concentrations of apoB-48 of the patients was 26.36 $\pm$ 0.354455 which was significantly higher than the control (8.46 $\pm$ 0.254093). Nevertheless, non-significant comparisons were observed between males and females ($P < 0.01$) (Figure 1). Our results are in accordance to those of serum ApoB marker test, where there is an abnormal rise in the levels of ApoB among the subjects than the control suggestive of CKD.

However, the mean level of apoA among the patients was 8.26 $\pm$ 0.13477 which was less than the control (8.46 $\pm$ 0.254093). However, there is no significant comparison seen between the male and female subjects ($p < 0.01$) [Figure 1]. Average concentration of CysC of the subjects was found to be 17.84 $\pm$ 0.449478 which was less than the control (8.46 $\pm$ 0.254093). Nevertheless, non-significant comparisons were observed between males and females ($p < 0.01$).

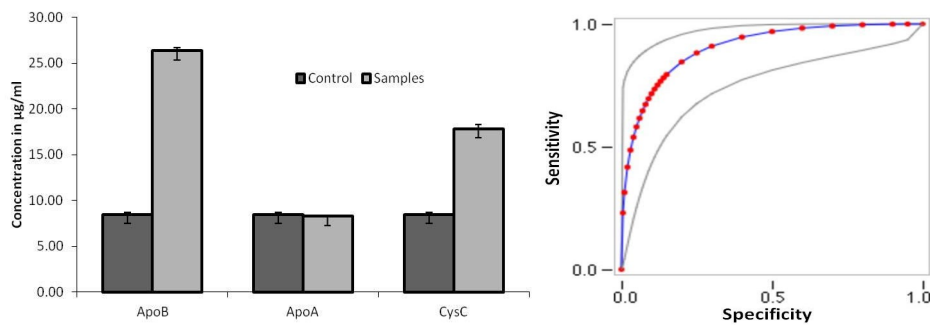


Figure 1: A Graph Shows Serum Concentrations of ApoB, ApoA and Cys C in Serum of both Control and Subjects. The Values are Triplicate Averages and Expressed as Value $\pm$ SD. Right: ROC Curve of ApoB and CysC for Predicting CKD.

Association between ApoB and Cys C: We found there is a strong correlation between ApoB and CysC ($P < 0.01$). There was a directly proportional relation between plasma Apo-B and CysC levels ($r = 0.552836$, $P < 0.001$) as shown by Spearman rank correlations analysis. Serum CysC and ApoB levels were found to be elevated in all the patients ($P < 0.01$). Mean absorbance values of apoB was found to be 0.9996 $\pm$ 0.134777 which was significantly lower than the control (2.1584 $\pm$ 0.449478).

Nevertheless, non-significant comparisons were observed between males and females ($p < 0.01$). From data points as calculated with ROC curve, we could figure out a strong association between ApoB and CysC. Overall, with the highest AUC for CysC, this factor was observed to be significant in predicting CKD (AUC = 0.652, $p < 0.001$), with the best

cut-off being 17.84, and it had a sensitivity of 88% and specificity of 80%. The highest AUC for ApoB was found to be significant in predicting CKD (AUC = 0.783, $P < 0.01$). (Fitted ROC Area: 0.905; Empiric ROC Area: 0.892). Real time Quantification: ApoA and ApoB mRNA expression levels were assayed in serum samples of both subjects and control using real time PCR method. Expression of each gene member was indicated as ratio of relative expression to that of control.

The relative expression of ApoA (Figure 2) was found to be downregulated in subjects in comparison with the controls ($P < 0.01$). However, relative expression of ApoB was found to be upregulated (Figure 3) in subjects when compared to control ($p < 0.01$). Similar reports were found, wherein ApoB levels increased among the subjects having CKD [17].

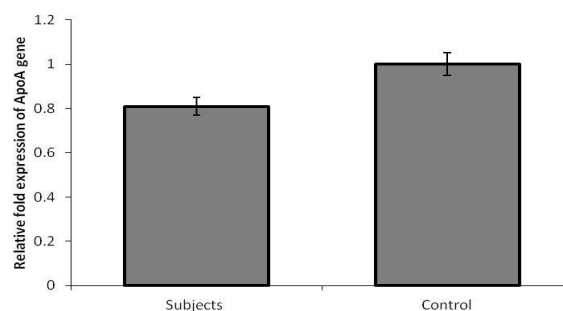


Figure 2: Graph Showing the Relative Fold Expression of ApoA Gene Member of Both Subjects and Control. The Values are Triplicate Averages and Expressed as Value $\pm$ SD. The Mean Ct of GAPDH is 13.44. Control Expression was Assumed to be 1 or 100%.

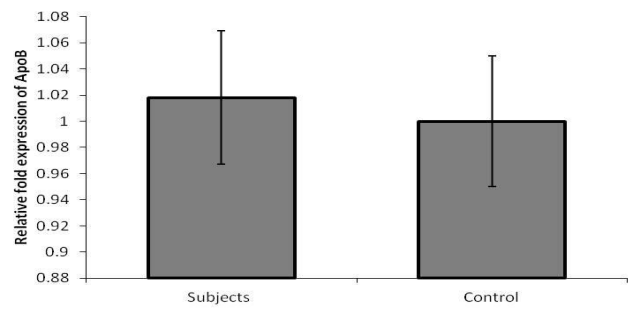


Figure 3: Graph Showing the Relative Fold Expression of ApoB Gene Member of both Subjects and Control. The Values are Triplicate Averages and Expressed as Value $\pm$ SD. Mean Ct of GAPDH is 13.44. Control Expression was Assumed to be 1 or 100%.

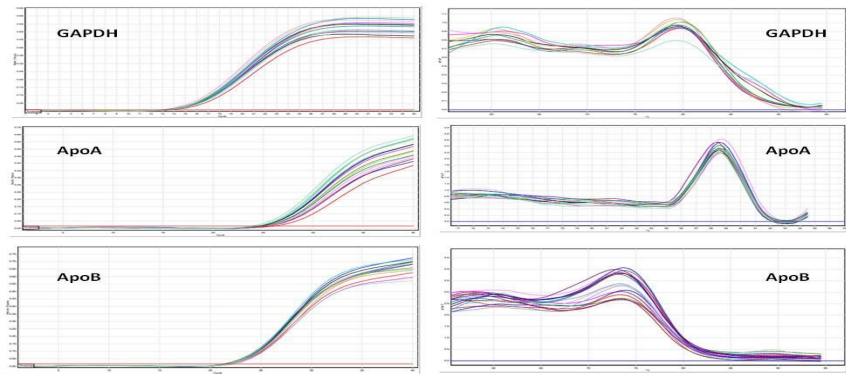


Figure 4: Real Time Ct (left) and Melt Curves (right) of GAPDH, ApoA and ApoB Genes of both Subjects and Controls. The Values are Triplicate Averages and Expressed as Value $\pm$ SD.

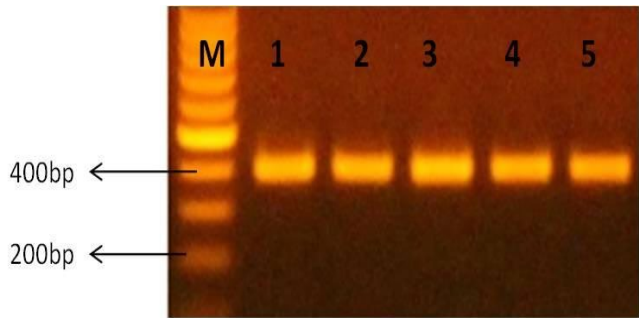


Figure 5: 1.2% Agarose Gel Showing the Amplified Bands of ApoB of the Samples. M: Molecular Marker (100bp); 1,2,3,4 and 5 are the Samples. Bands were Seen Approximately at 395bp.

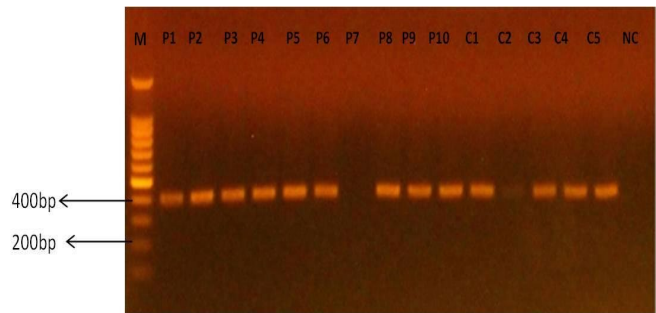


Figure 6: 1.5% Agarose Gel Showing the Amplified ApoB Specific Gene Region of Human Samples. M: 100bp DNA Ladder; Lanes P1-C5 Shows 395bp PCR Products.

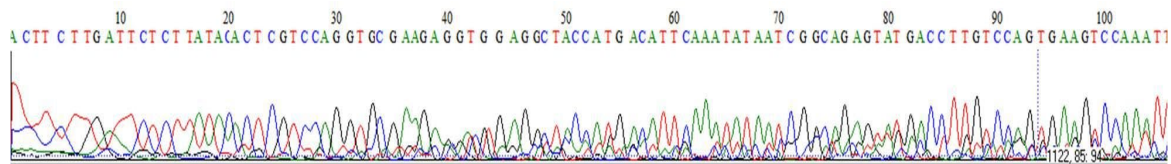


Figure 7: Chromatograph of the Sequenced PCR Product.

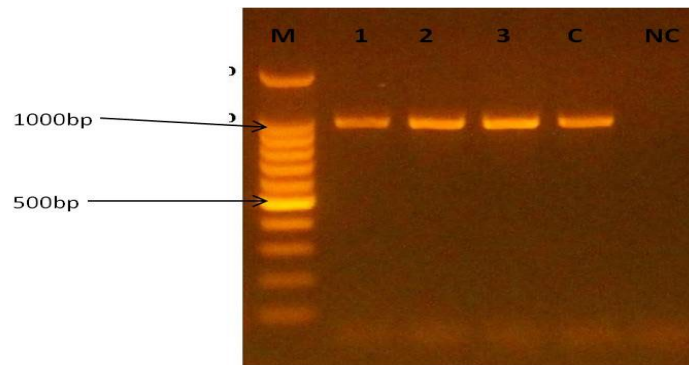


FIGURE 8: 1.2% Agarose Gel Showing the Amplified Bands of ApoA of the Samples. M: Molecular Marker (100bp); 1,2 and 3 are the Samples. C: Control; NC: Negative Control. Bands were Seen Approximately at 968bp.

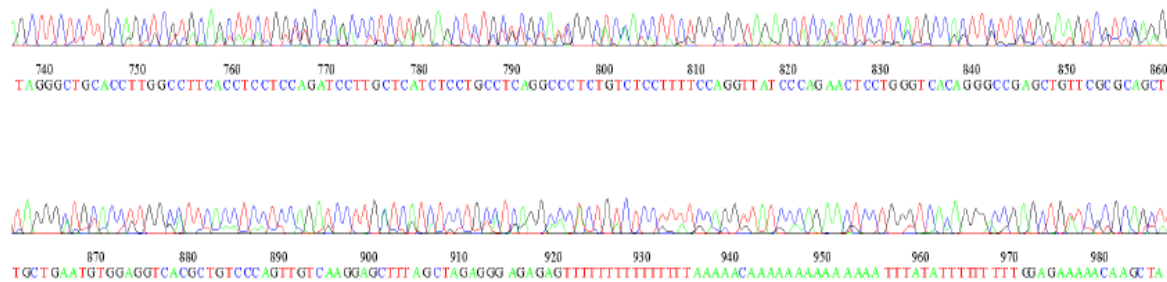


FIGURE 9: Chromatograph of the Sequenced (ApoA) PCR Product.

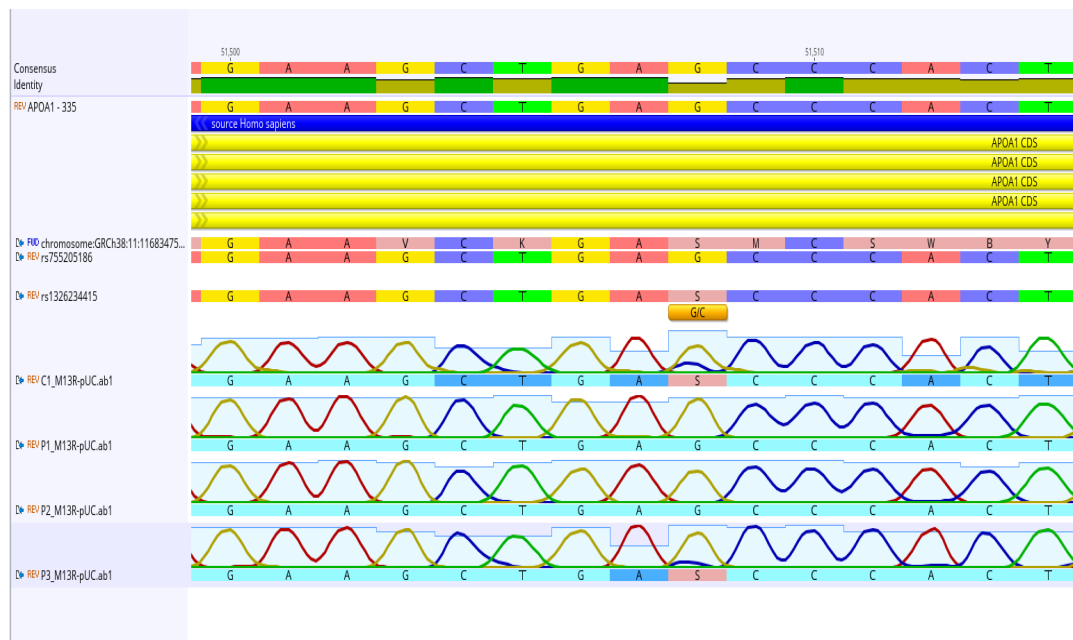


FIGURE 10: Sequence Similarity of the ApoA Sequences of the Control and the Samples as Seen on MEGA7.

Using the standard nucleotide BLAST's default settings, about 25 sequenced products were examined to determine the most significant alignments. All of the sequences (Figure 7) in this instance had high levels of homology (97–99%) to *Homo sapiens* apolipoprotein B (APOB), RefSeqGene on chromosome 2 (Accession no: NG_011793.2) and *Homo sapiens* apolipoprotein A (ApoA), RefSeqGene on chromosome 2 (Accession no: NC_000006.12).

Discussion

A major financial burden on healthcare systems, chronic kidney disease (CKD) is a leading cause of cardiovascular disease (CVD)

worldwide. Increased risks of cardiovascular morbidity, early mortality, and/or worse quality of life are linked to CKD in all stages. A precise prevalence estimate for CKD is unclear, as symptoms typically don't manifest until later stages [18]. In both cross-sectional studies and retrospective cohorts, our research found a positive correlation between the serum ApoB level and the level of cysC. To the best of our knowledge, this is the first study in China to clarify correlations using a 100-person data sets. We discovered that ApoB passed CKD in terms of correlation coefficients, and that an increase in ApoB is correlated favourably with the value of cysC. Additionally, we found a link between CysC and high serum ApoB levels. Through the following processes, ApoB, a significant component of the lipid profile, may

influence the onset and course of CKD. High concentrations of lipoproteins containing ApoB may cause and exacerbate atherosclerosis. The amount of lipoprotein particles containing the majority of ApoB penetrating the artery wall increases when plasma LDL-C and very low-density lipoprotein levels rise. The portion of the lipoprotein particles that are unable to escape back into the circulation binds to the arterial wall proteoglycans [19]. Proteoglycans and ApoB interact to drive lipid particle accumulation in the subendothelium, which can hasten vascular wall oxidation and inflammation. High levels of ApoB can cause oxidative stress, inflammation, and glomerular endothelial cells in renal vessels. It can also cause arteriosclerosis in small and medium-sized capillaries, which lowers eGFR and slows the progression of chronic kidney disease [20]. High levels of ApoB can cause oxidative stress, inflammation, and glomerular endothelial cells in renal vessels. It can also cause arteriosclerosis in small and medium-sized capillaries, which lowers eGFR and slows the progression of chronic kidney disease [21]. We found creatine levels to highly increase in comparison to the controls ($P < 0.01$). Although serum creatinine is frequently thought of as just a measurement of renal function, it actually also measures the production, uptake, and metabolism of creatinine [22]. Elevated creatinine levels are among the most frequent causes of nephrology consultations. The glomerular filtration rate (GFR) typically decreases when the serum creatinine concentration rises [23]. Contradictory data exist about the contribution of hyperuricemia to the development of chronic kidney disease (CKD), despite mounting evidence supporting the association between hyperuricemia and incident CKD. Tsai C-W et al demonstrated that, particularly in patients without proteinuria, increased uric acid levels are linked to a considerable and rapid fall in eGFR and a higher likelihood of renal failure [24]. According to their findings, hyperuricemia may be a component that can be changed to slow the progression of CKD. We found in all of our subject's uric acid level seems to be significantly less in comparison with the controls. However, urea levels were very abnormally elevated when compared to control. It is debatable whether hyperuricemia plays a part in the course of CKD [25]. While some research [26] found no link between hyperuricemia and CKD development. Another study by [27] revealed hyperuricemia might speed the decline of kidney functions as evaluated by eGFR or raise the chance of end stage renal disease (ESRD) development. It is still not apparent if the links between low blood albumin levels and declining kidney function are caused by chronic disease, underlying inflammation, urine albumin, or a combination of these variables. In patients with CKD, serum albumin content is a well-known predictor of death [28]. Serum albumin levels were discovered to be closely related to eGFR decline, fast eGFR decline, and incident CKD. These results were unaffected by albuminuria, self-reported health, indicators of inflammation, demographic risk factors, and clinical risk factors for the advancement of renal disease. In each fully adjusted model, urine ACR was also strongly correlated with eGFR decline, fast decline, and incident CKD. Serum albumin has not been

extensively studied as a potential risk factor for the onset of ESRD [29]. Especially in environments with limited resources, abnormalities in serum electrolytes and renal function are frequently disregarded and are linked to a higher risk of morbidity and mortality [30]. We found, potassium and phosphate to be elevated and calcium and sodium reduced in comparison with the controls ($P < 0.01$). All nucleated cells consistently produce cystatin C, a 13-kDa cysteine proteinase inhibitor protein, which is freely filtered by the kidney with almost complete reabsorption and catabolism in the proximal tubule and minimal urine excretion [Chew JS, 2008]. It has been well established for the past 15 years that cystatin-C is a reliable predictor for morbidity and death in a number of groups. It is linked to mortality in individuals with diabetes [31], acute kidney injury, CKD [32], and dialysis, as well as the development of end-stage renal disease (ESRD). Accelerated atherosclerosis and a high frequency of cardiovascular disease are linked to ESRD. Arteriosclerotic cardiovascular disease (ASCVD) is linked to higher levels of very low density lipoproteins cholesterol (VLDL-C), apolipoprotein-B (Apo-B) and lower level of high density lipoprotein cholesterol (HDL-C) and Apo-A1 [33]. Although the relation between Apo B level and change in kidney functions has been studied, it is unclear how Apo-B levels and CKD stages are related [34]. Previous research' findings are also conflicting. ApoB and CysC levels were increased in the subjects in comparison to the controls (8.46 ± 0.254093). But, ApoA concentration was found to be reduced ($p < 0.01$). We deduced a strong correlation between ApoB and CysC ($P < 0.01$). There was a directly proportional relation between plasma Apo-B and CysC levels ($r = 0.552836$, $P < 0.001$) as shown by Spearman rank. We are conscious of any previous descriptions of the connection between ApoB and the cysC in CKD. Our real time PCR results are also in accordance to our ELISA based assays of finding the concentration of ApoB. ApoB was found to be over expressed and ApoA was found to be downregulated when compared to control (Figure 2 & 3). Similar reports were found where there was an over expression of ApoB with CKD incidence and dysregulation of glomerular filtration [35].

Conclusion

Over 10% of people worldwide have chronic kidney disease (CKD), a condition that is becoming more prevalent. Serum creatinine, for example, has had its shortcomings as a conventional biomarker of renal failure well documented in the literature. Therefore, adding more recent biomarkers to clinical evaluation, like serum cystatin C or ApoB, has the potential to enhance disease monitoring and patient management. In conclusion, across all the participants, serum ApoB level had the greatest connection with CysC. Additionally, the rise in serum ApoB levels may occur before CKD development, indicating that lowering and monitoring of S. ApoB levels can offer a different strategy for the prevention and treatment of CKD. To sum up, serum cystatin C and ApoB are better indicators of early renal function than other markers.

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