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New Associations between Microsatellite Instability Loci and Chronic Kidney Disease Progression

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Abstract

Chronic kidney disease (CKD) is increasingly recognized as among the furthermost widespread communicable chronic diseases globally. Patients face an elevated risk of developing end-stage kidney disease (ESKD) and a heightened cardiovascular risk. It is known that CKD occurrence has a considerable genetic component, with heritability estimated to be high (25%–44%). This study aims to identify genomic changes in CKD and ESRD patients through microsatellites to forecast disease progression. Five milliliters of fasting blood samples were collected from 47 patients with ESRD on regular hemodialysis (group 1), 25 with CKD not on hemodialysis (group 2) and 25 healthy individuals were participated in the study as controls (group 3). Laboratory parameters were measured. Transthoracic echocardiogram was done for all participants. DNA was extracted from all blood samples and microsatellite instability (MSI) status was determined by polymerase chain reaction (PCR) using 5 microsatellite loci (BAT-25, BAT-26, NR-21, NR-22 and NR-24). The findings of our study indicated that the genomic instability observed in all analyzed microsatellites was statistically linked to the genetic predisposition to develop CKD and ESRD disease, but only BAT-26 and NR-22 have higher risks to develop CKD more than developing ESRD, suggesting a strong correlation between MSI and impairment of renal functions of the patients. The results of the present study could indicate the microsatellite DNA instability of CKD is associated with the genetic predisposition to develop renal impairment and ESRD in patients.

Keywords: Chronic kidney disease, Microsatellite instability, DNA sequencing.

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1. Introduction

Chronic Kidney Disease (CKD) ranks as the 16th leading global contributor to years of life lost. Effective screening, accurate diagnosis, and the comprehensive management by primary care clinicians are crucial for mitigating adverse CKD-related outcomes, which include cardiovascular disease, end-stage kidney disease (ESRD), and mortality [1]. While the etiologies of CKD are diverse and multifactorial, the early stages of disease frequently present with minimal or no overt symptoms, often progressing silently. It can be characterized as progressive loss of kidney function. Symptoms of ESRD become apparent and kidney transplantation or maintenance dialysis is essential for survival [2]. The continuous rise in global occurrence of chronic kidney disease (CKD) and its most severe manifestation, end-stage renal disease (ESRD), is a deeply concerning trend. This alarming increase is predominantly fueled by the surging rates of diabetes mellitus (DM) and hypertension, which stand as two most significant underlying causes of kidney failure. Consequently, ESRD has transitioned from a localized medical issue to a pervasive global health crisis [3], placing immense strain on healthcare systems and economies worldwide.

This escalating burden highlights an urgent need for enhanced preventative strategies and more effective management of these pervasive chronic conditions to mitigate the relentless progression of kidney disease. Patients afflicted with chronic kidney disease (CKD) are characterized by a significantly heightened cardiovascular risk, which manifests through a spectrum of conditions including coronary artery

disease, heart failure, various arrhythmias, and an increased propensity for sudden cardiac death. While the incidence and prevalence of adverse cardiovascular events are already substantially elevated in patients even at early stages of CKD compared to the general population, this risk becomes markedly more pronounced in individuals with advanced CKD stages [3]. This escalating risk highlights critical and pervasive impact of kidney dysfunction on cardiovascular health across the continuum of CKD progression [4]. End-stage renal disease (ESRD) represents a critical medical state characterized by the irreversible cessation of kidney function, mandating continuous renal replacement therapy, either through long-term dialysis or kidney transplantation, to sustain life [2]. The global burden associated with ESRD patients has escalated significantly, posing a formidable challenge to public health infrastructures worldwide.

Among therapeutic modalities, hemodialysis (HD) persistently stands as the predominant treatment intervention for ESRD patients across the globe [5]. Crucially, trajectory from the onset of chronic kidney disease (CKD) to its progression into this terminal condition remains a profound determinant of diminished patient quality of life and a substantial contributor to premature mortality [2]. This highlights an urgent and persistent need for more effective strategies in prevention and management of CKD to avert its devastating progression to ESRD. In realm of genetic disorders, single-gene disorders, referred to as "monogenic diseases," arise from a mutation within a solitary gene, which is sufficient to precipitate the disease state [6]. In contrast, polygenic disorders necessitate mutations across multiple distinct genes to manifest as a disease [6]. Notably, patients afflicted with chronic kidney disease (CKD) frequently exhibit elevated genomic instability [7]. This inherent instability can manifest as increased levels of genetic damage, quantifiable by incidence of chromosomal aberrations (such as micronuclei), particularly when their cells are exposed to ionizing radiation. This phenomenon may represent either a causative factor or a consequence of renal pathologies [8].

Furthermore, observations have indicated that CKD patients demonstrate diminished efficiency in repairing DNA damage. Consequently, variations within genes that regulate crucial physiological pathways could significantly influence an individual's susceptibility to, and incidence of, CKD [9]. Genetic testing offers substantial support in both identifying and, in many cases, definitively confirming a diagnosis of chronic kidney disease (CKD), thereby enabling more precise and tailored patient care [10]. Despite comprehensive clinical evaluations, the underlying etiology of CKD remains elusive in a significant proportion of patients [11]. In such ambiguous cases, genetic testing emerges as a powerful tool. It holds the potential to facilitate a formal diagnosis, which in turn can guide targeted therapeutic interventions and, crucially, contribute to a reduction in the empirical use of medications that carry a risk of considerable adverse effects [12]. This underscores the transformative potential of genetic insights in refining diagnostic clarity and optimizing treatment strategies the for CKD patients. Patients who present with chronic kidney disease (CKD) in conjunction with manifestations outside of the renal system are at a notably elevated risk of having an underlying genetic etiology.

A classic illustration of this association involves simultaneous occurrence of visual and hearing disturbances in individuals diagnosed with nephronophthisis [13]. Therefore, establishing a specific genetic cause for CKD can serve as a crucial trigger for initiating early referrals, which in turn facilitates timely identification and subsequent prevention of both known and potential extrarenal complications [14]. This proactive approach can significantly impact patient management and outcomes. Microsatellites are short, repetitive DNA sequences highly susceptible to errors like misalignment and frame shift mutations during cell division. This susceptibility leads to microsatellite instability (MSI), a molecular hallmark indicating defects in mismatch repair system (dMMR). dMMR prevents cells from correcting spontaneous mutations that occur in the microsatellites during DNA replication, resulting in insertions or deletions of sequence units [15]. Normally, the MMR system corrects these insertions, deletions, and base-base mismatches. However, in presence of dMMR, these replication errors remain uncorrected and are passed on to daughter cells, leading to MSI [16]. The foundational principles for MSI evaluation were established in 1997 by Dietmaier et al., who characterized sensitivity and specificity of 31 microsatellite markers in colorectal carcinomas [17]. They categorized results into:

- Microsatellite stability (MSS): No instability detected at any tested loci.
- Low-frequency MSI (MSI-L): Instability detected at one or two loci.
- High-frequency MSI (MSI-H): MSI detected at two or more loci.

Polymerase Chain Reaction (PCR) and sequencing-based systems have become viable methods for standard MSI testing in specific contexts. Advances in these molecular techniques have even extended MSI determination to liquid biopsies, allowing for analysis of cell-free DNA (cfDNA) in plasma [18]. In the context of renal diseases, mutational analysis is crucial for identifying the primary cause and holds significant diagnostic and prognostic value. Molecular genetic diagnostics typically involve examining genes for disease-causing DNA sequence changes. Mutation analysis is commonly performed via PCR followed by bidirectional sequencing, as approximately 85% of all disease-causing mutations in single-gene disorders are estimated to be located within coding exons [19]. This study is groundbreaking as it offers the first comprehensive overview of how dMMR and MSI impact the initiation and progression of non-malignant chronic kidney disease. It also explores the relevance of these alterations as diagnostic and predictive biomarkers. Developing standardized, highly sensitive, and specific tests for MSI in non-malignant diseases could not only improve prognostic evaluation and treatment success across various conditions but also help control disease progression and exacerbation.

1.1. Study Aim and Design

The overarching goal of this study is to identify genomic alterations in patients with CKD and ESRD using PCR followed by sequencing, comparing these findings with those from healthy volunteers. This aims to predict disease progression and determine the value of MSI in predisposing individuals to CKD, as well as its association with patients' clinical features.

Study Design: This is a cohort longitudinal study. We will regress the patient values against control values. Based on prior data, which indicated a standard deviation of 0.8 for the

control group and 1.7 for the regression errors, we determined that if the true slope of the regression line (patient vs. control) is 1.4, we would need to enroll 50 subjects per group. This sample size ensures we can reject the null hypothesis that this slope equals zero, with a probability associated with the test of this null hypothesis set at 0.05.

2. Subjects and Methods

2.1. Ethical Conduct

This study strictly adhered to ethical guidelines, having received full approval from the Ethics Committee of the Theodor Bilharz Research Institute (TBRI, Egypt). Before any involvement in the research, all participants provided written informed consent, explicitly authorizing use of their biological specimens for research. This was done in complete compliance with established institutional guidelines. Importantly, all experimental procedures carried out in this study were meticulously designed and executed to conform most current version of the Declaration of Helsinki and the overarching principles of good clinical practice, ensuring the highest standards of ethical research.

2.2. Patients and samples

This is a longitudinal study included 47 patients with ESRD (group 1) who received three, 4 hours dialysis sessions /week regularly for at least six months at the Nephrology Department, TBRI. In addition to 25 subjects who matched the ages, genders, and demographics of the study with the CKD not on hemodialysis were also included (group 2). Patients with congestive heart failure, malignancy and sepsis, and/or liver, autoimmune, severe infectious disease, and acute kidney injury and participants subjected to multiple blood transfusions were excluded from the study. Furthermore, patients who received antibiotics and anti-inflammatory drugs or corticosteroids during the study period were excluded from the study. To enhance validity, 25 healthy individuals without any kidney disease were included as controls (group 3). A full through history and clinical examinations were performed for all patients and control group included in study. Demographic and the clinical characteristics, including age, gender, blood pressure, duration of dialysis, etiology of ESRD and the CKD, recorded.

2.3. Laboratory Parameters

Five milliliters of fasting blood samples were gathered from all participants and controls. Laboratory parameters were performed: hemoglobin (Hb), kidney function tests (serum creatinine "Creat", urea), total cholesterol, serum albumin, aspartate transaminase (AST) and alanine transaminase (ALT) were measured.

Cardiology: Transthoracic echocardiogram was done for all participants to detect: heart rate (beat/minute), interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPWT), left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), left ventricular mass (LV mass), fractional shortening (FS), Ejection fraction (EF), intima media thickness (IMT). For molecular testing, genomic DNA was extracted from blood samples using the Qiagen DNeasy kit (Hilden, Germany), following the manufacturer's specified recommendations. The purified DNA was then dissolved in 50 µl of water, quantified using a Nanodrop ND-2000c (Thermo Fisher Scientific, Waltham, MA, USA), and subsequently stored at -20°C for later analysis. For MSI status analysis, five quasi-monomorphic mononucleotide microsatellite loci were utilized: BAT-25, BAT-26, NR-21, NR-22, and NR-24. The specific sequences for these primers are detailed in Table 1 (22). DNA amplification was carried out using Taq DNA Polymerase (Qiagen).

The process began with an initial denaturation step at 95°C for 5 minutes. This was followed by 40 cycles, with each cycle consisting of a denaturation step at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final elongation step was performed at 72°C for 10 minutes. Each reaction was prepared in a 25 µl volume, and positive controls (DNA from healthy volunteers) were included in every PCR reaction. The resulting PCR products were then separated on a 3% agarose gel via electrophoresis using a Bio-RAD electrophoresis chamber. A 5 µl aliquot of 100-1000 bp DNA ladder RTU served as a size marker, and the DNA bands were visualized by ethidium bromide staining. The gel images were analyzed using a Cleaver micro DOC gel documentation system. Selected bands from the gel, identified through a UV imaging system, were purified and subsequently sequenced using 3500/3500xL Genetic Analyzer (Applied Biosystems) at Colors for Research, Medical Laboratories, Egypt. Finally, the sequencing results were meticulously analyzed, reviewed, and aligned with those obtained from the control samples for comparative assessment.

2.4. Statistical analysis

Data analysis was rigorously carried out using the Statistical Package for Social Science (SPSS), specifically IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). For continuous variables that demonstrated a normal distribution, results are presented as mean ± standard deviation (SD). Categorical variables, on the other hand, are expressed through their frequencies and corresponding percentages. The statistical significance threshold was pre-defined as a P value less than 0.05, indicating that results falling below this value were considered statistically significant. To facilitate comparisons between the means of normally distributed variables across different groups, Student's t-test was employed. For assessing the distribution of categorical variables between groups, either the Chi-square (χ^2) test or Fisher's exact test was utilized, depending on data's characteristics. Furthermore, to quantify the risk associations, logistic regression analysis was performed, yielding odds ratios (OR) with their respective 95% confidence intervals (C.I.). Beyond conventional statistical methods, Molecular Evolutionary Genetic Analysis (MEGA-X) software, version 10.2.4, played a crucial role. This specialized software was used for sequence alignment to precisely identify any insertion/deletion events or nucleotide substitutions, providing a detailed molecular perspective on the genetic variations observed.

3. Results and discussion

3.1. Results

3.1.1. Patient characteristics

A total of 97 participants were recruited in this study, including 47 ESRD patients on hemodialysis (Group 1), 25 patients with CKD (Group 2), and 25 healthy individuals with no history of kidney disease (Group 3) were

included as a control group. Group 1 included 31 (66%) males and 16 (34%) females with mean age (56.53±14.43 years). Group 2 included 14 (56%) males and 11 (44%) females with mean age (59.56±8.27 years), and group 3 included 13 (52%) males and 12 (48%) females with mean age (52.80±9.67 years). As regards the demographic data, there is no significant difference between the three groups in terms of age and sex with P=0.407, 0.467 respectively. There is a statistically highly significant increase in creatinine, urea, AST, ALT and cholesterol and a highly significant decrease in albumin between groups with P=0.001. A highly significant increase was observed in IVST, LVPWT and LV mass and a highly significant decrease in IMT between groups with P=0.001. Individual demographic and clinical data of the studied groups are shown in (Table 2).

3.1.2. The microsatellite instability frequencies among patients

Instability frequency of the studied microsatellite loci varies according to the type of tested locus. Chi- square test (X2) was used to analyze the data. Regarding CKD patients, it was observed that 5 patients (20%) were instable in BAT-25, 21 (84%) instable in BAT-26, 11(44%) with instability in NR-21, 9 (36%) instable in NR-22 and 9 (36%) instable in NR-24 (Fig. 1A). While, for ESRD patients, the distribution of instability differs from that of CKD patients, 11(23.4%) were instable in BAT-25, 15(31.9%) instable in BAT-26, 15(31.9%)instable in NR-21, only 3 patients (6.4%) instable in NR- 22 and 9 (19.1%) instable in NR-24 (Fig. 1B).The statistical analysis revealed that 24 patients (33.3%) do not have instability in all the studied microsatellites i.e. they were completely MSS, 22 (30.6%) were instabe in only one microsatellite locus, 13 (18.1%) instable in 2 loci, 8 (11.1%) instable in 3 loci, 5 (6.9%) instable in 4 loci and finally none of the patients showed instability in the 5 studied loci (Fig. 1C).

3.1.3. Univariate logistic regression analysis

Logistic regression analysis of the studied microsatellites was carried out to evaluate their instability risk assessment to the CKD versus (Vs) controls and the P value. The analysis revealed that instability in all of the studied microsatellites BAT-25, BAT-26, NR-21, NR-22 and NR-24 were statistically associated with the disease, with odd ratio (95% Confidence Interval) of 2.3(1.6- 3.1), 7.3(2.9- 18.0), 2.8(1.8- 4.2), 2.6(1.7- 3.8) and 2.6(1.7- 3.8) respectively and with P =0.025 for BAT-25 and <0.001 for all the other satellites and no instability was detected in all samples of the healthy volunteers (Table 3). The microsatellites included in this study are presented as frequency and percentage, with their data analyzed using the Chi-square (χ^2) test. Odds Ratios (OR), along with their 95% Confidence Intervals (C.I.), were calculated, and the P-values were derived from logistic regression analysis. P value=<0.05, **P=<0.001.While the evaluation of the instability risk assessment of the studied microsatellites BAT-25, BAT-26, NR-21 and NR-24 among the ESRD patients Vs controls, revealed a statistical association with disease with OR(95% CI)= 1.7(1.4- 2.1), 1.8(1.4- 2.2), 1.8(1.4- 2.2) and 1.8(1.4- 2.2) respectively, but regarding NR-22, instability risk assessment does not reach the significance level with P=0.197 because 3 patients (6.4%) only were with MSI. No instability was detected in all samples of the healthy volunteers (Table 4). Regarding instability risk assessment for studied microsatellites according to ESRD patients Vs CKD group, only BAT-26 and NR-22 showed a statistical significance increase in the percentage instability in CKD group than ESRD group with OR (95% CI) = 0.1(0.0- 0.3), P=0.001 and 0.1(0.0- 0.3), P=0.05 (Table 5).

3.1.4. Associations between the MSI Levels with patient outcomes

Statistical analysis using Student's t test revealed that, patients with MSI in NR-21 showed a highly significant association with high levels of urea and cholesterol with P= 0.006 and 0.004 respectively, and highly significant decrease in FS and EF with P=0.003 and 0.004 respectively. Patients with MSI in NR-22 showed a highly significant association with high levels of AST with P=0.005 and a significant increase in ALT and cholesterol with P=0.047 and 0.024 respectively, while a highly significant decrease in albumin was observed with P=0.003, patients with MSI in NR-24 showed significant association with high levels of cholesterol with P=0.03, these results suggesting a strong correlation between MSI and impairment of renal function (Table 6). For interpretation purposes, microsatellite instability at ≥ 2 loci was defined as MSI-high (MSI-H), instability at a single and double loci was defined as MSI- low (MSI-L), and no instability at any of the tested loci was defined as microsatellite stable (MSS) (5).

Based on the above classification, it has been observed that only 8% of the CKD patients were MSS, 48% were MSI-L and 44% were MSI-H (Fig. 2A). While regarding the ESRD patients, the MSS was 36% which is much greater than that in CKD patients, 51% were considered as MSI-L and only 12.8% were MSI-H (Fig. 2B). Regarding the ESRD patients, the MSI-H were significantly associated with lower levels of albumin with P=0.008, and those of the CKD group, the MSI-H was significantly associated with higher levels of creatinine, urea, AST and cholesterol with P= 0.011, 0.049, 0.034 and 0.011 respectively, associated with lower levels of albumin with P=0.013, CKD patients with MSI-H were also associated with increased IMT with P= 0.047 (Table 7). The software Molecular Evolutionary Genetic Analysis (MEGA-X) (version 10.2.4) was employed to identify nucleotide insertions, deletions, or substitutions through alignment of the sequenced DNA of the patients with that of controls.

Regarding the CKD patients, an instability was detected by alignment of the sequencing results of the patient with that of control in NR-24, the alignment results showed a single nucleotide polymorphism at positions 28,39, and 46, while a double nucleotide polymorphism were detected at 67 and 68 positions, in addition, a nucleotide deletion at position 29 (Fig. 3A). Regarding the ESRD patients, an instability was detected in BAT-26 and the alignment results showed a single nucleotide polymorphism at positions 2, 4, 45 and 48 (Fig. 3B). An instability was observed in NR-21and the alignment results showed a single nucleotide polymorphism at positions 20, 27, 30 and 38, and a double nucleotide polymorphism were detected at positions 34 and 35 in ESRD case than control (Fig. 3C). Finally, the alignment results of instability of ESRD case in NR-24 showed a nucleotide deletion at position 29, in addition to single nucleotide polymorphism at positions 46, 49, 50, 64, 67, 74 and 77 (Fig. 3D).

3.2. Discussion

The trajectory from the initial development of chronic kidney disease (CKD) to its culmination in end-stage renal disease (ESRD) profoundly impairs an individual's quality of life and represents a significant driver of premature mortality. Given its debilitating nature, CKD mandates proactive and aggressive medical surveillance to detect early signs of disease progression, thereby facilitating timely referrals to specialized care for potential dialysis initiation or renal transplantation [20]. Although CKD exhibits a higher prevalence within the elderly demographic, it is noteworthy that approximately 30% of patients over the age of 65 years may experience a relatively stable disease course. Conversely, younger individuals diagnosed with CKD typically face a more relentless and progressive loss of kidney function [21]. Etiology of CKD is diverse and multifactorial, with hypertension, diabetes mellitus, and various glomerular diseases standing as the most frequently identified causes. Beyond these, polycystic kidney disease (PKD) emerges as a prominent genetic disorder contributing to CKD.

Furthermore, other inherited forms of kidney disease manifest with distinct glomerular injury patterns, including conditions such as genetic nephrotic syndrome, focal segmental glomerulosclerosis, APOL1-related kidney disease, and atypical hemolytic uremic syndrome [22]. This broad spectrum of underlying causes underscores the complex nature of CKD and the need for comprehensive diagnostic approaches. To effectively lower the incidence of CKD, it's crucial to identify high-risk patients. To shed light on the genetic underpinnings, we then investigated the top five microsatellite loci for their association with CKD development in an Egyptian population. In this study, we specifically examined microsatellite DNA instability in both CKD and ESRD patients undergoing regular hemodialysis. Our findings indicate that the frequency of this instability differs between these two groups, depending on the specific microsatellite phenotype observed. But, to a large extent, the frequency is considered higher in the CKD than in the ESRD group. These results suggest an association of MSI with the genetic predisposition to suffer renal impairment.

Logistic regression analysis was used to evaluate the risk assessment of the instability to both of the CKD and the ESRD groups, it was noted that all microsatellite loci are supposed to be associated with the risk of developing CKD with the same $P<0.001$ for all loci except for BAT-25, the $P=0.025$. While, for the risk assessment of the MSI in ESRD patients, it was observed that all the studied loci are supposed to be associated with the risk of developing ESRD with significance for all of them except for NR-22 that has no risk to develop ESRD when compared to the healthy volunteers group that were all MSS and no instability was detected in any of them. But surprisingly, NR-22 has higher risk to develop CKD than developing ESRD with $P=0.05$, and similarly, BAT-26 has the same higher risk to develop CKD than developing ESRD with $P=0.001$ when comparing the two groups together regarding the risk assessment. Because the frequency of these microsatellite loci was high to large extent, this MSI is enough reflect the large part of genetic predisposition to CKD and ESRD. This is supposed that MSI contribute to the development of the disease.

In most cases of this study, renal function showed a tendency to increase regardless of the MSI loci phenotype. Some microsatellite loci have been shown to affect levels of studied patient parameters, like patients with MSI in NR-21 have higher levels of urea and cholesterol, and those with MSI in NR-22 have higher levels of ALT, AST and cholesterol, while patients with MSI in NR-24 have higher levels of cholesterol. Indeed, the levels of these parameters were significantly different among patients with MSS in NR-21, who have an almost negligible increase in FS and EF, and patients with MSS in NR-22 have higher levels of albumin and on contrary, patients with MSI have reduced albumin levels with inverse correlation observed between the number of MSI loci and the albumin level, $r= -0.484$ and $P=0$, This observation significantly expands upon existing body of literature that posits a direct correlation between decreased serum albumin levels and an increased risk for chronic kidney disease (CKD) progression. Our findings are notably congruent with groundbreaking work of Cheng et al. (2023). Their study was pioneering in demonstrating that serum albumin exhibits a non-linear relationship with both overall renal prognosis and the rate of renal function decline [23].

This convergence of evidence underscores the critical role of albumin as a prognostic indicator in CKD and highlights the complexity of its relationship with kidney health, moving beyond a simple linear correlation. It suggests that therapeutic interventions targeting albumin levels might need to consider this non-linear dynamic for optimal patient outcomes. Upon classification of the genomic instability, it has been observed that only 8% of the CKD were MSS, 48% were MSI-L and 44% were MSI-H. While regarding the ESRD patients, the MSS was 36% which is much greater than that in CKD patients, 51% were considered as MSI-L and only 12.8% were MSI-H. Several microsatellite loci have been indicated to have relation with the predisposition to develop CKD. The greater degree of genomic instability observed in the chronic kidney disease (CKD) cohort relative to end-stage renal disease (ESRD) group can be mechanistically ascribed to a confluence of factors. Specifically, the profound uraemic state inherent to CKD, coupled with persistent chronic inflammation, is hypothesized to orchestrate a breakdown in intrinsic DNA damage repair pathways, concurrently leading to an escalation in chromosomal damage. This is likely mediated, at least in part, by the exacerbated generation of reactive oxygen species [24].

Although the precise detrimental impact of reactive oxygen species and reactive nitrogen species (collectively termed RONS) within the context of CKD has yet to be unequivocally elucidated, there is a clear understanding that elevated concentrations of RONS can instigate various forms of DNA damage. These include the formation of DNA strand breaks, the induction of point mutations, and the promotion of aberrant DNA cross-linking. Critically, these cumulative molecular aberrations are direct contributors to the overall development and perpetuation of genomic instability within affected cells [25]. This suggests a complex interplay where the uremic milieu and inflammatory processes synergistically impair genomic integrity in CKD. The identification of microsatellite markers in close proximity to the *PKD1* and *PKD2* loci has led to the increasing application of PCR amplification of these markers for presymptomatic and prenatal diagnosis of Autosomal Dominant Polycystic Kidney Disease (ADPKD). ADPKD stands as one of the most prevalent genetic diseases in humans, characterized by the progressive development of numerous large renal cysts.

Figure 1. Distribution of MSI frequency regarding studied microsatellites among patients.

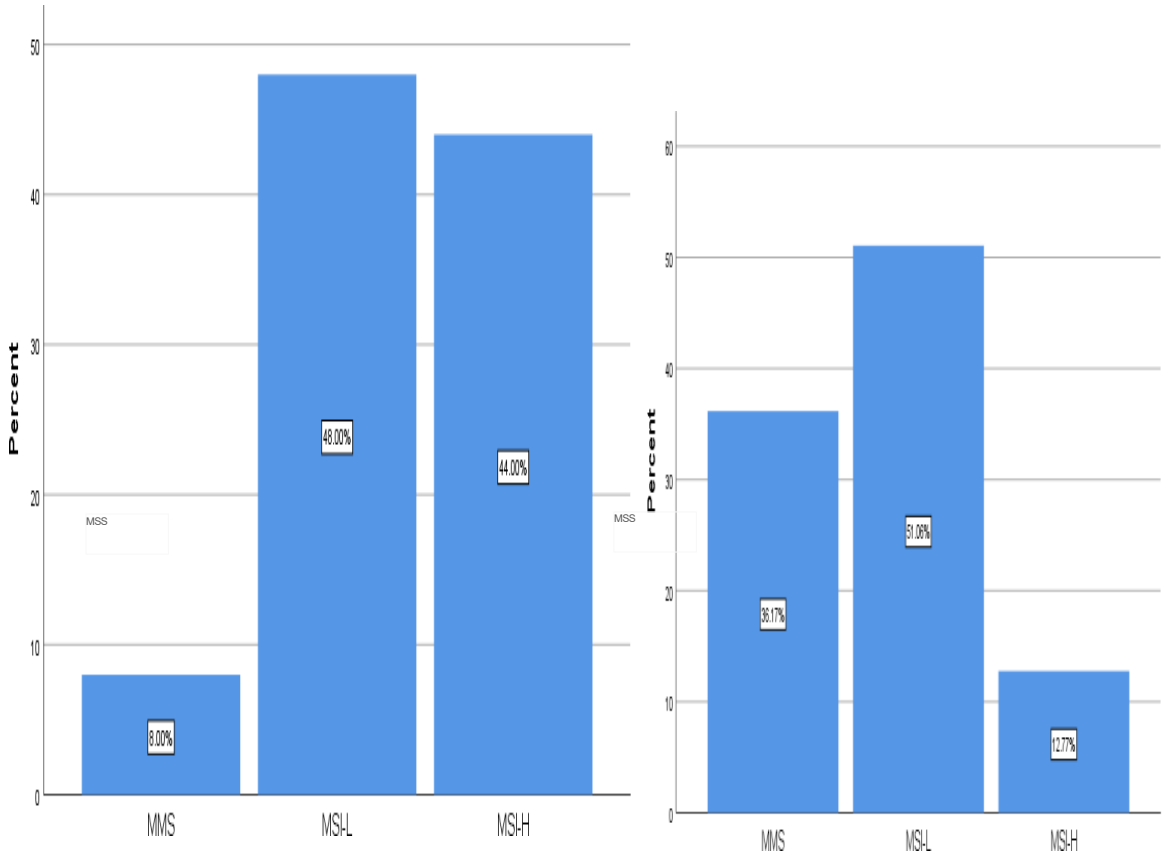
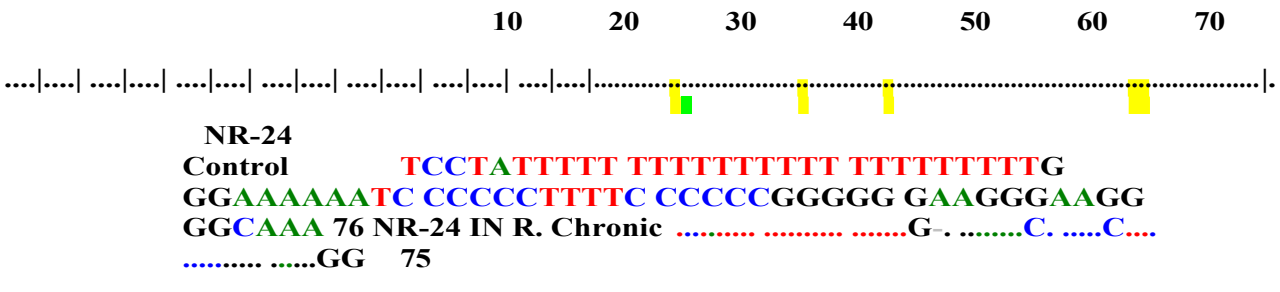
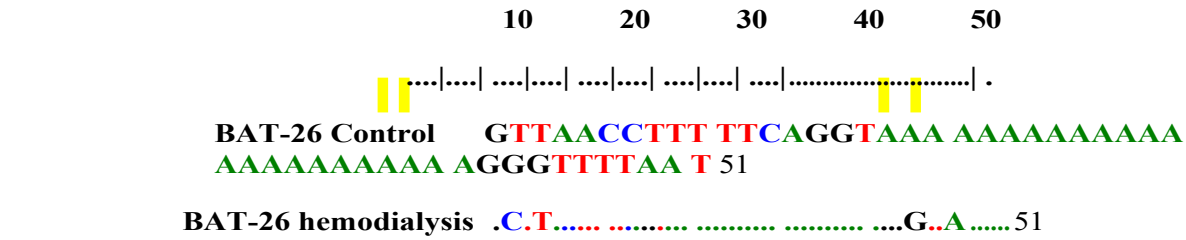


Figure 2. (A): MSI frequency in CKD patients, (B): MSI in ESRD patients.

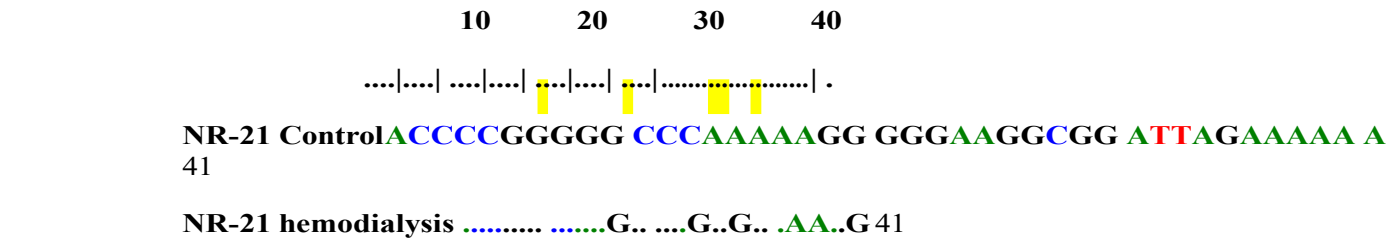
(A)



(B)



(C)



(D)

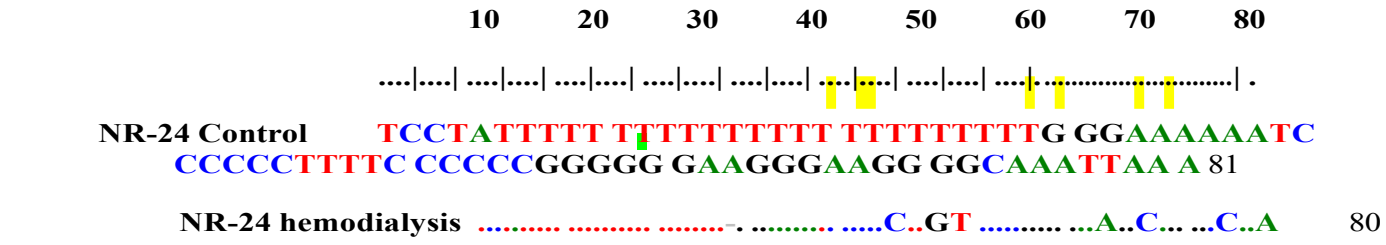


Figure 3. Results of Sequence Alignment of patients with controls.

Table 1. Primer sequence of the used loci.

Marker	Sense primer(′3→′5)	Antisense primer(′3→′5)	PCR product size
BAT-25	TCGCCTCCAAGAATGTAAGT	TCTGCATTTTAACTATGGCTC	120
BAT-26	TGACTACTTTTGACTTCAGCC	AACCATTCAACATTTTTAACCC	124
NR-21	TAAATGTATGTCTCCCCTGG	ATTCCTACTCCGCATTCACA	103
NR-22	GAGGCTTGTCAAGGACATAA	AATTCGGATGCCATCCAGTT	142
NR-24	CCATTGCTGAATTTTACCTC	ATTGTGCCATTGCATTCCAA	132

Table 2. Demographic, laboratory and echocardiographic data of the three groups.

	Group 1 No.=47		Group 2 No.=25		Group 3 No.=25		P. value
	Mean	SD	Mean	SD	Mean	SD	
Age (Years)	56.53	14.43	59.56	8.27	52.80	9.67	0.407
Sex (Male) Female	31(66%) 16(34%)		14(56%) 11(44%)		13(52%) 12(48%)		0.467
Creatinine	7.7	2.2	4.2	1.7	0.8	0.1	0.001**
Urea	137.5	34.4	147.3	70.6	32.2	3.3	0.001**
Albumin	4.1	0.3	2.1	0.7	4.0	0.2	0.001**
AST	23.1	11.8	32.0	12.6	21.3	8.4	0.002**
ALT	17.4	6.3	29.1	13.7	16.0	5.2	0.001**
HB	10.6	1.5	11.4	8.8	12.7	0.9	0.190
Cholesterol	203.6	27.5	210.7	37.1	169.2	14.1	0.001**
Heart rate(beat/minute)	86.1	8.2	87.7	10.2	82.7	6.0	0.128
IVST (cm)	1.1	0.1	1.2	0.1	1.0	0.1	0.001**
LVPWT (cm)	1.1	0.2	1.2	0.1	1.0	0.1	0.001**
LVEDD (cm)	5.2	0.7	5.2	0.5	4.9	0.4	0.077
LVESD (cm)	3.2	0.7	3.2	0.6	2.9	0.5	0.098
LV mass	235.0	69.8	240.5	32.9	178.2	46.1	0.001**
FS %	37.8	7.6	38.7	5.7	40.1	7.4	0.451
EF%	66.8	10.0	68.6	6.9	70.4	8.2	0.250
IMT (cm)	1.1	0.2	1.1	0.1	0.5	0.1	0.001**

IVST=interventricular septal thickness, LVPWT= left ventricular posterior wall thickness, LVEDD=left ventricular end diastolic diameter, LVESD= left ventricular end systolic diameter, LV mass= left ventricular mass, FS=fractional shortening, EF=Ejection fraction, IMT=intima media thickness. P value=<0.05, **P=<0.001.

Table 3. Studied microsatellites according to the CKD Vs control group with the risk assessment.

		Studied groups			Risk assessment	
Loci	MSI	CKD group	Control group	P. value	OR (95% C.I)	P. value
BAT-25	Instable	5(20.0%)	0(0.0%)	0.018*	2.3(1.6- 3.1)	0.025*
	Stable	20(80.0%)	25(100.0%)			
BAT-26	Instable	21(84.0%)	0(0.0%)	<0.001**	7.3(2.9- 18.0)	<0.001**
	Stable	4(16.0%)	25(100.0%)			
NR-21	Instable	11(44.0%)	0(0.0%)	<0.001**	2.8(1.8- 4.2)	<0.001**
	Stable	14(56.0%)	25(100.0%)			
NR-22	Instable	9(36.0%)	0(0.0%)	<0.001**	2.6(1.7- 3.8)	<0.001**
	Stable	16(64.0%)	25(100.0%)			
NR-24	Instable	9(36.0%)	0(0.0%)	<0.001**	2.6(1.7- 3.8)	<0.001**
	Stable	16(64.0%)	25(100.0%)			

Table 4. Studied microsatellites according to ESRD group Vs control group of with the risk assessment.

		Studied groups			Risk assessment	
Loci	MSI	ESRD	Control group	P. value	OR (95% C.I)	P. value
BAT-25	Instable	11(23.4%)	0(0.0%)	0.006**	1.7(1.4- 2.1)	0.009**
	Stable	36(76.6%)	25(100.0%)			
BAT-26	Instable	15(31.9%)	0(0.0%)	<0.001**	1.8(1.4- 2.2)	0.002**
	Stable	32(68.1%)	25(100.0%)			

NR-21	Instable	15(31.9%)	0(0.0%)	<0.001**	1.8(1.4- 2.2)	0.002**
	Stable	32(68.1%)	25(100.0%)			
NR-22	Instable	3(6.4%)	0(0.0%)	0.272	1.6(1.3- 1.9)	0.197
	Stable	44(93.6%)	25(100.0%)			
NR-24	Instable	9(19.1%)	0(0.0%)	0.016*	1.8(1.4- 2.2)	0.019*
	Stable	38(80.9%)	25(100.0%)			

Studied microsatellites are represented as frequency and percent; the data were analyzed by X2 test. OR; Odd Ratio, C.I; Confidence Interval, P value calculated depend on logistic regression analysis.
P value=<0.05, **P=<0.001.

Table 5. Studied microsatellites according to the ESRD Vs CKD group with the risk assessment.

		Studied groups			Risk assessment	
Loci	MSI	ESRD	CKD	P. value	OR (95% C.I)	P. value
BAT-25	Instable	11(23.4%)	5(20%)	0.741	1.2(0.4- 4.0)	0.34
	Stable	36(76.6%)	20(80%)			
BAT-26	Instable	15(31.9%)	21(84%)	0.001**	0.1(0.0- 0.3)	0.001**
	Stable	32(68.1%)	4(16%)			
NR-21	Instable	15(31.9%)	11(44%)	0.309	0.6(0.2- 1.6)	0.314
	Stable	32(68.1%)	14(56%)			
NR-22	Instable	3(6.4%)	9(36%)	0.001**	0.1(0.0- 0.3)	0.05*
	Stable	44(93.6%)	16(64%)			
NR-24	Instable	9(19.1%)	9(36%)	0.116	0.4(0.1- 1.3)	0.765
	Stable	38(80.9%)	16(64%)			

Studied microsatellites are represented as frequency and percent; the data were analyzed by X2 test. OR; Odd Ratio, C.I; Confidence Interval, P value calculated depend on logistic regression analysis.
P value=<0.05, **P=<0.001.

Table 6. The Associations between the MSI Level with the studied parameters.

		Patients with MSS		Patients with MSI		P. value
Loci		Mean	SD	Mean	SD	
NR-21	Urea	128.43	44.52	163.04	51.60	0.006**
	Cholesterol	197.83	27.80	220.54	31.77	0.004**
	FS	39.50	6.72	35.73	7.00	0.003**
	EF	69.04	8.64	64.50	9.09	0.004**
NR-22	Albumin	3.62	0.93	2.31	1.14	0.003**
	AST	23.62	10.53	39.17	15.10	0.005**
	ALT	19.82	9.26	29.75	15.08	0.047*
	Cholesterol	200.50	25.00	233.67	43.39	0.024*
NR-24	Cholesterol	200.83	28.25	221.61	34.69	0.03*

The studied parameters are represented as mean and SD; the data were analyzed by t test.
P value=<0.05, **P=<0.001.

Table 7. Association of MSI-L and MSI-H CKD patients with patient's data.

	MSI-L No.=12		MSI-H No.=11		
	Mean	SD	Mean	SD	P value
Creat	3.25	1.29	5.14	1.84	0.011
Urea	120.17	71.64	179.55	64.40	0.049
Albumin	2.51	0.65	1.76	0.66	0.013
AST	26.33	6.92	38.00	15.08	0.034
Cholest	192.17	21.45	233.09	42.14	0.011
IMT	1.19	0.10	1.09	0.12	0.047

P value=<0.05, **P=<0.001.

Historically, DNA-based analysis employing flanking markers has served as the gold standard for differentiating between two forms of the disease (PKD1 and PKD2) [26]. Further solidifying this utility, Binczak et al. (2006) reported that DNA microsatellite analysis was instrumental in establishing specific type of ADPKD and concluded that disease was linked to *PKD1*. Their findings highlighted that DNA microsatellite analysis offers a valuable tool for early diagnosis and should be considered for at-risk families affected by the ADPKD [27]. Nowadays, and after more than twenty years, we used DNA microsatellite analysis to detect the genomic alterations in the CKD and ESRD patients by PCR followed by bi-directional sequencing and comparing the results with those of healthy volunteers group to predict disease progression. There is biological and genetic evidence suggesting that common genetic determinants modify kidney disease progression to ESRD, independent of primary renal disorder. Our study confirmed this fact by detecting a deletion mutation at position 29 in CKD case with MSI in NR-24, and the same deletion mutation was detected at same position 29 in ESRD case with MSI in NR-24.

In 2022, participants of the KDIGO Conference published recommendations highlighting the potential benefits of early genetic testing, particularly in the context of kidney transplantation. They advised that donors related to a recipient with a known genetic kidney condition should undergo genetic testing early in the donor-evaluation process [28]. This proactive approach is anticipated to significantly broaden the array of molecular diagnostic tools accessible to clinicians globally. Recent advancements in clinical genetic evaluation, such as next-generation sequencing, including targeted gene panels and whole genome sequencing, have played a pivotal role. These sophisticated platforms deliver DNA sequence reads with exceptional coverage across the entire genome, leading to identification of novel genetic causes of chronic kidney disease (CKD) [29]. In 2023, Han et al. conducted a genome-wide association study (GWAS) to pinpoint genetic variations linked to the decline in estimated glomerular filtration rate (eGFR), which serves as an indicator of CKD progression.

Their research identified specific risk single nucleotide polymorphisms (SNPs) within the TPPP and FAT1-LINC02374 loci among patients with CKD. This study's findings suggest a significant association between genetic variations in these particular loci and the progression of the chronic kidney disease [30]. Our current study is the first to prove that genomic instability in the studied microsatellite loci are associated with the CKD progression. Our study made it clear that we are not dealing with a single disease or its different looks, but we are targeting a disease progression. In molecular biology, sequencing allows researchers to study genomic variability and thus to identify changes in genes. The goal of the future therapy is to use the new therapeutic types depend on genetic basis to bring in new molecular approaches for treatment. Because of this, it is important to use what we know about microsatellites in the diagnostic evaluation process.

4. Conclusion

The results showed that the genomic instability in all of the studied microsatellites BAT-25, BAT-26, NR-21, NR-22 and NR-24 were statistically associated with the genetic predisposition to develop CKD and ESRD disease, but only BAT-26 and NR-22 have higher risks to develop CKD more than developing ESRD, suggesting a strong correlation between MSI and impairment of renal functions of the patients. Our MSI findings in established patients with CKD progression offer crucial insights into how genomic instability contributes to the development of CKD. This provides a strong foundation for future research, allowing for further studies based on diverse clinical outcomes of patients.

Recommendation

For future research endeavors, comprehensive studies focusing on genomic microsatellite instability (MSI) are imperative to deepen our understanding of genomic instability in chronic kidney disease (CKD). It is crucial to meticulously collect and thoroughly analyze data concerning the relationship between MSI and the chance of developing CKD and end-stage renal disease (ESRD). This comprehensive approach will pave the way for precisely evaluating the genetic risk associated with these conditions. In this regard, the DNA genomic instability identified in the present study appears to lay a foundational groundwork for future investigations, as it contributes valuable information toward the prediction and understanding of CKD and ESRD development.

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