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O r i g i n a l i n v e s t i g a t i o n s / c o m m e n t a r i e s

New Associations between Microsatellite Instability Loci and Chronic Kidney Disease Progression

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Abstract. Background: Chronic kidney disease (CKD) is becoming one of the world's most prevalent no communicable chronic diseases. The World Health Organization projects CKD to become the 5th most common chronic disease in 2040. Progression rates are highly variable, but patients encounter both an increased risk for end-stage kidney disease (ESKD) as well as increased cardiovascular risk. The occurrence of CKD is known to have a significant genetic component, with an estimated high degree of heritability (25%–44%). The aim of this study is to detect the genomic alterations in patients with CKD and ESRD using microsatellites to predict the disease progression. Patients and Methods: 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). Results: Our results showed that the genomic instability in all of the studied microsatellites 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. Conclusion: Our findings could point to the achievement that 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.

Introduction

Chronic kidney disease (CKD) is the 16th leading cause of life lost worldwide. Appropriate screening, diagnosis, and management by primary care, clinicians are necessary to prevent adverse CKD associated outcomes, including cardiovascular disease, end-stage kidney disease (ESRD), and death (Borg et al., 2023). Causes of CKD are multifactorial and diverse, but early-stage symptoms are often few and silent. 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 (Hashmi et al., 2022). The prevalence of CKD and ESRD is increasing continuously as a result of the dramatic growth in the prevalence of two main causes of ESRD which are diabetes mellitus (DM) and hypertension, hence, ESRD represents a global concern (Helmy et al., 2022).

Patients with CKD exhibit an elevated cardiovascular risk manifesting as coronary artery disease, heart failure, arrhythmias, and sudden cardiac death. Although the incidence and prevalence of cardiovascular events is already significantly higher in patients with early CKD stages compared with the general population, patients with advanced CKD stages exhibit a markedly elevated risk (Joachim et al., 2021).

End-stage renal disease is a medical condition in which a person's kidneys cease functioning on a permanent basis leading to the need for a regular course of long-term dialysis or a kidney transplant to maintain life (Hashmi et al., 2022). Patients with ESRD have become recognized as serious challenge in the global public health. Hemodialysis (HD) continues to be the predominant therapeutic approach for treatment of ESRD patients all over the world (Himmelfarb, et al., 2020). The development of CKD and its progression to this terminal disease remains a significant source of reduced quality of life and significant premature mortality (Benjamin, 2022).

In single-gene disorders, which are also known as "monogenic diseases", a mutation of a single gene is sufficient to cause the disease. Conversely, in polygenic disorders, mutations of multiple different genes are

necessary to result in a disease (Cerrone et al., 2019).Chronic kidney disease patients are also characterized by a high genomic instability (Moffitt et al., 2014). This instability could be translated to high levels of genetic damage measured by the incidence of chromosomal damage (micronuclei) when their cells are challenged with ionizing radiation and could be either the cause or the consequence of renal pathologies (Rodríguez et al., 2015). In addition, it has been observed that CKD patients repair less efficiently DNA damage (Stoyanova et al., 2014). Variations in the genes regulating physiological pathways might affect the incidence and predisposition to CKD (Corredor et al., 2020).

Genetic testing is supportive in identifying and possibly confirming diagnosis of CKD, thereby guiding care (Prasad et al., 2022). In many patients with CKD, the cause of the disease remains unclear despite a thorough evaluation (Cocchi et al., 2020). In these instances, genetic testing has the potential to enable formal diagnosis and targeted intervention, as well as reduce the utilization of medications with potential significant side effects (Hays et al., 2020).

Patients with CKD and extrarenal manifestations are also at a high risk of a genetic etiology, as exemplified by visual and hearing disturbances in nephronophthisis (Armstrong et al., 2019). As a corollary, identification of a genetic cause for CKD will prompt early referrals for early identification and prevention of known or potential extrarenal manifestations (Mann et al., 2019).

Microsatellites are DNA elements composed of short repetitive motifs that are prone to misalignment and frame shift mutations during cell division. Microsatellite instability (MSI) is a molecular fingerprint for defects in the mismatch repair system (dMMR) and is associated with higher risks of cancers (Gilson et al., 2021). DMMR is characterized by the inability of cells to repair spontaneous mutations in microsatellites that occur during replication. Mutations in microsatellites lead to deletions or insertions of sequence units (Schöniger et al., 2022). These insertions and deletions, as well as base–base mismatches are normally corrected by the MMR system. If dMMR exists, those spontaneous errors of replication remain unrepaired and transmitted to daughter cells, thus, the sequel is MSI (Pećina et al., 2020). The basic principles for the evaluation of MSI were set in 1997 when Dietmaier et al., characterized the sensitivity and specificity of 31 microsatellite markers in colorectal carcinomas and classified the results into three categories, i.e., microsatellite stability (MSS) when no instability detected at any of the loci tested, and low-frequency (MSI-L) when instability detected at a single or double loci and high-frequency microsatellite instability (MSI-H) when MSI detected at ≥ 2 loci.

Polymerase Chain Reaction (PCR) and sequencing based systems have emerged and represent viable methods to standard MSI testing in specific settings. The evolution of the standard molecular techniques has offered the opportunity to extend MSI determination to liquid biopsy based on the analysis of cell-free DNA (cfDNA) in plasma (Darbeheshti et al., 2022).

Mutational analysis in renal diseases reveals the primary cause of the disease, and has a very high diagnostic and prognostic value. When performing molecular genetic diagnostics, genes are examined for disease-causing DNA sequence changes. Mutation analysis is usually performed by PCR followed by bidirectional sequencing, as it is estimated that about 85% of all disease-causing mutations in single-gene disorders are positioned within a coding exon care (Prasad et al., 2022).

This entry study provides for the first time a comprehensive overview of the impact of dMMR and MSI on (non-malignant) chronic kidney disease initiation and progression as well as the relevance of these alterations as diagnostic and predictive biomarkers. The development of standardized tests to detect MSI with high sensitivity and specificity in non-malignant diseases will likely not only improve prognostic evaluation and treatment success of different disease entities but may also helps to control the disease from progression and exacerbation.

The aim of this study is to detect the genomic alterations in patients with CKD and ESRD by PCR followed by sequencing and comparing the results with those of healthy volunteers group using microsatellites to predict the disease progression and to determine the value of MSI in the predisposition to develop CKD and determine its association with the clinical features of patients.

Study design: We designed a cohort longitudinal study of subjects in which we are regress their values of the patients against controls. Prior data indicated that the standard deviation of control is 0.8 and the standard deviation of the regression errors was 1.7. If the true slope of the line obtained by regression 's patient against control is 1.4, we were needed to study 50 subjects for each group to be able to reject the null hypothesis that this slope equals zero with probability associated with the test of this null hypothesis is 0.05.

Subjects & Methods:

Ethics statement: The study was approved by the Ethics Committee of Theodor Bilharz Research Institute (TBRI, Egypt), and written informed consents were signed by all participants to use their specimens for research purposes, in accordance with the institutional guidelines. All the

experimental procedures in this study complies with the latest version of the Declaration of Helsinki and general guidelines for good clinical practice.

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 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 the control group included in the study. Demographic and clinical characteristics, including age, gender, blood pressure, duration of dialysis, etiology of ESRD and CKD, were recorded.

Laboratory Parameters:

Five milliliters of fasting blood samples were collected from all subjects 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).

Molecular testing: Genomic DNA was extracted from blood samples using Qiagen DNeasy kit (Hilden, Germany) according to the manufacturer’s recommendations. The purified DNA was dissolved in 50 µl water, measured on a Nanodrop ND-2000c (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20˚C for further analysis.

Molecular analysis for MSI status: MSI analysis was carried out using 5 quasi-monomorphic mononucleotide microsatellite loci (BAT-25, BAT-26, NR-21, NR-22 and NR-24). The set of primer sequences are shown in (Table 1) Mamdouh et al., 2015.
DNA was amplified using Taq DNA Polymerase (Qiagen), starting with an initial denaturation step at 95˚C for 5 minutes followed by 40 cycles, each cycle started with denaturation step at 95˚C for 30 sec, annealing at 55˚C for 30 sec and extension at 72˚C for 1 min, with a final elongation at 72˚C for 10 min. The reaction was performed in a 25 µl volume. Positive controls (DNA of healthy volunteers) were included in each PCR reaction.
PCR products were resolved on 3% agarose gel, electrophoresed on a Bio-RAD electrophoresis chamber, with 5 µl of 100-1000 bp DNA ladder RTU used as a marker and visualized by ethidium bromide staining. The gel image was analyzed using Cleaver micro DOC gel documentation system. Gel results obtained using a UV imaging system and selected bands were purified from the gel and sequenced using 3500/3500xL Genetic Analyzer (Applied Biosystems) at Colors for Research, Medical Laboratories, Egypt. The results of the sequence were analyzed, revised, lined up alongside those of 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

Statistical analysis

The data were analyzed using statistical package for social science ‘IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA)’. Continuous normally distributed variables were represented as mean ±SD. Frequencies and percentage for categorical variables were used; a P value < 0.05 was considered statistically significant. To compare the means of normally distributed variables between groups, the Student’s t test was

performed, χ2 test or Fisher's exact test was used to determine the distribution of categorical variables between groups. The risk assessment OR (95% C.I) was done by using logistic regression analysis. In addition, the Molecular Evolutionary Genetic Analysis (MEGA-X) software was used (Version 10.2.4) to determine the insertion/deletion or nucleotide substitution by alignment.

Results

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).

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.

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).

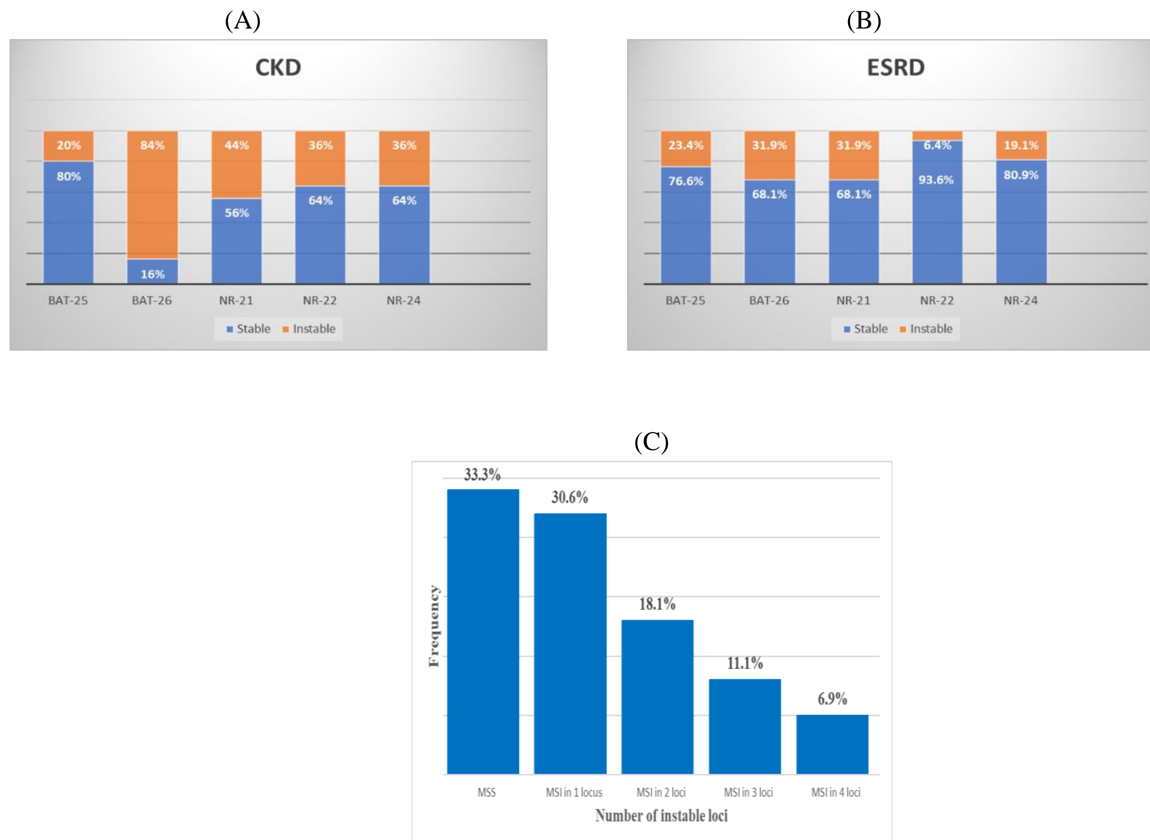

Figure 1. Distribution of MSI frequency regarding studied microsatellites among patients.

Univariate logistic regression analysis:

Logistic regression analysis of the studied microsatellites was carried out to evaluate their instability risk asessment 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).

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%)			

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.

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 the 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, the 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).

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.

Regarding the instability risk assessment for the 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 the 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).

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.

The 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).

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

		Patients with MSS		Patients with MSI		
Loci		Mean	SD	Mean	SD	P. value
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.

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) (Boland et al., 1998). 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).

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.

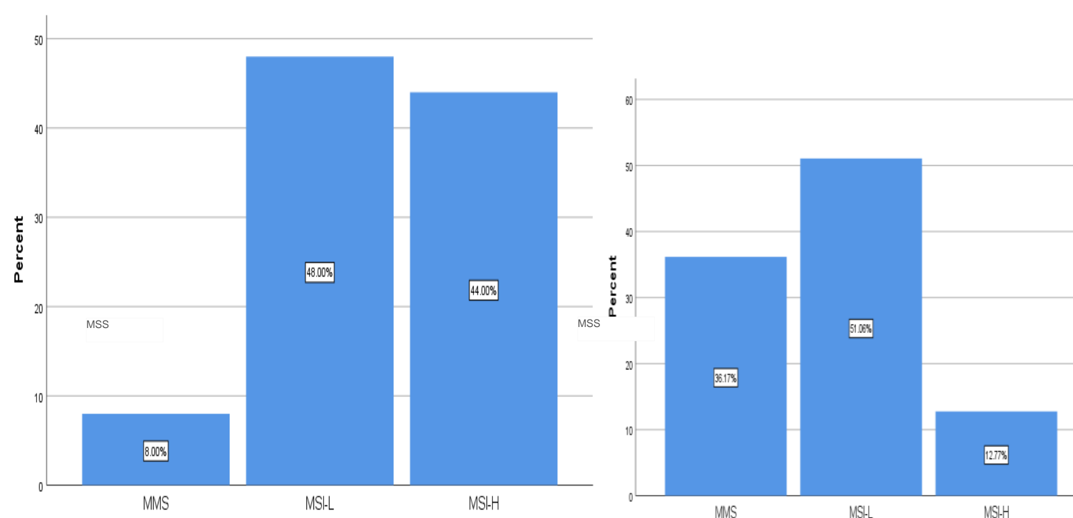

Figure 2. (A): MSI frequency in CKD patients, (B): MSI in ESRD patients.

Instability landscape of the studied microsatellites:

Molecular Evolutionary Genetic Analysis (MEGA-X) software (version 10.2.4) was used to detect the nucleotide insertion, deletion or substitution by 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 the 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).

(A)

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                              10         20         30         40         50         60         70
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|.
NR-24 Control        TCCTATTTTT TTTTTTTTTT TTTTTTTTTG GGAAAAAATC CCCCCTTTTC CCCCCGGGGG GAAGGGAAGG GGCAAA 76
NR-24 IN R. Chronic  .......... .......... .......G-. ........C. .....C.... .......... ......GG.. ...... 75
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(B)

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                              10         20         30         40         50
                     ....|....| ....|....| ....|....| ....|....| ....|....| .
BAT-26 Control       GTTAACCTTT TTCAGGTAAA AAAAAAAAAA AAAAAAAAAA AGGGTTTTAA T 51
BAT-26 hemodialysis  .C.T...... .......... .......... .......... ....G..A.. . 51
```

(C)

```
                              10         20         30         40
                     ....|....| ....|....| ....|....| ....|....| .
NR-21 Control        ACCCCGGGGG CCCAAAAAGG GGGAAGGCGG ATTAGAAAAA A 41
NR-21 hemodialysis   .......... .......G.. ....G..G.. .AA..G.... . 41
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(D)

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                              10         20         30         40         50         60         70         80
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| .
NR-24 Control        TCCTATTTTT TTTTTTTTTT TTTTTTTTTG GGAAAAAATC CCCCCTTTTC CCCCCGGGGG GAAGGGAAGG GGCAAATTAA A 81
NR-24 hemodialysis   .......... .......... ........-. .......... .....C..GT .......... ...A..C... ...C..A... . 80
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Figure 3. Results of Sequence Alignment of patients with controls.

Discussion

The development of chronic kidney disease (CKD) and its progression to this terminal disease ESRD, remains a significant cause of reduced quality of life and premature mortality. The CKD is a debilitating disease, and standards of medical care involve aggressive monitoring for signs of disease progression and early referral to specialists for dialysis or possible renal transplant (Hashmi et al., 2022). It is more prevalent in the elderly population. However, while younger patients with CKD typically experience progressive loss of kidney function, 30% of patients over 65 years of age with CKD have stable disease (Vaidya et al., 2022).

The most common causes of CKD include hypertension, diabetes mellitus, glomerular disease as well as polycystic kidney disease (PKD), which is a genetic disorder. Other forms of genetic kidney disease include those with glomerular patterns of injury such as genetic nephrotic syndrome, focal segmental glomerulosclerosis, APOL1-related kidney disease and atypical hemolytic uremic syndrome (Sawaf et al., 2023).

In order to reduce the incidence of CKD effectively, it is necessary to identify the patients at high risk. In order to delineate the genetic background, we have subsequently investigated the top five microsatellite loci for the association with the development of the disease in an Egyptian population. In the present study, we examined the microsatellite DNA instability in CKD and ESRD patients on regular hemodialysis, the frequency of instability varies between the two groups according to the microsatellite phenotype. 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.

However, because the frequency of these microsatellite loci was high to large extent, this MSI is enough to reflect the large part of genetic predisposition to CKD and ESRD.

It 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 the levels of the 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 the 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 finding extend the existing literature that supports the hypothesis of decreased serum albumin increases the hazard of CKD progression, our result goes parallel with that of Cheng et al., 2023 who was the first to observe that serum albumin has a non-linear relationship with renal prognosis and renal function decline.

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 logical explanation for the increase of instability in CKD than the ESRD group is, the failure of DNA damage repair and increased chromosome damage in CKD may be caused by the uraemic state as well as by chronic inflammation linked to increased formation of reactive oxygen species (Schupp et al., 2010; Tung et al., 2023). Even though a negative impact of reactive oxygen species and reactive nitrogen species (collectively denoted RONS) in CKD has not been clearly demonstrated, increased RONS lead to DNA strand breaks, point mutations and aberrant DNA cross-linking, thereby causing genomic instability (Hussain et al., 2007; Aranda et al., 2022).

With the discovery of microsatellite markers closely associated with the loci PKD1 and PKD2, PCR amplification of these markers is being increasingly applied for presymptomatic and prenatal ADPKD diagnosis (Autosomal dominant polycystic kidney disease), which is one of the most common genetic diseases in humans and characterized by the progressive development of large renal cysts. DNA-based analysis using flanking markers has been used as the gold standard method for the differentiation between both forms of the disease (Lin et al., 2002; Vouk et al., 2000). In 2006, Binczak et al., reported that DNA microsatellite analysis was established the type of the disease and concluded that the disease was linked to PKD1 and DNA microsatellite analysis provides an early diagnosis and may be considered in ADPKD families.

Nowadays, and after more than twenty years, we used the DNA microsatellite analysis to detect the genomic alterations in CKD and ESRD patients by PCR followed by bi-directional sequencing and comparing the results with those of healthy volunteers group to predict the disease progression. There is biological and genetic evidence suggesting that common genetic determinants modify kidney disease progression to ESRD, independent of the 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 the same position 29 in ESRD case with MSI in NR-24.

In 2022, KDIGO Conference Participants published that, early genetic testing may be advantageous especially in cases of kidney transplantation as donors related to a recipient with a known genetic condition should be tested early during the donor-evaluation process. This approach will expand the repertoire of molecular diagnostic tools available for physicians around the world. Advances in the clinical genetic evaluation include next-generation sequencing with targeted gene panels and whole genome sequencing. These platforms provide DNA sequence reads with excellent coverage throughout the genome and have identified novel genetic causes of CKD (Prasad et al., 2022).

In 2023, Han et al., performed GWAS to identify genetic variation associated with eGFR decline, as a measure of CKD progression, they identified the risk SNPs in TPPP and FAT1-LINC02374 loci among patients with CKD, the study suggests that genetic variations in the mentioned loci are associated with CKD progression. Our current study is the first to prove that genomic instability in the studied microsatellite loci are associated with 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 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.

In summary, our 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 results of MSI for CKD progression in established patients provided imperative insights into the genomic instability contribution in developing CKD and a credible basis for future studies based on various clinical outcomes of patients.

In the future direction, informative studies regarding genomic MSI are required to enhance our knowledge and understanding all about CKD genomic instability. Information as the relation between MSI and the risk of developing CKD and ESRD should be gathered and analyzed comprehensively to construct a way to evaluate the genetic risk of them precisely. In this sense, The DNA genomic instability examined in this study seems to be a basis for the future studies as it adds information for the prediction and the development of CKD and ESRD.

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