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Research Article

IL1 β exon5 3954 C/T Polymorphism: A Potential Genetic Risk Factor of Heart Diseases' predisposition in Sudanese Patients

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Abstract

Background: IL-1β was known to cause an inflammation in heart tissue leading to progressive loss of contractile tissues. The aim of this study was to evaluate "for the first time" the relationship between IL-1β polymorphism (rs1143634) and the risk of heart diseases (HDs) in Sudanese patients.

Methods: Fifty patients with HD and 65 healthy controls were enrolled in this cross-sectional study. The IL-1β (rs1143634) polymorphism was detected by PCR-RFLP using TaqI restriction enzyme.

Results: About 82% of the HD cases were aged >40 years. No gender difference was reported between the two groups (P = 0.28). 24% of the cases had a previous history of heart attack. Family history of HD was associated with a six-fold increased risk of HD.

The analysis provides evidence that the mutant genotype (CT + TT) of the IL1β polymorphism is significantly associated with HD, with up to four-fold increased risk of the disease (P = 0.015, OR = 3.8). The mutant allele T was significantly higher in HD patients as compared to the controls (P = 0.023). The frequency of the CT genotype among patient who have family history, previous attack of HD, hypertension, and diabetes was 79%, 33%, 81%, and 90% respectively.

Conclusion: The IL1β (rs1143634) polymorphism was associated with the increased risk of HD in our samples. The carriage of the mutant allele among those who have family history of HD, previous attack, hypertension, and diabetes might be a predictive factor for the onset of clinical manifestation of HD in Sudanese patients.

Keywords: heart diseases' risk factors, gene polymorphisms, Interleukin (IL)-1 β

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1. Introduction

Heart diseases (HDs) are the number one cause of death worldwide. The magnitude of HDs continues to accelerate globally and it is expected to rise more rapidly in developing countries [1] not only because many people in these countries have been exposed to unhealthy life habits, high prevalence of hypertension, dyslipidemia and diabetes, but also because this situation is coupled with the lack of prevention and control regimes [1]. HDs are multifactorial, where both environmental and genetic factors contribute to

the disease. To date, many genes are thought to contribute to the risk of HD, however, the results have been inconclusive [2–10].

The role of the cytokines in the alterations of vascular endothelial cells functions has been documented [11]. Such alterations occur as a result of the exposure of the vascular endothelial cells to cytokines which involve the downregulation of the endothelial nitric oxide synthase, production of new proteins, and induction of endothelial cell apoptosis [11]. Based on this, a series of studies have been conducted to investigate the contribution of different cytokines in the development and progress in cardiovascular diseases; however, the results have been contradictory [12–16].

L-1 is one of the inflammatory cytokines with pleotropic effects consisting of two distinct ligands – IL-1α and IL-1β. The release of IL-1β results in widespread inflammation, leading to further death of cardiomyocytes, progressive loss of viable contractile tissue, and development of cardiomyopathy and heart failure [17]. The contribution of *IL-1* gene to developing the risk of HDs have been studied among different ethnic groups with contradictory outcomes [18–23].

IL1β polymorphism is not only being presented as a risk factor for susceptibility to HD [24, 25], but it also associated with the severity and worse prognosis of the disease [26, 27].

In Sudan, while several studies have focused on the pathophysiology and progression of chronic heart failure [28–30], relatively little attention has been paid to unravel the genetic association with HD. Based on the above, this study aimed to investigate a possible association of the 3954C/T IL-1β polymorphism (rs1143634) with HD susceptibility in our patients.

2. Materials and Methods

This case–control retrospective study included 50 heart patients and 65 controls. The samples were collected from the ALShorta Hospitals and Modern Medical Center in Khartoum State. The demographic data of all subjects were obtained using a well-designed questionnaire. DNA quantification and purity were assayed using a UV spectrophotometer at 260 and 280 nm.

In this study, we conducted a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of IL-1β exon 5. As describe in Table 1, the polymorphism (rs1143634) was done using the iNtRON Biotechnology, Inc. pre-mix, followed by incubation in Taq1 restriction enzyme.

The homozygous wild type genotype (CC) of the 3954C/T polymorphism (rs1143634) in exon 5 of IL-1β was indicated by the presence of 249 bp band. The homozygous mutant genotype was cleaved giving a band of 136 and 113 bp. The heterozygous genotype was indicated by the presence of three fragments, 249 bp, 136 bp, and 113 bp.

Table 1: The RFLP–PCR method used.

Primer sequence	Forward primer 5'GTTGTCATCAGACTTTGACC 3' Reverse primer 5'TTCAGTTCATATGGACCAGA3'
PCR mix contains	(i) iNtRON Biotechnology, Inc. pre-mix (ii) 1 µL of 10 pmol/µL of each primer (iii) 3–5 µL of 50 ng/µL of template DNA (iv) Volume completed to 12.5 µl
PCR program	(i) 95°C for 5 min for initial denaturing (ii) 30 cycles of denaturing at 95°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 30 sec (iii) Final extension at 72°C for 10 min
RFLP: Restriction mix containing	(i) 2 µl of the PCR product (ii) 0.5 U of Taq1 restriction enzyme (New England Biolabs) (iii) 1 µL Tango buffer (iv) The mix was completed to 10 µL (v) The restriction mixture was incubated for 3 hr

2.1. Statistical Analysis

The SPSS, version 21, was used to count the genotype, Chi square, *P*-value, odds ratio (OR). $P < 0.05$ was considered as statistically significant. In addition, ORs were used to assess the strength of the association of the tested variables with the risk of HDs in Sudanese patients.

3. Result

No significant gender difference was observed between the cases and the controls ($P = 0.28$). About 82% of the HD patients were aged >40 years with a significant difference when compared with the controls ($P = 0.001$). Atherosclerosis was observed in 42% of the HD cases. In addition. low frequency of other types of HDs including the dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) was observed (4% and 2%, respectively). Previous attack of HD had been encountered in 24% of the patients (Table 2).

Although not all patients and controls were able to answer with certainty whether they have family history of HD or not, among those who answered, a six-fold increased risk of HDs were reported.

Also, of the total HD patients, 42% and 38% had hypertension and diabetes mellitus, respectively, of which 48% and 21%, respectively, developed HD within less than five years of disease onset (Table 2).

The genotype analysis of the IL1β C > T (rs1143634) polymorphism (Figure 1) considering the mutant genotype versus the wild genotype (CT + TT vs CC) showed a significantly higher frequency in HD patients compared to the controls and was associated with about four-fold increased risk of HD ($P = 0.015$, OR = 3.8). Carriage of the mutant IL-1 β (+3954) allele T was higher (62%) in HD patients as compared to the controls, with a significantly high difference between the two groups ($P = 0.023$) (Table 3).

The mutant TT and CT genotypes were reported in atherosclerotic patients with frequencies of 38% and 48%, respectively (Table 4). Moreover, 33% and 42% frequencies of the CT and TT genotypes were observed in HD patients who encountered previous attack of the disease.

Considering the cofounding factors, the family history of HD, hypertension, and diabetes mellitus, the mutant heterozygote CT genotype was observed with frequencies of 79%, 81%, and 90% in HD, respectively (Table 4), and the frequency of the mutant T allele ranged 40–58%, as shown in Table 4.

Table 2: The demographic data of the HD cases and healthy controls.

		Case N = 50	Control N = 65 N	P-value
Sex	Male	27 (57.4%)	32 (49.2%)	*P* = 0.28
	Female	20 (42.6%)	33 (50.8%)	
Age	<40 years	9 (18%)	30 (46.2%)	*P* = 0.001
	40–60 years	41 (82%)	35 (53.8%)	
Type of HDs	Atherosclerosis	21 (42%)	0%	
	DCM	4 (8%)		
	HCM	2 (4%)		
	Other	23 (46%)		
Previous attack	Yes	12 (24%)		
	No	38 (76%)		
Family history of HDs	Yes	19 (40.4%)	3 (9.7%)	*P* = 0.003
	No	28 (59.6%)	28 (90.3)	OR = 6.3
	Missing data	3	34	
Hypertension	Yes	21 (42%)	0%	
	No	29 (58%)		
Diabetes mellitus	Yes	19 (38%)		
	No	31 (62%)		
Hypertension for <5 years	10/21 (47.6%)			
Diabetes mellitus for <5 years	4/19 (21.1%)			

4. Discussion

HDs are the number one leading causes of death and a serious public health problem worldwide [31]. HDs are multi-factorial and polygenic where not more than one gene contributes to the disease outcomes. To the best of our knowledge, the current research is the first genetic study aimed to investigate the association of gene polymorphism with the risk of HDs in Sudanese patients.

Table 3: Comparison of the genotypes and allele frequency of IL1β C > T (rs1143634) polymorphism between HD cases and controls.

Genotypes	Cases (HDs) N=50	Controls N=65	P value Odd ratio
CC	7 (14.0%)	25 (38.5%)	P=0.015, OR=3.8
TC	24 (48.0%)	22 (33.8%)	
TT	19 (38%)	18 (27.7%)	
Allele frequency			
C T	0.38 0.62	0.55 0.45	P=0.023

Table 4: Frequencies of genotypes and the mutant allele of IL1β C > T (rs1143634) polymorphism and the confounding factors in HD patients.

	CC	CT	TT	Mutant T
Atherosclerosis (*n* = 21)	3 (14.3%)	10 (47.6%)	8 (38.1%)	61.9%
Previous attack (*n* = 12)	3 (25%)	4 (33.3%)	5 (41.7%)	58.3%
Family history of HD (*n* = 19)	4 (21.1%)	15 (78.9%)	0%	39.5%
Hypertension (*n* = 21)	4 (19.0%)	17 (81%)	0%	40.5%
Diabetes mellitus (*n* = 19)	2 (10.5%)	17 (89.5%)	0%	44.7%

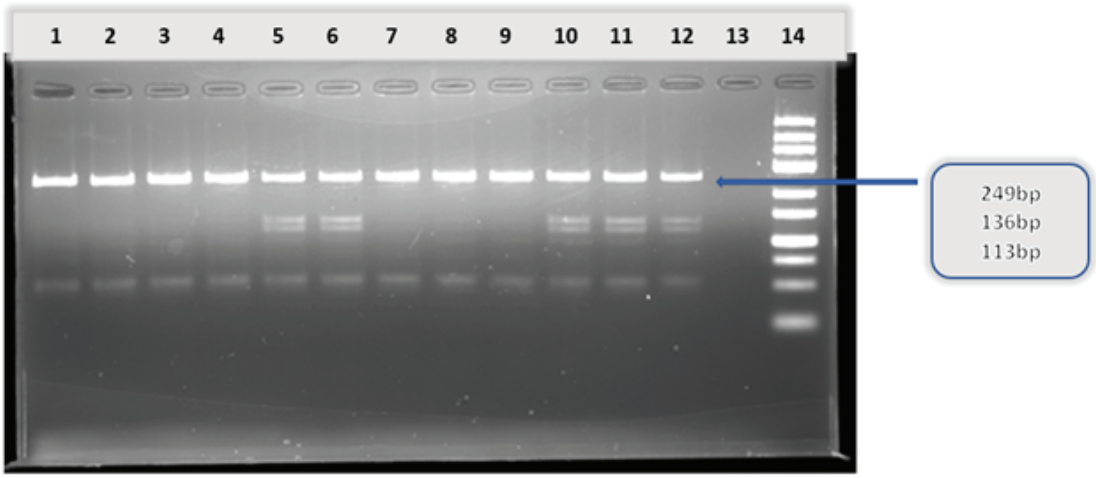

Figure 1: Lanes 1–4 and 7–9 are uncleaved wild-type homozygous CC (249 bp), lanes 5, 6, and 10–12 are heterozygous CT mutant (249, 136, 113 bp), lane 13 is negative control, and lane 14 represents the 25 bp DNA ladder.

The statistical genotype analysis provided evidence for the association of the IL1β C > T (rs1143634) polymorphism with HDs' susceptibility (P = 0.015) with a four-fold increased risk of HDs. Although, this result is in line with several previous studies [25, 26, 32], it disagree with some [33–35], which can be explained by the differences in the genetic makeup of the varied ethnic groups enrolled in these studies. The significantly higher frequency of the mutant T allele among cases (P = 0.023), the 42% frequencies of TT genotypes in HD patients who encountered previous attack of the disease, and the ≥40% frequency of the mutant T allele in HD patients who had a family history of HD,

hypertension, and diabetes mellitus, supports the important role of polymorphism in HD predisposition in these patients.

The analysis of the other demographic data showed that the previous attack of HD had been encountered in 24% of the patients and 82% of the HD patients aged 40–60 years with a significant difference when compared with the controls (P = 0.001). This reflects the adverse effect of HDs on the quality of life of a large scale of the patients at the reproductive age.

Although not all patients and controls are able to answer with certainty whether they had a family history or not, a six-fold increased risk of HDs was reported among patients with a family history of HD, indicating a lack of awareness about the disease and off course about the disease risks among the general population.

Hypertension and diabetes mellitus were considered as risks for HD [36, 37]. This study revealed that 42% and 38% of the HD patients had hypertension and diabetes mellitus, respectively, of which 48% and 21%, respectively, develop HD within less than five years of the disease onset. This study also showed that the mutant heterozygote CT genotype was reported with high frequencies among hypertensive and diabetics – 81% and 90%, respectively. All these not only suggest an important role of the IL1β C > T (rs1143634) polymorphism with HD susceptibility, but also indicate a possible role in hypertension and diabetes outcomes.

5. Conclusion

The results of this study demonstrate that IL1β C > T (rs1143634) polymorphism was associated with an increased risk of HD in our samples. The carriage of the mutant allele and/or the heterozygote CT genotype among those who had a family history of HD, previous attack, hypertension, and diabetes might be a predictive factor for the onset of clinical manifestation of HD in Sudanese patients.

Recommendation

Further genetic studies targeted more polymorphisms in IL1 and in other candidate genes are needed. Such studies enable the identification of people at risk of developing the disease and also enables a provision of targeted therapy. In addition, a cost-effective public awareness and early detection programs are desperately needed to decrease the burden of the HDs in the general population.

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Ethical approval

This study has been approved by the Ethical Committee at the AlNeelain University, Faculty of Medical Laboratory Science. A verbal consent was obtained from all participants after explaining the purpose of the study.

Competing Interest

The authors declare that there is no conflict of interest.

Availability of Data and Material

Data will be available from the authors upon reasonable request.

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Not applicable.

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