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Original Research Paper

# Immunological and Comparative Study of Serum HSP-70 Level and MicroRNA-21 Expression with Clinical Findings in Patients with Diabetic Nephropathy

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**Abstract:** Diabetic nephropathy is an important cause of morbidity and mortality in all types of diabetes. MicroRNA-21 expression can be induced by hypoxic conditions such as kidney injury and regulated in kidney diseases and may be used as a diagnostic biomarker in kidney diseases. On the other hand, in most people with nephropathy, heat shock protein (HSP 70) has been investigated from different aspects in experimental studies. HSP-70 is the most abundant form of HSP in cells. HSP-70 is involved in the response to various acute and chronic conditions in the kidney as well as other urinary tract infections. The purpose of this study is to compare the immunological and comparative study of HSP-70 serum level and MicroRNA-21 expression with clinical findings in diabetic nephropathy patients.

**Keywords:** Diabetic Nephropathy, HSP-70, MicroRNA-21, Biomarkers, Renal Stress

## 1. Introduction

Serum Level of HSP-70 and MicroRNA-21 Expression with Clinical Findings in Diabetic Nephropathy Patients," provides a comprehensive overview of diabetic nephropathy (DN), its significance as a global health issue, and the molecular mechanisms underlying its pathogenesis [1]. It also introduces the roles of heat shock protein 70 (HSP-70) and microRNA-21 (miR-21) as potential biomarkers and contributors to the disease, setting the stage for the research objectives [2].

### *Diabetic Nephropathy*

Diabetic nephropathy (DN) is highlighted as a major cause of morbidity and mortality in patients with both type 1 and type 2 diabetes [3]. Globally, over 150 million people are affected by diabetes, a number projected to double by 2025, underscoring the growing burden of associated complications like DN [4]. The prevalence of DN increases with the duration of diabetes, peaking at 21% after 20-25 years and decreasing to 10% after 40 years, with a cumulative incidence reaching 45% over four decades [5]. The disease predominantly affects men and is characterized by progressive morphological and ultrastructural changes in the kidney, such as the expansion of the glomerular basement membrane matrix [6]. DN is a multifactorial, progressive condition with a complex pathogenesis involving various cells, molecules, and factors [7].

The interplay of metabolic and hemodynamic factors is central to DN's development, activating shared pathways that lead to renal damage [8]. Hyperglycemia, hypertension, and genetic predisposition are identified as primary risk factors, with additional contributions from elevated serum lipids and dietary protein intake [9]. Genetic and environmental factors also play significant roles, with studies suggesting that only about one-third of type 1 diabetes patients develop DN, indicating a strong genetic component [10]. In type 2 diabetes, fewer

patients progress to end-stage renal disease (ESRD), yet DN affects approximately 40% of both type 1 and 2 diabetic populations [11]. The rising incidence of type 2 diabetes amplifies the clinical importance of this subgroup [12].

Clinical trials like the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) demonstrate that while strict metabolic control and antihypertensive therapy can slow DN progression, they cannot fully prevent it [13]. This limitation emphasizes the need to understand the molecular mechanisms of DN to develop novel therapeutic strategies and diagnostic markers [14]. The earliest sign of DN is increased urinary protein excretion (albuminuria), linked to defects in the glomerular filtration barrier, comprising endothelial cells, podocytes, and the glomerular basement membrane [15]. DN progresses through stages of microalbuminuria and macroalbuminuria, often leading to glomerulosclerosis and a declining glomerular filtration rate (GFR). If uncontrolled, this culminates in ESRD, necessitating dialysis [16,17].

Current management focuses on controlling blood pressure and maintaining hemoglobin A1c below 7%, yet challenges persist due to the multifactorial nature of hypertension [18]. Screening for DN relies on albuminuria assessment, though recent evidence suggests renal dysfunction may precede albuminuria, highlighting limitations in its use as a sole marker [19]. Alternative biomarkers, such as serum TNF-α receptor levels, are emerging as promising predictors of chronic kidney disease (CKD) and ESRD progression in diabetes [20].

### *Heat Shock Proteins (HSPs)*

Heat shock proteins (HSPs) are a family of highly conserved proteins produced by cells in response to stressors like heat, oxidative stress, and hypoxia [21]. Classified by molecular weight (e.g., HSP-27, HSP-60, HSP-70), they play critical roles in protein folding, cellular protection, and stress response [22]. HSP-70, the most abundant and temperature-sensitive member, is found in 60-80% of eukaryotic cells and is vital for cell survival under stress [23]. It is induced by various stimuli, including hypoxia, acidosis, reactive oxygen species, and infections, and exerts antioxidant and anti-inflammatory effects while preventing apoptosis [24].

### *Role of HSP-70 in Diabetic Nephropathy*

In DN, HSP-70 responds to acute and chronic renal stressors and is present in the kidney and urinary tract [25]. Although not a specific biomarker for any single kidney disease, its levels in body fluids may serve as a general indicator of renal stress [26]. Extracellular HSP-70 exhibits dual roles as a cytokine and molecular chaperone, detectable in serum under conditions like diabetes, atherosclerosis, and renal disease [27]. Its exact extracellular source remains unclear, but it is biologically active and recognized by the immune system, potentially acting as an autoantigen [28]. Experimental studies underscore HSP-70's protective role against renal injury, making it a candidate for further investigation in DN [2].

### *MicroRNAs in Diabetic Nephropathy*

MicroRNAs (miRNAs) are small, non-coding RNAs (22-25 nucleotides) that regulate gene expression by binding to messenger RNA (mRNA), leading to its degradation or translational repression [29]. Discovered over a decade ago, miRNAs modulate at least 30% of the human protein-coding genome and are implicated in numerous diseases, including DN [30]. They are transcribed from DNA into primary miRNAs (pri-miRNAs), processed into precursor miRNAs (pre-miRNAs) by Drosha and DGCR8, and matured into duplex miRNAs by Dicer [31]. One strand integrates into the RNA-induced silencing complex (RISC) to target mRNAs, influencing cellular processes like inflammation and apoptosis [32].

### *Role of miR-21 in Diabetic Nephropathy*

MiR-21, inducible by hypoxic conditions such as renal ischemia-reperfusion injury, is upregulated in kidney diseases and proposed as a diagnostic biomarker [33]. It exerts anti-apoptotic effects in tubular epithelial cells via TGF-β signaling, a key player in renal injury [34]. However, miR-21's overexpression is also linked to pathological states, including cancer and cardiac injury, where it may interact with endothelial nitric oxide synthase (eNOS) and HSP-70 [35]. In DN, miR-21 is associated with high glucose levels, activating intracellular phosphate-dependent pathways that exacerbate renal cell damage and contribute to disease

onse [36]. Its role in stimulating inflammatory molecules like HSP-70 and promoting apoptosis further complicates its function in DN [37]. The aim of the present study was to compare the immunological and comparative study of HSP-70 serum level and MicroRNA-21 expression with clinical findings in diabetic nephropathy patients.

## 2. Methodology

*Samples collection*

The sample included 55 DN patients and 40 healthy controls, selected from 1,401 patients referred to therefore mentioned hospitals. This yielded a total of 95 participants for analysis.

## 3. Results and discussion

Analyzing serum HSP-70 levels, MicroRNA-21 (miR-21) expression, and clinical parameters in 55 DN patients and 40 controls. The findings are supported by statistical tests, graphs, and tables, revealing significant differences between groups and correlations among variables.
maximum, mean, standard deviation (SD), and t-test results (t=-2.50, P=0.014).

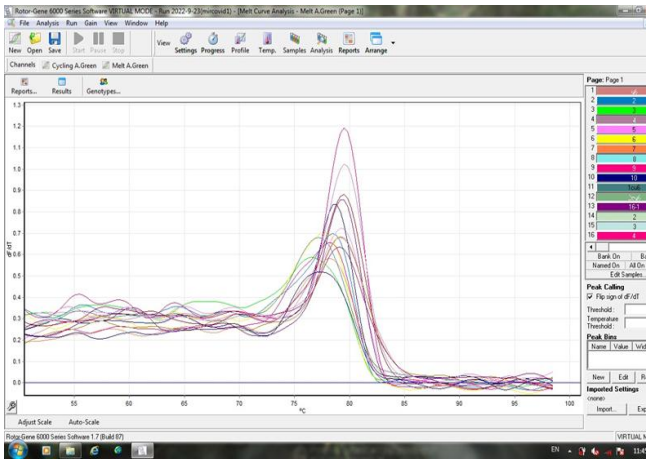

Fig 1.Ct Plot: Amplification began exponentially at cycle 21, with higher initial template amounts in patient samples reducing Ct values (Figure 3-4).

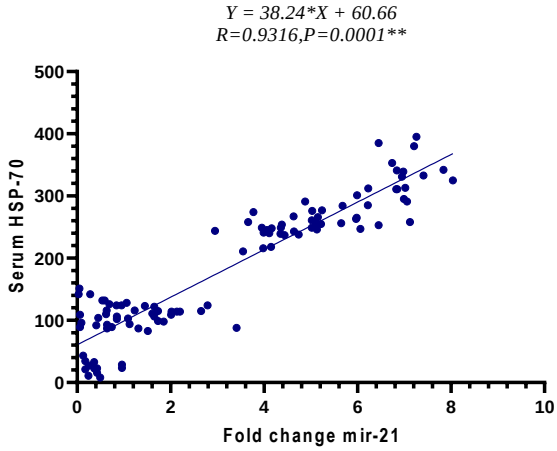


Fig 2. Correlation of miR-21 with Serum HSP-70.

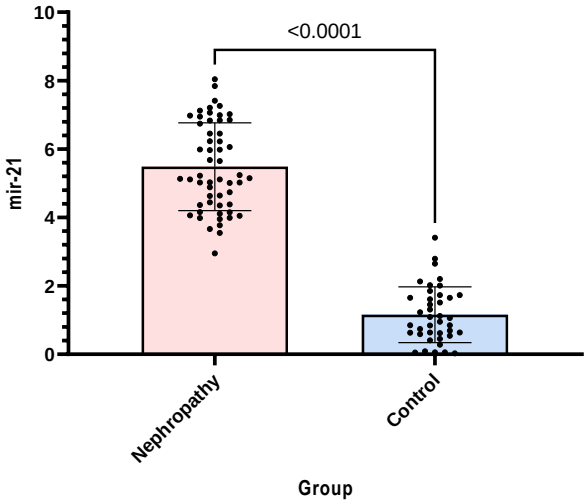


Figure 4-4: "Distribution of miR-21 in Patients with Nephropathy and Control Group" - Visualizes miR-21 expression differences.

MicroRNA-21 (miR-21), an anti-apoptotic miRNA, is implicated in several processes triggered by ischemia-reperfusion injury (IRI). The induction of hypoxia or ischemia in tubular epithelial cells leads to the upregulation of miR-21, which may play a protective role by modulating cellular responses to stress [38]. This miRNA has been shown to influence key pathways, such as the PI3K/AKT signaling pathway, promoting cell survival and proliferation under stress conditions, as observed in studies involving extracellular vesicles derived from human umbilical cord mesenchymal stem cells [38]. However, its overexpression can also

exacerbate inflammatory responses, potentially contributing to the progression of renal damage in conditions like diabetic nephropathy, where genetic variations in inflammatory cytokines amplify disease risk [39].

In contrast, heat shock protein 70 (HSP70) levels are inversely correlated with tubular injury, apoptosis, and renal dysfunction following renal ischemia (IR). HSP70 is thought to exert a protective effect by mitigating cellular damage and supporting renal recovery post-ischemia. This protective role aligns with findings that interventions targeting proteinuria, a hallmark of renal injury, can slow the progression of diabetic nephropathy, suggesting a potential interplay between HSP70 and renoprotective mechanisms [40]. Together, the opposing dynamics of miR-21 and HSP70 highlight their complex roles in renal pathophysiology, warranting further investigation into their therapeutic potential.

In this study, miR-21 expression in diabetic nephropathy with ROC curve analysis to test the validity of MicroRNA-21 gene expression in patient samples compared to the control group shows that based on the area under the curve, P=0.0001 AUC= 0.9995, Std= 0.0007759 The validity of this test is evaluated very well in relation to diabetic nephropathy, miR-21 expression was positively correlated with glycosylated hemoglobin (HbA1c), fasting blood sugar (FBS). There is a significant difference (Student's test) between the two groups in terms of Serum HSP-70 (P-Value=0.0001**). HSP70 serum level was positively correlated with glycosylated hemoglobin (HbA1c), fasting blood glucose (FBS), age and UREA-Creatinine-eGFR.

## Conclusion

1The results of ROC curve analysis for testing the validity of MicroRNA-21 gene expression in patient samples compared to the control group show that based on the area under the curve, P=0.0001, AUC=0.9995, Std=0.0007759, the validity of this test in relation to diabetic nephropathy is very high. It is evaluated well. The correlation coefficient obtained between the expression of the mir-21 gene and serum HSP-70 of the patients shows that a significant and inverse relationship was observed between the percentage index of serum HSP-70 and the expression of the mir-21 gene (R=0.9316, P=0.0001). Thus, with the increase of mir-21 gene expression, the percentage of Serum HSP-70 increased. 3- The results show that FBS, HBA1C and Creatinine, eGFR, Creatinine have a significant relationship between blood indices and Serum HSP-70 and MicroRNA-21

## Ethics

The study adhered to ethical guidelines, ensuring participant confidentiality and obtaining informed consent. No additional costs were imposed on patients, and the research complied with the ethical standards of the Islamic Azad University Medical Faculty.

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