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# Molecular Study Of Insulin-Like Growth Factors-1 Gene In Patients With Various Stages Of Chronic Kidney Disease

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**Implication for health policy/practice/research/medical education:**

The study's findings have important implications for health policy, medical practice, research, and medical education. In health policy, integrating insulin growth factor -1 (IGF-1) gene, expression as a biomarker can enhance early screening and prevention strategies for chronic kidney disease (CKD). In medical practice, IGF-1 testing can improve diagnosis and enable personalized treatment plans. For research, these results encourage further studies on CKD-related genes and new therapeutic developments. In medical education, incorporating these findings can update curricula and provide continuous training for healthcare professionals, improving CKD management and patient outcomes.

***Abstract***

***Introduction****: Genetic research worldwide has been carried out to discover the genes that influence the prevalence of chronic kidney disease (CKD) in individuals. Insulin-like growth factor 1 (IGF-1) is one of these genes involved in CKD emergence.*

***Objectives****: Given the likely role of IGF-1 in advanced stages of CKD, we sought to examine its expression levels and their impact on renal function in patients with varying stages of renal disease.*

***Patients and Methods:*** *This case-control study comprises 55 patients diagnosed clinically and serologically with CKD, their age varies from (18 to 75 years) and 20 individuals as control. Blood samples were collected to extract RNA, then converted to cDNA, and the gene expression was detected by amplifying using a real-time polymerase chain reaction technique. The levels of mRNA IGF-1 genes were normalized by amplifying the endogenous control gene B-actin. The Excel and SPSS were among the statistical programs and software utilized for quantitative polymerase chain reactions.*

***Results:*** *IGF-1gene has significant overexpression in CKD patients, also the expression in males increases 2-fold in females. IGF-1 gene folding elevated gradually with the progression of stages of CKD. Moreover, the highest value of expression was registered in the advanced stages of disease. The folding was assessed in different age groups and showed elevated in the early ages and decreased in elderly patients.*

***Conclusion****: We concluded that IGF-1 overexpression at the level of a biomarker could potentially assist in identifying those who are most at risk of developing CKD and help prevent and treat them early.*

***Keywords:*** *Insulin-like growth factors gene, chronic kidney disease, gene expression, growth factor*

## Introduction

Currently, chronic kidney disease (CKD) represents a major challenge for the healthcare system (1). It's considered the main cause of early death in younger patients aged between 50 and 70 years. This condition is referred to when kidney damage is established as irreversible and thus results in lost nephrons, along with a decrease in the estimated glomerular filtration rate to less than 60 ml/min/1.73 $m^2$ for more than three months

(2).

The origin of CKD is different all over the world, and CKD is largely due to primary diseases like diabetes, hypertension, glomerulonephritis, obesity, and metabolic syndrome. This complex pathogenesis of CKD brings about changes in hormones and metabolism, including the growth hormone (GH)–insulin-like growth factor (IGF)-I axis. Among several complications of CKD, the disturbances in somatotropic hormone regulation lead to growth retardation, catabolism, and disease progression (3). Insulin-like growth factors are worth noting in more scientific studies due to their bioeffects and therapeutic applicability. IGF-1(insulin-like growth factor1) is involved in cell growth, differentiation, and transformation as a master regulator of the process. The gene for IGF-1 is located at chromosomal locus 12q22-24.1. This gene encodes a protein consisting of 70 amino acids with a single-chain polypeptide with a molecular weight of 7.6 kDa(4).

In kidney, there are two sources of IGF-1, first; circulating IGF-1 that is primarily synthesized in the liver and acts on target tissues through endocrine pathways, and its production by the liver is regulated by insulin, protein intake, and growth hormone. These processes are further influenced by race, age, gender, and genetic susceptibility (5) and secondly, locally produced IGF-1 within the kidney acts as an autocrine or paracrine factor that helps regulate renal cell metabolism. It can be inferred that biosynthesis of IGF-1 occurs in the kidneys, since its levels are higher in renal venous blood compared to renal arterial blood (6). In adult human kidneys, IGF-1 mRNA was found throughout the nephron, including the glomerulus, thick ascending limb of Henle's loop, distal nephron, and collecting duct, with the least observed levels in proximal tubules (7).

One of the primary roles of growth factors is known to be associated with the development of renal vascular complications, which are likely to be genetic-dependent mechanisms (8). Late microvascular complications have been shown to be IGF-1-related. Since circulating IGF-1 levels may not accurately reflect bioactivity due to interference by binding proteins, particularly in pathological cases, genetic studies may be an alternative avenue (9).

Within the period of two to three years after the initiation of the disease, patients diagnosed with severe CKD will ultimately transition into uremia, which becomes a source of many devastating complications and leads to death (10). Pathophysiological studies state that genetic predisposition, hemodynamic changes, and metabolic abnormalities are all major contributing factors in the development of CKD(11).

Several kidney diseases have been connected to IGF-1/IGF-1 receptor signaling (12). Specifically, IGF-1 overexpression has been shown to have direct effects on renal hemodynamics that interact with the intrarenal renin-angiotensin-aldosterone system (RAAS), resulting in a decrease of renal vascular resistance and a rise of intraglomerular hypertension which promotes glomerular filtration (early stages of CKD) and progression of proteinuria (13). It has been demonstrated that, the produced IGF-1 by podocytes is causing extracellular matrix protein stimulation and hence contributes to glomerulosclerosis. Additionally, it acts on tubules to increase salt and water reabsorption, leading to fluid retention(14).

## Patients and Methods

### Study design

The case-control study enrolled 55 patients diagnosed with CKD: 31 males and 24 females, aged between 18 and 75 years. These patients were selected from those admitted to Al-Zahraa teaching hospital, Wasit, during the period from February 2022 to March 2023. Furthermore, the CKD cases were classified into five stages based on the National Kidney Foundation's classification system outlined in its Kidney Disease Outcomes Quality Initiative (NKF KDOQI) clinical practice guidelines (15), take into account severity which is determined by kidney function levels estimated through estimated glomerular filtration rate (eGFR). The eGFR was calculated using the MDRD equation as recommended by Levey in 1999, this equation estimates GFR based on serum creatinine levels, age, gender, and race, providing a reliable measure of kidney function. The formula used is as follows (16);

$$GFR\left(\frac{\frac{mL}{min}}{1.73\ m^2}\right) = 186 \times (serum\ creatinine)^{-1.154} \times (age)^{-0.203} \times (0.742\ if\ female) \times (1.210\ if\ Black)$$

The stages of CKD were classified according to the division stages of the National Kidney Foundation (NKF) guidelines. These guidelines categorize CKD into five stages based on the estimated glomerular filtration rate (eGFR) as following:

- Stage 1: With normal or high (GFR > 90 mL/min/1.73 m²)
- Stage 2: Mild CKD (GFR= 60-89 mL/min/1.73 m²)
- Stage 3A: Moderate CKD (GFR= 45-59 mL/min/1.73 m²)
- Stage 3B: Moderate CKD (GFR= 30-44 mL/min/1.73 m²)
- Stage 4: Sever CKD (GFR= 15-29 mL/min/1.73 m)
- Stage 5: End stage CKD (GFR < 15 mL/min/1.73 m²). These stages help assess the severity of kidney damage and guide treatment strategies (17).

Furthermore, 20 healthy people were enrolled in the study to serve as age and sex controls, and all participants gave written informed consent. Both groups were subjected to a comprehensive clinical examination, and detailed medical history was recorded. Blood pressure measurements were taken with patients sitting down. This study was conducted on CKD patients with

kidney disease. For the assessment of biochemical parameters, such as serum urea and creatinine, venous blood samples were collected.

***Molecular study***

Reverse transcription- quantitative polymerase chain reaction (RT-qPCR) was conducted to determine the expression of IGF-1 gene, and 200 µL EDTA anticoagulated blood was conducted to extract RNA. Total RNA from all samples was extracted using AccuZol reagent according to the manufacturer's instructions and then converted into complementary DNA (cDNA) by reverse transcription technology (СИНТОЛ Company/Russia). According to the instructions, the procedure was performed in a reaction volume of 25 µL. Then apply EVAgreen real-time quantitative PCR technology. Each type of double-stranded DNA, including cDNA, is detected by fluorescent dyes, and the amount of amplification is quantified as cycle threshold (Ct). The mRNA levels of the IGF-1 gene were normalized by amplification of the endogenous control gene B-actin. The primers ordered from Alpha DNA Canada are as illustrated in Table 1.

**Table 1.** The sequences of primers used in RT-qPCR

| | | |
|---|---|---|
| *IGF-1* | Forward | 5′-TTGGGCACATAGTAGAGCTCAC-3 (4) |
| | Reverse | 5′-CAAAAGCCCAGAGCAGACAT-3′ |
| *B*- actin<br>ference gene) | Forward | 5’-CTGGAACGGTGA AGGTGACA-’3 (18) |
| | Reverse | 5’-CGGCCACATTGTGAACTTTG - ‘3 |

**Statistical analysis**

A computerized system was conducted to translate the data into a format that is easier for analysis and manipulation. Statistical analysis was performed using SPSS version 25 and Microsoft Excel 2019 software. An unpaired T-test was conducted to evaluate differences between the patients and control subjects, as the data were normally distributed. Results were deemed statistically significant if the P-value was < 0.05. In addition, gene expression was calculated using Equation ($2^{-\Delta\Delta Ct}$).

**Result**

The groups of patients and healthy controls are approximately similar in age, with averages of 46.22 years for the patients and 43.03 years for the controls. However, patients exhibited higher mean levels of serum creatinine and urea compared to the healthy individuals, indicating a notable difference in renal function. Additionally, the glomerular filtration rate (GFR) was significantly lower in the patient group, as detailed in Table (2)

T**able 2.** Features of the study groups' biochemical and clinical profiles

| **Subjects** | **CKD patients** | | **Control cases** | |
|---|---|---|---|---|
| | No | % | No | % |
| Male | 31 | 56.37% | 12 | 60% |
| Female | 24 | 43.64% | 8 | 40% |
| Age (year) ±SD | 46.22±13.2 | | 43.03±15.1** | |
| Serum creatinine (mg/dl) ±SD | 3.96 ±3.02* | | 0.89±0.45 | |
| Serum urea (mg/dl) ±SD | 120.56±32* | | 32.55±2.4 | |
| GFR (ml /min/1.73ml$^2$) | 32±12.5* | | 118±32.1 | |

*significant, **non-significant, p-value <0.05 CKD: chronic kidney disease

**Gene expression study**

Several genes are involved in the cause of this chronic disease, where some expression variations have shown effects on the initiation and progression of the disease, increasing the overall risk of it. Hence, genetic susceptibility has been identified as a crucial risk factor for CKD.

According to the data in Table 3, relative gene expression was determined by RT-qPCR using the formula ($2^{-\Delta\Delta Ct}$). The levels of mRNA of the IGF-1 genes were normalized by amplifying the endogenous control gene *B*-actin. In patients, IGF-1 is overexpressed [3.22] compared with healthy volunteers [1.00], (p-value less than 0.05), which illustrates the notable difference between the two groups. Moreover, we focused on the affect the sex on expression IGF-1 gene, the result in Table 3 shows overexpression in both patients' sex. However, the folding in male [5.85] appeared higher than females [2.71] significantly.

**Table 3.** IGF-1 mRNA expression in the study groups, and comparison according to sex in CKD patients.

| Subjects | Mean Ct gene | Mean Ct *B* actin | Mean Δ Ct test | Mean ΔΔ Ct | Folding |
|---|---|---|---|---|---|
| Patients | 17.21 | 16.38 | 0.83 | -1.68 | 3.22* |

| | | | | | |
|---|---|---|---|---|---|
| Control | 15.76 | 16.38 | - 0.62 | 0.00 | 1.00 |
| Female | 17.68 | 16.38 | 1.30 | -1.44 | 2.71* |
| Male | 16.85 | 16.38 | 0.47 | -2.55 | 5.85* |

* : significant

**IGF-1 gene expression with CKD**

According to the five steps of renal disease detected by NKF KDOQI, our result revealed IGF-1 gene folding elevated gradually with progression of steps of CKD as shown in the curve in Figure 1. Moreover, the highest value of expression registered in advanced stages of disease (5 stage, which considered the final step of the disease in which the kidney loses its function permanently and irreversibly) (Table 4).

**Table 4. IGF-1 gene expression with CKD stages**

| **Subjects** | **GFR (mL/min)** | **Mean Ct gene** | **Mean Ct actin** | **Mean Δ Ct test** | **Mean ΔΔ Ct** | **Folding** |
|---|---|---|---|---|---|---|
| **Stage 1** | > 90 | 18.09 | 16.24 | 1.85 | -0.67 | 1.59* |
| **Stage 2** | 60-89 | 17.96 | 16.24 | 1.72 | -0.79 | 1.73* |
| **Stage 3 A** | 45-59 | 17.55 | 16.24 | 1.30 | -1.21 | 2.31* |
| **Stage 3B** | 30-44 | 16.92 | 16.24 | 0.67 | -1.84 | 3.59* |
| **Stage 4** | 15-29 | 16.88 | 16.24 | 0.64 | -1.88 | 3.68* |
| **Stage 5** | >15 | 16.71 | 16.24 | 0.46 | -2.05 | 4.14* |
| **Control** | 118 | 18.76 | 16.24 | 2.52 | 0.00 | 1.00 |

*Significant at p-value <0.05

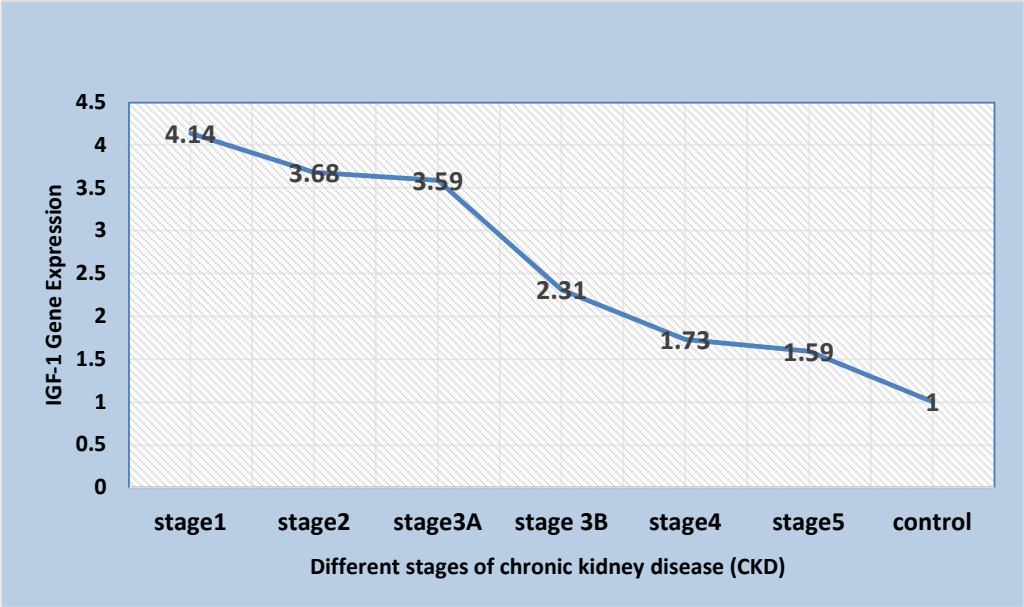
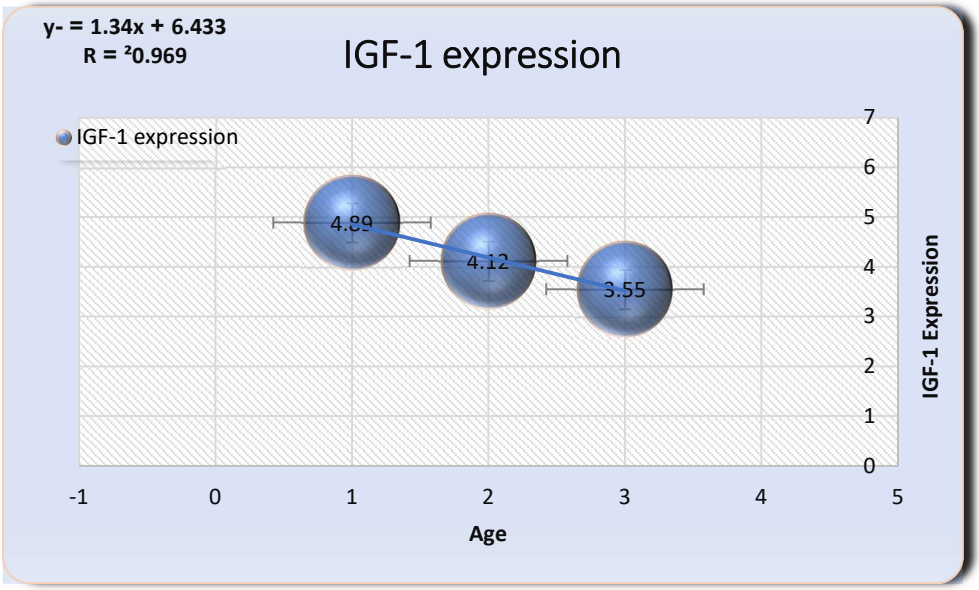



**Figure 1. The curve of IGF-1 gene expression with CKD stages**

**IGF-1 gene expression and age range**

In this study, different age groups were examined to see if variation in age had an effect on IGF-1 folding. Three groups were employed in this study, starting from the youngest < 25, >25-55<, to the oldest group > 55 years old. The results found that IGF-1 gene expression is greatly affected by age, since it was seen an overexpression in all age groups. Furthermore, this increase was highest in the early ages and decreased as patients aged, as follow 4.89, 4.12, 3.55 respectively (Figure 2).



**Discussion**

In current result, IGF-1 expression was found to be abnormal in CKD patients and the expression increased significantly, however, it is still unknown how the IGF-1 contributes to progression this disease. Our findings related to the expression of IGF-1 was in line with previous results which has been found a substantial correlation in upregulated IGF-1 levels and some important considerations like increase the destruction of renal glomeruli in kidney disease (19).

There is a conflict opinion among researchers about IGF-1 gene overexpression in patients with CKD, between its efficacy to enhance renal function to improve glomerular filtration rate, or its role for kidney damage and development of glomerulosclerosis.

As described in several studies, an abnormal increase in IGF-1 levels has been found to be associated with renal hypertrophy in both humans and rodents (20). Transgenic mice that have been created to carry excessively high IGF-1 concentrations are reported to exhibit the giant phenotype as well as increased size of organs, including kidney weight even after adjusting it with respect to total body weight (21). In addition, these animals suffer from glomerulosclerosis and progressive albuminuria as part of a sequence starting from glomerular hypertrophy (22). On the other hand, transgenic mice overexpressing IGF-1 are larger than their wild-type controls (23). Moreover, CKD complications also lead to changes in IGF-1 production. For example, metabolic acidosis raised secretion and expression of hepatic IGF1 mRNA (24). Additionally, GFR is significantly affected by IGF1, and it was also found that transgenic mice overexpressing IGF-1 develop glomerulosclerosis; hence, high levels of IGF1 may have adverse effects on CKD progression (25).

On the contrary, a different investigation suggests that long-term administration of IGF-1 for patients diagnosed with end-stage CKD contributed slightly to improve renal function, raising renal blood flow and GFR. It is also potently anabolic, can improve creatinine clearance, and display minimal lift-off inulin clearance and nitrogen balance, with not expected side effects (26). Treatment with IGF-1 in children and adults with CKD resulted in increases in urinary calcium excretion, whereas serum calcium levels remained constant. These effects are most likely due to IGF-1-induced increased calcitriol synthesis, resulting in enhanced intestinal and renal calcium absorption (27).

Using our genetic expression assay, we measured the levels of IGF-1 in CKD patients and found that males had significantly higher levels compared to females. These findings support previous studies which indicated that serum IGF-1 levels are doubled in males compared to females (28). It has been suggested that, this difference contributes to variations in body weight and skeletal properties between sexes. Notably, this sexual dimorphism is influenced by the somatotropic (GH/IGF-1) axis. Additionally, a separate investigation demonstrated an upregulation of IGF-1 gene expression in placentas associated with male fetuses (29).

Moreover, it was revealed that IGF-1 gene folding increased sequentially along CKD stages; the maximum expression level was seen in patients with an advanced stage of this pathology. At different stages of CKD, changes in IGF-1 expression alter the control of endocrine, paracrine, and autocrine actions within the GH-IGF-IGFBP axis (3). The GH-IGF1 axis causes early residual renal hypertrophy and then gradually leads kidneys to a more advanced stage (30).

Another finding of the present research paper shows that IGF-1 gene expression dramatically reduces with age in older patients, which might further deteriorate renal function during the elderly years (31). Aging is accompanied by a decline in growth hormone secretion as well as decreased plasma IGF-1 levels in humans and rodent models. Specifically, nearly undetectable levels of growth hormone can be found in human subjects older than 60 years (32). The fact that hepatic IGF-1 production is controlled by growth hormone helps explain why IGF-1 levels tend to decrease with age in humans and animal models of human aging (33).

Moreover, higher expression levels of IGF-1 can led to a reduced renal filtration rate, increasing susceptibility to early-stage renal problems (34). By using in situ hybridization studies, it has been found that in the medullary thick ascending limb of Henle's loop, IGF-1 causes a reduction in renal vascular resistance and increases

perfusion of renal glomeruli, resulting in hyperfiltration by the resorption of both water and sodium. Subsequently, this contributes to glomerular hypertrophy followed by soft tissue enlargement (35). Various aspects such as the extracellular domain, matrix creation, and the relationship between structure and function are influenced by changes in IGF-1 gene expression (36).

**Conclusion**

Genetic predisposition has been identified as a key cofactor in order for CKD to develop and progress. IGF-1 has a marked upregulation and can develop CKD. In light of this, we came to the conclusion that identifying this overexpression at the level of a biomarker might help identify those who are at a high risk of developing CKD and identify them for early prevention and treatment. Due to the probability of variation in ethnicity, further studies are needed to confirm our findings.

**Limitations of the study**

1. Sample Size; the study was conducted on a limited sample of 55 CKD patients and 20 controls, which may affect the generalizability of the results.
2. Geographic and ethnic diversity; this study focused on patients from a specific geographic area (Al-Zahraa teaching hospital, Wasit), limiting the applicability to other regions and ethnic groups.
3. Environmental and lifestyle factors; this study did not consider the impact of different environmental and lifestyle factors, such as diet and physical activity, on IGF-1 levels.
4. Other genetic factors; the focus was solely on the IGF-1 gene, without considering other potential genetic contributors to CKD.
5. Cross-sectional design; the cross-sectional nature of the study means data were collected at a single point in time, preventing the determination of causal relationships between IGF-1 expression and CKD progression.

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**Authors' contribution**

Conceptualization:Alyaa Abdulhadi Salih

Data curation:Alyaa Abdulhadi Salih

Formal analysis:Alyaa Abdulhadi Salih

Funding acquisition/: Alyaa Abdulhadi Salih

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Methodology: Alyaa Abdulhadi Salih

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Supervision:Alyaa Abdulhadi Salih

Validation:Alyaa Abdulhadi Salih

Visualization:Alyaa Abdulhadi Salih

Writing–original draft:Alyaa Abdulhadi Salih

Writing–review & editing: Alyaa Abdulhadi Salih

**Ethical statement**

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and obtained ethical approval from the Ethics Committee of Wasit university, college of science (No#24134 in 2/2022). Accordingly, written informed consent was taken from all participants before any intervention.

**Conflicts of interest**

The authors declare that they have no competing interests.

**Funding/support**

None.

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