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RESEARCH ARTICLE

Assessment of New Chemical Markers for Renal Function after Hemodialysis

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ABSTRACT

Chronic kidney disease (CKD) is a broad term that refers to several conditions that impact the structure and function of the kidneys. Disease and management are classified according to stages of disease severity for patients with end-stage renal disease, hemodialysis (HD) becomes a crucial therapeutic intervention to sustain life by filtering waste products and excess fluids from the bloodstream. This study aimed to assess the impact of HD on the levels of activin and Zinc-α2-glycoprotein (ZAG) in patients with chronic renal disease. The study carried out in Kirkuk city from November 2023 to April 2024. The study included 70 patients with CKD undergoing regular HD at Alamal center of dialysis in Kirkuk city, using 40 healthy persons as a comparison group. Pre- and post- HD blood samples, together with samples from the control group, were collected and examined for multiple parameters. The blood samples were taken to assess the levels of serum activin and ZAG using Enzyme-Linked Immunosorbent Assay. The study shows that activin level was highly significant increase ($p < 0.01$) in HD patients before dialysis as compared to control group. The mean of activin level in HD patients before dialysis was 3.104±1.172 ng/ml, while in the control group, it was 1.745±0.068 ng/ml. Similarly, ZAG concentration was highly significant increase($P<0.01$) in HD patients before dialysis compared to control group, The mean of ZAG level in HD patients before dialysis was 312.761±67.85 ng/ml, while in the control group, it was 152.03±25.53 ng/ml. The study observed a rise in serum activin and ZAG levels after HD. Specifically, the average serum activin level before HD increased from 3.104±1.172 ng/ml to 3.670±1.255 ng/ml after HD, while the average serum ZAG level before HD increased from 312.761±67.85 ng/ml to 356.003±42.59 ng/ml after HD. The study found that HD patients had elevated levels of biomarkers. Elevated activin levels suggest HD may contribute to chronic inflammatory state, and increased ZAG levels may be a compensatory response to CKD and dialysis-related inflammation.

INTRODUCTION

Chronic kidney disease (CKD) is a broad term used to describe a diverse ailment that impacts the structure and function of the kidney (Padberg et al., 2014). The diversity in disease manifestation is partially linked to etiology, pathogenesis, intensity, and pace of advancement(Sterling et al., 2012). Since the inception of the conceptual framework, definition, and classification of chronic renal disease 10 years ago(Charles and Ferris, 2020), (CKD) is characterized by the presence of kidney damage, specifically albuminuria, or impaired kidney function, known as glomerular filtration rate (GFR), below 60 mL/min per 1·73 m^2 for a period of at least 3 months, regardless of clinical

diagnosis(Schwartz and Furth, 2007). The condition is classified into five phases based on the Glomerular Filtration Rate (GFR) due to its substantial influence on the development of problems.

Hemodialysis (HD) is the most prevalent method of kidney replacement therapy (KRT) globally, making up over 69% of all KRT and 89% of all dialysis procedures(Kalantar-Zadeh et al., 2014; Thurlow et al., 2021). The main objective of HD is to reestablish the normal fluid environment found in intracellular and extracellular, which is typically seen in healthy kidney function(Eriksen et al., 2010). This is achieved by transferring substances like urea from the blood to the dialysate, and substances like bicarbonate from the dialysate to the blood(Murdeshwar and Anjum, 2024). The rate of diffusion is primarily influenced by the concentration and molecular weight of the solutes. Activins are a type of dimeric polypeptides that consist of two subunits of inhibin connected by a disulfide bridge(Wang et al., 2016). Activins are a type of growth and differentiation factors that are part of the transforming growth factor (TGF-) superfamily. There are five different types of subunits that have been identified, and the naming of activin is based on the specific type or types of subunits that make it up. Mammals have isolated activin A (A/A), activin B (B/B), activin AB (A/B), activin C (C/C), and activin E (E/E). However, only the dimers activin A, activin B, and activin AB have clearly defined biological action. Act A has a crucial role in regulating the correct development of the kidney, and its expression is easily recognized in growing fetal kidneys. Expression of this factor is absent in healthy adult kidneys.

Zinc-alpha-2-glycoprotein (ZAG) is a glycoprotein with a molecular weight of 41-43 kDa. It is recognized for its ability to promote the breakdown of fats by activating adenylate cyclase through a mechanism that depends on guanosine triphosphate. This activation occurs by binding to the b3 adrenoreceptor. ZAG is found in various types of epithelial tissues and is released into multiple bodily fluids. ZAG is present at elevated levels in individuals with CKD and those with end-stage renal disease. The elevated concentration of ZAG in the serum of this population is believed to be caused by a decrease in the pace at which the kidneys filter blood and/or a decrease in the breakdown of ZAG by the kidneys.

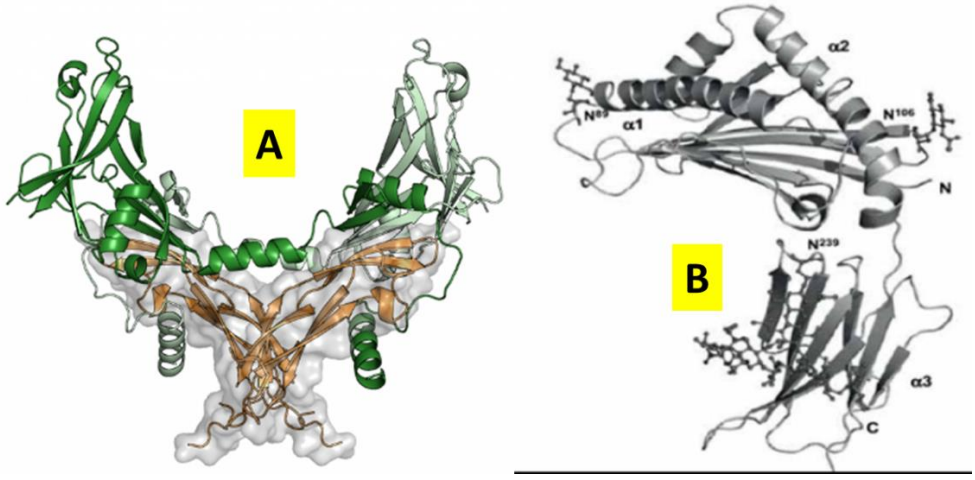

Figure 1: A representative images for the structure of (B) activin (Wang et al., 2016) and (A) ZAG (Hassan et al., 2008).

MATERIAL AND METHODS

Study Design: The cross-sectional study included 180 sample, 70 patients with CKD and 40 controls. The first group represented as cases group consisting of 70 patients with CKD who underwent regular HD. The second group comprised a control group consisting of 40 healthy subjects.

Ethical Approval: The research protocol obtained official authority from the Scientific Committee of the Faculty of Medicine at Tikrit University, which had previously granted approval for the approach. The Kirkuk Health Department authorized the gathering of patient samples.

Exclusion Criteria: Patients with diabetes mellitus, obesity, cancer, hepatitis, heart failure, and neuromuscular disease

Sample collection: Five ml of blood sample was collected from control groups and patients before and after HD process in plane tube then let stand for about (20–30) minute to clot formation and centrifuged by using macrocentrifuge for about (15) minutes on speed of 3000 rpm then fresh non hemolysis serum collected in epindroof tubes and kept in deep freeze (-20C^{o}) Thawing of the samples allowed to take place at 25˚C for 1 hour before conducting the assay for more accurate test results to be obtained

Statistical analysis: The study utilized SPSS v29 and Prism GraphPad for computerized statistical analysis, with one-way ANOVA T-Test probability (P value) for comparison. A P value < 0.05 was considered statistically significant, while a P value > 0.05 was non-significant. The correlation coefficient was interpreted as negative or positive.

RESULTS AND DISCUSSION

The serum activin before dialysis was 3.104±1.172 ng/ml, whereas after dialysis, it increased to 3.670±1.255ng/ml, the difference in activin levels before and after dialysis is statistically significant at (P-value:<0.001), The mean of control group was 1.745±0.068ng/ml, which is statistically significant than both the before and after HD groups at (P-value:<0.001) (Table 1).

The serum ZAG before dialysis was 312.761±67.85ng/ml, whereas after dialysis, it increased to 356.003±42.59ng/ml, the difference in serum ZAG levels before and after dialysis is statistically significant at (P-value:<0.01), The mean of control group was 152.03±25.53ng/ml, which is statistically significant than both the before and after HD groups at (P-value:<0.01) (Table 1).

Table 1: Comparison serum level of activin and ZAG in patients with CKD in the studied groups

Measured parameters	HD group (n=70)		Control group (n=40)
	Before	After	
activin (ng/ml)	3.104±1.172*	3.670±1.255*^	1.745±0.068
ZAG (ng/ml)	312.761±67.85*	356.003±42.59*^	152.03±25.53
Data expressed as mean±SD, *^ indicate significant difference at p value less than 0.05, * as compared to control group, ^ as compared to before diualysis			

In previous study found that the activin level increased after dialysis, The study revealed that the activin A/follistatin system is both activated and disrupted in chronic HD patients, leading to a decrease in the amount of activin A stored in tissues(Borawski et al., 2003). The type and dosage of heparin used during HD operations have a significant impact on this multifaceted system, and can thus modify essential bodily functions and the progression of severe illnesses(Gozdzikiewicz et al., 2009). The elevation of circulating activin A is caused by its displacement from the proteoglycans on the cell surface and the subsequent disintegration of the complex(Heawchaiyaphum et al., 2018).

Another study discovered that the amount of activin significantly increases after HD(Borawski et al., 2003; Nordholm et al., 2023; Yonata et al., 2020). The researchers suggest that this occurrence is likely caused by the displacement of cell surface proteoglycans and the disintegration of the complex(Hesse et al., 2020; Padberg et al., 2014). Additionally, it has a crucial function in regulating the immune system by controlling the production of other proinflammatory cytokines(Lonnemann et al., 1990; Pertosa et al., 2000; Rios et al., 2017). Consequently, inhibiting its effects may have therapeutic potential in treating inflammatory disorders. Eliminating the use of heparin during

dialysis effectively inhibited the activation of activin(Gozdzikiewicz et al., 2009; Rydzewska-Rosołowska et al., 2009). Enoxaparin facilitated the release and maintained stable levels of the substance(Green et al., 2017; Pon et al., 2014).

A prior study reported that the average concentration of serum ZAG was 125±65 μg/ml before the HD session and 145±43 μg/ml after the session (+18%, P<0.05). The study indicates that the plasma used for HD patients resulted in a comparable rise in ZAG content in fat cells. This suggests that the component responsible for stimulation was not eliminated throughout the HD procedure. Conversely, plasma obtained from patients with neurodegenerative diseases did not cause any alteration in the concentration of ZAG(Chang et al., 2024; Heawchaiyaphum et al., 2018; Kheirouri et al., 2019). Protein-bound toxins are not effectively eliminated by traditional HD methods, even when using dialysis membranes with large pores, due to their great affinity for proteins(Niwa, 2013). Unlike HD, which is an intermittent dialysis method, peritoneal dialysis involves the gradual and continuous elimination of solutes(Bersenas, 2011; Mehrotra et al., 2016). Peritoneal dialysis is additionally linked to a decrease in albumin in the peritoneum and an enhanced preservation of the remaining kidney function(Andreoli and Totoli, 2020; Krediet and Struijk, 2013). While the clearance of protein-bound solutes was higher in HD patients compared to peritoneal dialysis patients, it is paradoxical that the plasma concentration of these solutes was lower in peritoneal dialysis patients than in HD patients(Asano et al., 2019; Bammens et al., 2003; Sirich et al., 2014).

In addition, another study discovered that patients with HD demonstrated a subsequent elevation in plasma ZAG concentration following the dialysis session. The average plasma ZAG concentration was 132.3 (113.3–159.2) μg/ml before the haemodialysis session and 196.1 (168.8–217.9) μg/ml after the session, representing a 48% increase(Pelletier et al., 2014). This difference was statistically significant with a p-value of 0.002. The study found that the level of plasma ZAG increased during HD, regardless of changes in blood volume. This indicates that additional ZAG is secreted within the body during the process of HD. ZAG release may increase in persons with HD due to the presence of inflammation, shear stress, oxidative stress, catecholamine generation, or lack of biocompatibility(Romauch, 2020, p. 3). There was a significant increase in plasma ZAG concentration in patients who underwent HD compared to patients who underwent peritoneal dialysis (P=0.004). This discovery suggests that the increase in circulating ZAG during the HD procedure is linked to factors that are not present in peritoneal dialysis(Bouchara et al., 2018). Studies have demonstrated that glucocorticoids increase the release of ZAG. Studies have shown that HD sessions can result in elevated cortisol levels(Hassan et al., 2008; Russell and Tisdale, 2005; Wei et al., 2019). These findings indicate that glucocorticoids may enhance the formation of ZAG and have a role in the increase of ZAG levels in the blood during dialysis(Zhang et al., 2020). Conversely, it has been shown that inflammatory cytokines such as TNF-alpha decrease the secretion of ZAG(Mracek et al., 2010).

CONCLUSION

The study found that HD patients had elevated levels of activin and ZAG, suggesting potential involvement in inflammation, metabolic processes, and renal function. Elevated serum activin levels, associated with inflammatory responses and tissue damage, suggest that HD may influence the inflammatory milieu in CKD patients, potentially contributing to the chronic inflammatory state observed in this population. Increased levels of ZAG were found in HD patients compared to the control group, suggesting an attempt by the body to counteract inflammation and improve lipid metabolism in response to CKD and regular dialysis sessions. These findings contribute to understanding the pathophysiology of CKD and emphasize the importance of monitoring activin and ZAG levels in comprehensive patient care.

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