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Correlation Between the 8-hydroxy-2'-deoxyguanosine Level in the Peritoneal Solution of Patients Undergoing Peritoneal Dialysis and the Peritoneal Equilibration Test, Kt/V, Ferritin, and Albumin Levels

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Original Paper

Introduction. Peritoneal dialysis (PD) is an effective treatment modality for advanced kidney failure, offering patients a significant degree of independence. However, the long-term use of PD is limited due to the degeneration of the peritoneal membrane, resulting in reduced dialysis adequacy. Evaluating the peritoneal membrane condition in patients with advanced kidney failure who are undergoing PD is challenging with existing methods. Therefore, this study aimed to investigate the correlation between 8-hydroxy-2'-deoxyguanosine (8OHDG) levels in the peritoneal solution of patients undergoing PD and various factors, such as peritoneal equilibration test (PET), dialysis adequacy (Kt/V), underlying diseases, serum ferritin, and albumin levels. 8OHDG is a sensitive marker of oxidative stress caused by DNA damage.
Methods. A total of 56 patients were included in this cross-sectional study. Five milliliters of PD fluid were collected from the patients, and 8-OHdG levels were measured using ELISA method. Then, they were compared with PET, Kt/V, albumin, and ferritin markers in the patients' files, and the results were analyzed by statistical tests.
Results. The study examined the correlation between 8OHDG and other markers. It was found that this index had significant associations with PET and underlying HTN ($P < .05$), whereas no significant associations were identified with the other markers.
Conclusion. The results of the present study demonstrate that the level of 8OHDG, as one of the oxidative stress markers, could be used to evaluate the function of the peritoneum in patients undergoing PD.

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INTRODUCTION

Chronic kidney disease (CKD) is a condition caused by progressive and irreversible kidney damage, which leads to the body's inability to maintain proper metabolism, water, and electrolyte balance, resulting in uremia. In recent years, the number of patients with end-stage kidney disease (ESKD) undergoing hemodialysis has been increasing worldwide, including Iran, and the mortality rate associated with this disease is significant and on the rise globally.[1-3] The etiology of chronic kidney disease across different countries varies, affected by the factors such as the prevalence of the disease in the region, lifestyle, familial

predisposition, sex, racial background, and the availability of health facilities for the diagnosis and treatment of kidney diseases.[4-6] The disease is classified according to the glomerular filtration rate into five stages. Stage five is the terminal phase of kidney failure referred to as ESKD, which leads to uremic syndrome and ultimately death in the absence of dialysis or transplantation.[7]

Peritoneal dialysis (PD) is a kind of dialysis that uses the peritoneal membrane to exchange liquids and solutes with the blood.[8] This method removes excess fluid and uremic toxins, and corrects electrolyte disorders in patients with kidney failure. PD is a successful method of treating and controlling patients with ESKD and is also a suitable method as the first therapeutic choice.[9] However, over time, it results in destruction of the peritoneal membrane, characterized by a progressive increase in the peritoneal membrane thickness, especially in the submucosal collagen layer. Thus, this increase in thickness results in reduced filtration.[10]

One of PD's most important long-term complications is encapsulated peritoneal sclerosis, characterized by an increased fibrogenic response in peritoneal tissue.[11,12] Usually, the peritoneal solute transfer rate is measured as a criterion in estimating peritoneal damage by peritoneal equilibration test or PET (a semiquantitative assessment of peritoneal membrane transport function in PD patients). However, this test is invasive and time-consuming, and requires blood sample preparation. Therefore, accurately determining the extent of peritoneal damage by noninvasive techniques is of utmost importance.[13] Another method used to evaluate the adequacy of PD and its degradation is measuring the Kt/V index (a well-known index of removal adequacy per dialysis session). However, the flaws of this method include the complexity and tediousness of the calculations, as well as its results, which are difficult to understand.[14,15]

Serum ferritin level and iron saturation ratio are the most common markers of iron status in dialysis patients.[16] However, serum ferritin is considered an acute phase reactant and increases in inflammatory conditions.[17,18] 8OHdG is a product of guanine oxidation by reactive oxygen species, which occurs during oxidative damage of DNA and is a highly effective and sensitive marker for detecting oxidative stress as well.[19,20] Considering that it has been shown in previous publications that dialysis can lead to oxidative stress and inflammation,[21-24] this study aimed to investigate the correlation between 8OHDG levels in the peritoneal solution of PD patients and peritoneal equilibration test (PET), dialysis adequacy (Kt/V), underlying diseases, ferritin, and albumin levels.

MATERIALS AND METHODS

Study Population

Fifty-six PD patients referred to the PD centers of Hajar Hospital in Chaharmahal and Bakhtiari province, as well as Alzahra Hospital in Isfahan province, Iran; were included in this cross-sectional study. Inclusion criteria were PD cases and willingness to participate in the study. Factors that disrupt inflammatory and oxidative markers were also excluded. In this case, patients undergoing anti-inflammatory agents were not included in the study.

Data Collection and Experimental Analysis

At the beginning of the study, all eligible patients were provided with the essential information regarding the long-term complications of PD and the necessity of their evaluation. In addition, they received instructions on how to conduct experiments and testing as well as the procedure. Five ml of PD fluid was taken from the returned dialysis fluid and before re-dialysis from all the study participants, as the tested sample. All samples were stored at -70°C and were transferred to the laboratory at the same time. 8OHDG level was measured using a human enzyme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm CO., LTD.; Hangzhou, China; CK-E90285), according to the manufacturer's instructions. Other clinical and biochemical variables, including PET, Kt/V values, albumin, and ferritin levels, were collected from the patients' files. After measuring the mentioned markers, the data were compared. The assumed relationship was evaluated based on increased oxidative markers during dialysis.

Ethical Considerations

All steps of the study and its conditions were explained to the participants, and informed consent was taken from them. Codes identified medical records and information for all individuals. Thus, no information was disclosed, and the patients'

history and related information were preserved. Indeed, the study participants were assured that their information would be reported generally and used for research purposes without sharing it with anyone else. Human rights were also respected according to The Declaration of Helsinki (1975), revised in 1983. The Ethics Committee of Shahrekord University of Medical Sciences (Code No. IR.SKUMS.REC.1398.029) approved this study.

Statistical Analysis

Quantitative data were described as mean and standard deviation, and qualitative data as frequency and percentage. To check the normality of the distributions, the Kolmogorov-Smirnov test was used. Statistical analyses were performed using Kruskal-Wallis, Mann-Whitney, and Spearman tests. SPSS-23 software was used for statistical analysis, and a P value < .05 was considered statistically significant.

RESULTS

Demographic Characteristics of Participants

In this study, 24 female (42.9%) and 32 male participants (57.1%) with a mean age of 58.02 ± 14.29 years were included. The participants had five primary underlying diseases: hypertension (29 patients), diabetes mellitus (23 patients), trauma (one patient), pyelonephritis (one patient), and Systemic Lupus Erythematosus (one patient). The mean level of 8OHDG in the peritoneal solution of patients was 194.65 ± 40.36 ng/mL. The mean dialysis duration in these patients was 44.55 ± 36.963 months (Table 1). The average range of PD adequacy (Kt/V) was 2.05 ± 0.87 mmol/L. Meanwhile, the PET, its division, and the number of cases is shown in Table 2. The albumin and ferritin levels were 248.82 ± 202.69 g/L and 3.43 ± 0.56 ng/mL, respectively (Table 1).

Table 1. Details of the Study Participants

Variable	Mean ± SD
Age, y	58.02 ± 14.29
Duration of Dialysis, mo	44.55 ± 36.96
Dialysis adequacy, mmol/L	2.05 ± 0.87
Albumin level, g/dL	3.43 ± 0.56
Ferritin level, ng/mL	248.82 ± 202.69
8OHDG level, ng/mL	194.65 ± 40.36

Abbreviations: 8OHDG, 8-Hydroxy-2'-deoxyguanosine

Table 2. The Peritoneal Equilibration Test (PET) includes the Number of Patients and How to Divide it

Peritoneal Equilibration Test (unit)	D/P Creatinine	Number of Patients
Low	< 0.62	3
Low average	0.62 to 0.72	13
High average	0.72 to 0.82	32
High	> 0.82	8

Abbreviations: D/P, dialysate to plasma solute concentration

Relationship Between 8OHDG Levels and PET, Kt/V, Ferritin, and Albumin Levels

Evaluation of the correlation between 8OHDG level and other markers according to Spearman correlation and Kruskal-Wallis tests demonstrated a statistically significant relationship of this index only with PET results (P = .03) (Table 3) and no significant relationship was observed with other markers (P > .05). Also, only the results of PET showed a statistically significant negative relationship with serum albumin levels in PD patients (r = -0.31) (P = .02). However, the measurement results of other markers did not show any statistically significant relationship with each other (P > .05) (Table 5).

Association of 8OHDG Level with Underlying Causes and Other Characteristics of the Participants

The findings of this study showed that there was a relationship between hypertension (HTN) and 8OHDG level, while no relationship was observed

Table 3. Correlation Between 8OHDG Marker and Other Factors Including Sex, PTE, and Underlying Causes

Variable	Mean of 8OHDG	*P*
Sex		
Male	191,67	.52
Female	198,62	
Peritoneal Equilibration Test (unit)		
Low	169,35	.03
Low Average	195,75	
High Average	201,31	
High	221,50	
The Underlying Causes		
HTN		
Positive	205,43	.02
Negative	183,07	
DM		
Positive	198,55	.69
Negative	191,93	

Abbbreviations: HTN, hypertension; DM, diabetes mellitus

Table 4. Correlation Analysis of Age, Dialysis Duration, Dialysis Adequacy (Kt/V), Albumin, and Ferritin Levels with 8OHDG Markers

Variable	*r*	*P*
Age, y	-0.08	.55
Duration of Dialysis, mo	-0.04	.76
Dialysis Adequacy, (Kt/V), mmol/L	0.05	.65
Albumin Level, g/L	0.03	.82
Ferritin Level, ng/mL	-0.07	.60

r: correlation coefficient

between 8OHDG level and diabetes mellitus (DM) (Table 3). Although the mean levels of 8OHDG in female and male participants were 198.62 ± 47.45 ng/mL and 191.67 ± 34.61 ng/mL, respectively; this difference was not statistically significant (Table 3). However, no significant relationship was observed between this factor and dialysis duration. It should be noted that there was no significant relationship between age and 8OHDG level (Table 3).

DISCUSSION

Relationship Between PET and 8OHDG Level

The main finding of this study revealed a significant association between the amount of 8OHDG, an inflammatory and oxidative component, in patients undergoing PD and the PET results. Therefore, the 8OHDG level increases with an increased dialysis/plasma creatinine (D/P creatinine) ratio in PET examination and progression of peritoneal ultrafiltration failure. These findings indicate that evaluating 8OHDG levels in dialysis fluid can serve as a reliable method for evaluating peritoneal damage. In this regard, in 2012, Morishita *et al.*, in Tochigi, Japan, conducted a study on the level of 8OHDG in PD as a marker of peritoneal injury. They evaluated 28 patients undergoing PD for 8OHDG levels, PET, and matrix metalloproteinase-2 (MMP-2). Their data demonstrated a significant correlation between 8OHDG levels and PET and MMP-2 levels. Thus, it could be used as an effective marker in assessing peritoneal injury during dialysis.[25] Our data matches their findings.

8OHDG is one of the markers of oxidative stress derived from DNA structure. Numerous studies have proposed this marker as a practical indicator for assessing oxidative stress induced by free radicals in ESKD patients.[24,26] The mechanisms related to the altered structure and function of the peritoneum have not yet been fully determined. Still, investigations have suggested the presence of oxidant compounds as an effective factor in peritoneal damage.[27-29] Noh *et al.*, 2006, reported limited amounts of transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF), and type 1 collagen in peritoneal dialysis fluid by application of antioxidants.[27,30] Kihm *et al.*, 2011, reported an increase in TGF-β1, alpha-smooth muscle actin, VEGF, and peritoneal angiogenesis were reduced using antioxidant compounds.[28] Gotloib *et al.*, 2004, demonstrated the fundamental role of oxidative stress in developing peritoneal fibrosis and sclerosis characterized by peritoneal adhesions, wrapping of intestinal loops and the formation of a fibrous tissue layer in the animal model of PD.[29] Oberacker *et al.*, 2022, showed that PD patients had elevated thioredoxin-interacting protein (TXNIP) levels compared to healthy controls. They demonstrated that PD patients had reduced thioredoxin (TRX) activity, thereby increasing oxidative DNA damage.[30] These results indicated the importance of oxidative stress in inflammation, fibrosis, revascularization, and sclerosis in PD. Thus, measurement of 8OHDG as a marker of oxidative stress might be beneficial in evaluating the function of peritoneal membrane in individuals undergoing PD. Generally, the relationship between 8OHDG level and PET as a semi-quantitative test to evaluate peritoneal membrane function indicates that 8OHDG measurement is as effective as PET in evaluating peritoneal membrane function.

The Relationship Between HTN and 8OHDG Level

Another important finding of this study was a significant correlation between 8OHDG levels and HTN. This revealed higher levels of this marker in hypertensive individuals. This data could imply a relationship between oxidative stress and HTN. Although a more detailed investigation of such a relationship is required in the future, this relationship has been discussed previously in some cases. Pouvreau *et al.* demonstrated that 8-iso-PGF2α and erythrocyte GSH may be clinically useful for assessing HTN and T2DM as a comorbidity, while significant changes in the inflammatory profile were also observed with HTN progression.[31]

The Relationship Between Other Factors and 8OHDG Level

The absence of correlation between the 8OHDG

level and the dialysis adequacy index (Kt/V) does not contradict its correlation with PET. These tests provide two different concepts that are used in the evaluation of PD adequacy. To the best of our knowledge, this study is the first one to compare the 8OHDG values with Kt/V results, and further research is necessary. Regarding ferritin, the results of previous studies have demonstrated no relationship between the level of this substance and oxidative stress in ESKD patients treated with erythropoietin. Besides, no significant correlation was observed between serum ferritin and the 8OHDG levels.[32,33] Our data also appears in the same line. Serum albumin level is effective in predicting the mortality rate in dialysis patients. It is used to evaluate the health status and quality of care for these patients. It has been demonstrated that serum albumin level predicts mortality in dialysis patients and is used to assess their health status and the quality of delivered care. However, the threshold for increased mortality varies according to the dialysis method.[34] Our results also indicate this relationship and suggest that there is a significant negative relationship between the albumin level and the D/P ratio in PET, as well as ultrafiltration failure of the peritoneal membrane. Previous studies show serum 8OHDG and albumin levels were not significantly correlated. Accordingly, the increase in 8OHDG levels in dialysis patients does not depend on nutritional status and micro-inflammation.[24,35] Our findings, however, are inconsistent with the studies mentioned above.

Limitations of the Study

Despite our efforts to collect samples from two regional hospitals to achieve an adequate sample size, the limited number of peritoneal dialysis patients in Chaharmahal and Bakhtiari and Isfahan provinces posed a limitation on the number of samples available for the current investigation. Nevertheless, there has been a rise in the number of participants in comparison to the study conducted by Morishita *et al*, in 2012.

CONCLUSION

The present study demonstrates the practical utility of 8OHDG, an oxidative stress marker, in assessing peritoneal membrane function in patients receiving peritoneal dialysis. Since not all aspects of using this marker are known, additional investigation is necessary to fully understand its potential. Also, this study demonstrated a significant correlation between 8OHDG level and HTN, as a higher level of this marker was observed in this group of patients. This finding demonstrates the correlation between inflammation and the development of HTN. It is recommended to conduct additional studies with larger sample sizes to investigate alternative markers of inflammation and oxidation that contribute to peritoneal damage and to explore the relationship between these markers and 8OHDG levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ETHICAL CONSIDERATION

The Ethics Committee of Shahre-kord University of Medical Sciences approved this study (Code No. IR.SKUMS.REC.1398.029). The informed consent to use the patient's information for research was taken from patients, at the time of admission. The information was recorded anonymously and by coding the patients.

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