URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE LEVELS IN CANCER PATIENTS TREATED WITH CISPLATIN

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ABSTRACT

Objective: *Cisplatin is a widely used chemotherapeutic agent. The most important side effect due of cisplatin is nephrotoxicity. N-acetyl-beta-D-glucosaminidase (NAG), is a lysosomal enzyme that has low urine levels in healthy individuals. The urine NAG excretion increases in renal disorders. The increases of serum NAG levels in cases like diabetes mellitus, proteinuria due to the renal involvement of systemic lupus erythematosus, some nephrotoxic medications like cisplatin, are demonstrated in several studies. In this study, we aimed to evaluate the acute nephrotoxicity due to cisplatin.*

Material and Method: *Thirty patients were included in the study. The urine NAG levels are measured 24 hours before and 24 hours after the administration of 75 mg/m^2 cisplatin in the 24 hours urine sample. The serum urea, creatinine levels are also measured before and after the medication.*

Results: *The median age of the study group was 54 (26-75). The median administrated cisplatin dosage was 130.13±8.01 mg. The urine NAG excretion was 6.98±4.49 U/L before the treatment and 10.09±5.50 U/L after the treatment (p=0.001). The blood urea level was 28.33±9.48 mg/dl before the treatment and 30.03±7.19 mg/dl after the treatment (p=0.107). The blood creatinine level was 0.75±0.27 mg/dl before the treatment and 0.78±0.29 mg/dl after the treatment (p=0.229). After therapy, there was no significant correlation between the total dosage of cisplatin and NAG level (r=-0.048, p=0.80) and creatinine level (r=-0.218, p=0.23), and also after therapy, there was no significant correlation between NAG and creatinine levels (r=0.301, p=0.10).*

Conclusion: *For the evaluation of acute nephrotoxicity of cisplatin we suggest, to use the measurement of 24 hours urine NAG excretion, as a non invasive test.*

Key Words: *Acetylglucosaminidase, urine, cisplatin, toxicity* ***Nobel Med 2013; 9(1): 56-60***

SİSPLATİN İLE TEDAVİ EDİLEN KANSERLİ HASTALARDA İDRARDA N-ASETİL BETA-GLUKOZAMİNİDAZ DÜZEYLERİ

ÖZET

Amaç: Sisplatin sıklıkla kullanılan bir kemoterapotik bir ajandır. Sisplatine bağlı en önemli yan etki nefrotoksisitedir.N-asetil β-D glukozaminidaz (NAG), lizozomal bir enzimdir ve sağlıklı insanlardaki düzeyi düşüktür. İdrar NAG düzeyi böbrek patolojilerinde artar. Çalışmalarda diabetes mellitus, sistemik lupus eritomatozusa bağlı proteinüride, sisplatin gibi nefrotoksik ilaç uygulamalarında serum NAG düzeyinin arttığı gösterilmiştir. Bu çalışmada sisplatine bağlı akut nefrotoksisiteyi değerlendirmeyi amaçladık.

Materyal ve Metod: Çalışmaya 30 hasta alındı. İdrar NAG düzeyi sisplatin öncesi ve 75 mg/m^2 sisplatin verildikten 24 saat sonra 24 saatlik idrarda ölçüldü. Birlikte serum üre, kreatinin değerleri de tedaviden önce ve sonra ölçüldü.

Bulgular: Hastaların medyan yaşı 54 (26-75) idi. Verilen sisplatin dozu ortalama 130,13±8,01 mgr'dı. Tedavi öncesi idrar NAG düzeyi 6,8±4,49 U/L ve tedavi sonrası idrar NAG düzeyi 10,09±5,50 U/L idi (p=0,001). Tedavi öncesi üre 28,33±9,48 mg/dl ve tedavi sonrası 30,03±7,19 mg/dl idi (p=0,107). Tedavi öncesi kreatinin düzeyi 0,75±0,27 mg/dl ve tedavi sonrası plazma kreatinin düzeyi 0,78±0,29 mg/dl idi (p=0,229).

Sisplatin total dozları ile tedavi sonrası dönemdeki NAG düzeyi (r=-0,048, p=0,80) ve kreatinin (r=-0,218, p=0,23) düzeyleri arasında ve tedavi sonrası dönemde NAG düzeyi ile kreatinin arasında da anlamlı bir korelasyon yoktu (r=0,301, p=0,10).

Sonuç: Sisplatinin akut nefrotoksisitesini değerlendirmek için biz noninvaziv bir test olan 24 saatlik idrarda NAG seviyelerinin tetkikini öneriyoruz.

Anahtar Kelimeler: Asetil glukozaminidaz, idrar, sisplatin, toksisite **Nobel Med 2013; 9(1): 56-60**

INTRODUCTION

Cisplatin is an antineoplastic alkylating agent and is used to treat various forms of cancer including lung, ovarian, germ cell and gastric cancer. Although it has been used for curative aims in various forms of malignancies, the dosage has significant limitations because of its adverse effects on kidney and nerves.[1-3] Cisplatin is a molecule formed as the encirclement of platinum atom by chloride and ammonium.

The chloride interchanges with the water inside the cells and forms highly reactive platinum complexes.[1,3] These complexes directly bind to DNA and cause apoptosis in lower doses, while in higher doses cause necrosis in proximal tubules.[3,4] Following intravenous application, the half-life of the drug and its metabolites are 30 minutes and 5 days respectively. The drug accumulates in proximal tubules of kidney about 5 fold increased than the serum level.[4-10] Therefore, increased N-acetyl-beta-D-glucosaminidase (NAG) and Beta-2 microglobulin levels indicates proximal tubular damage.[11-18]

NAG is a lysosomal enzyme with a molecular weight of 130,000 dalton and under physiological conditions it exists in the urine with very low levels.[19-22] It is found in proximal tubules, and its urinary excretion increases in following situations; exposure to nephrotoxic agents such as chemotherapeutical drugs and antibiotics including vancomycin and amikacin, deformation of the entire proximal tubules or the lysosomes, in the diseases such as diabetes mellitus or glomerulonephritis.
There are tubular proteins such as N-acetyl-beta-D-glucosaminidase and beta-2 microglobulin, urinary excretions of which are increased in comparison with healthy subjects in case of renal injuries as a result of exposure of kidneys to chemotherapeutic drugs (such as cisplatin) and some antibiotics, presence of diseases such as glomerulopathies or diabetes mellitus.[12-14,23-37] These proteins are divided into two groups as low and high molecular weight proteins. The amount of them increases, as the kidney exposes to the toxic effects.[19-22] In proximal renal tubules, there are organelles containing acid hydrolases called as lysosomes. When the cells are exposed to toxic effects, urinary enzymes such as NAG increase if these effects disturb the integrity of the cell or the lysosomes. Although this enzyme exists in high amounts in the blood, due to its high molecular weight (130,000 Dalton) it does not passes through glomerulus, therefore its existence in the urine has been thought to be a marker of early proximal tubular damage.[20,22]

It has been reported that the beginning of the increase in the urinary excretion varies between 48th hours and 7th days, however, the exact time has not been established yet. Since the half- life of cisplatin is 30 minutes and approximately 50-67% of the drug is detected in the urine after 24 hours of medication,[2,6-10] we decided to measure the urinary NAG levels at 24 hours after the treatment. →

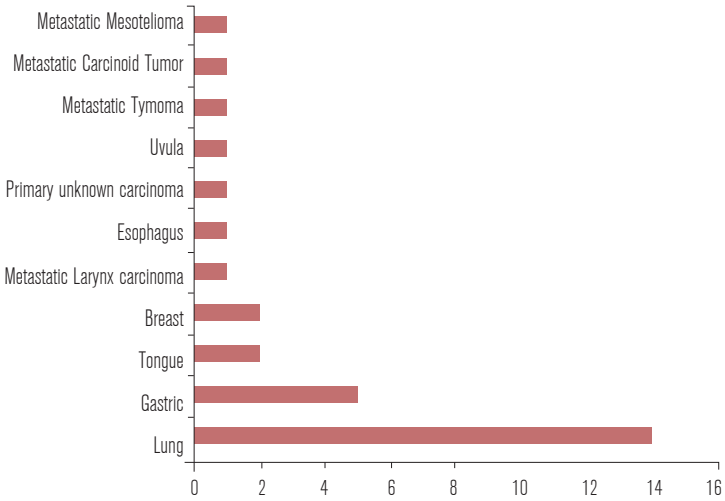

Figure 1: Distribution of cancer types

Table 1: The biochemical data of the patients

	Mean±SD	Normal Ranges
Na^{++}	136.56±4.50 mmol/L	136-144 mmol/L
K^{+}	4.02±0.64 mmol/L	3.6-5.1 mmol/L
Total Bilirubin	0.77±0.45 mg/dl	0.3-1.2 mg/dl
Direct Bilirubin	0.32±0.23 mg/dl	0.1-0.5 mg/dl
Total protein	7.67±0.43 gr/dl	3.69±0.46 gr/dl
Albumin	3.69±0.46 gr/dl	3.5-5.4 gr/dl
AST	20.80±8.33 IU/L	5-40 IU/L
ALT	22.83±11.16 IU/L	7-40 IU/L

Table 2: Pre and post-treatment urinary NAG, blood urea nitrogen (BUN) and creatinine levels

	Before Cisplatin	After Cisplatin*[a]	p
NAG	7.13±4.50	9.91±5.49	0.001
UREA	28.29±9.32 mg	24.44±12.11 mg	0.107
CREATININ (mg/dl)	0.68±0.28 mg	0.77±0.29 mg	0.229

*: The cisplatin is administrated in 75 mg/m^2 dosage to all of the patients; a: The mean dose of cisplatin is 130.13±8.01 mgr

MATERIAL and METHOD

Thirty consecutive cancer patients, admitted to oncology clinic of Adana Numune Training and Research Hospital and given medication including cisplatin with a 75 mg/m^2 dosage between 1st April and 31st October of 2007, were included in the study. Patients with known kidney diseases were excluded from the study. Patients were taken into study on their own permission. Written informed consent was signed by all of the patients included in the study.

Cisplatin dosage was calculated according to the body surface area. Twenty-four hours urine collection was carried out before and 24 hours after the cisplatin administration. The urine NAG excretion, the serum creatinine BUN and the other biochemical parameters were detected. The NAG was measured by spectrophotometry using Cobasmire device. Blood samples were collected after 10-12 hours fasting, blood glucose, hemoglobin, hematocrit, platelet, bilirubine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), sodium (Na), potassium (K), BUN (blood urea nitrogen), creatinine levels were analyzed. 24 hours after cisplatin therapy, the serum creatinine and BUN levels were reanalyzed. Biochemical measurements were performed by Cobas Integra autoanalyzer.

Before starting cisplatin treatment, patients were hydrated by 1000 ml 0.9% sodium chloride (NaCl) infusion in 90 minutes. Cisplatin was given in 500 cc NaCl during 120 minutes. Twenty for hours exactly after drug administration, patients started to collect 24 hour s urine. We aimed to investigate the changes in the levels of urine NAG excretion and to evaluate its relation with the cisplatin nephrotoxicity.

Statistical Analysis

The relationship among the numeric variables were analyzed by Pearson correlation tests, while NAG, BUN and creatinine changes before and after the treatment were compared by paired samples T test. The p levels of <0.05 were accepted as statistically significant. Statistical analyses were performed by using 11.0 version of SPSS statistic software program.

RESULTS

Of the total 30 patients, 23 (77%) were male and 7 (23%) were female. The median age was 54 (26-75) years. The CBC, and the serum AST, ALT, total bilirubin, and direct bilirubin levels were between normal ranges (Table1). The distribution of cancer types among the patients were as follows; 14 (46.7%) lung, 5 (16.7%) gastric, 2 (6.7%) tongue, 2 (6.7%) breast, 1 (3.3%) metastatic larynx, 1 (3.3%) oesophagus, 1 (3.3%) carcinoma with an unknown primary, 1 (3.3%) uvula, 1 (3.3%) metastatic thymoma, 1 (3.3%) metastatic carcinoid, 1 (3.2%) metastatic mesothelioma cancer (Figure 1). The patients were given 130.13±8.01 mg cisplatin with a mean dosage of 75 mg/m^2.The mean NAG level was 6.98±4.49 U/L at the beginning and 10.08±5.50 U/L after the medication (p=0.001). In 23 (77%) of patients, we detected increase in NAG levels after treatment, while decrease in 6 (20%) patients while no change in 1 (3%) patient (Table 2). The mean serum creatinine level before treatment was 0.75±0.27 and 0.78±0.29 after treatment (p=0.229) (Table 2).

After therapy, there was no significant correlation between the total dosage of cisplatin and NAG level (r=-0.048, p=0.80) and creatinine level (r=-0.218, p=0.23), and also after therapy, there was no significant correlation between NAG and creatinine levels (r=0.301, p=0.10). →

DISCUSSION

Cisplatin is a widely used drug for the treatment of cancers such as germ cell ovarian cancer, head and neck cancers and lung cancers. The platinum core inside the cisplatin converts into highly reactive compounds by aquation reaction and therefore, binds to cellular DNA and causes apoptosis.[1,3,4]

It is a very effective drug; however, it has some important limitations such as its adverse effects on kidneys and neurons. These include followings; decrease in the renal blood flow, cytotoxicity, stimulation of some cytokine secretions and direct toxicity on proximal renal tubules.[1-5] The effect on proximal tubules results from the accumulation of cisplatin on renal proximal tubules approximately 5 times higher than it does in systemic circulation and its stimulation of necrosis or apoptosis at proximal renal tubules.[1,2,4]

It has been reported that kidney failure after cisplatin administration occurs in subsequent 48 hours-5 days.[3,4] In our study, 24 hours after treatment, we detected a significant increase in urinary NAG levels in 77% of patients and this increase was independent of serum creatinine levels. This finding was similar to study results of Verplenke et al., but the study population of our study was higher than the mentioned study and NAG increase was detected only 77% of patients, so we suggest that cisplatin toxicity does not occur in every patient.[14]

There was no increase in serum creatinine levels of our patients. We suggest that appropriate pretreatment via hydration might have caused this situation. Serum creatinine level is not significant alone, because it may be normal unless the 50% of nephrons are ineffective and is affected by the age and muscle mass, as well.[3] We suggest that the early increase in urinary NAG might be due to proximal cell necrosis in early stages, not to the apoptosis that have been thought to be responsible from renal toxicity, or to the anti-tumor effect of cisplatin, as frequently reported in the literature. Interestingly,

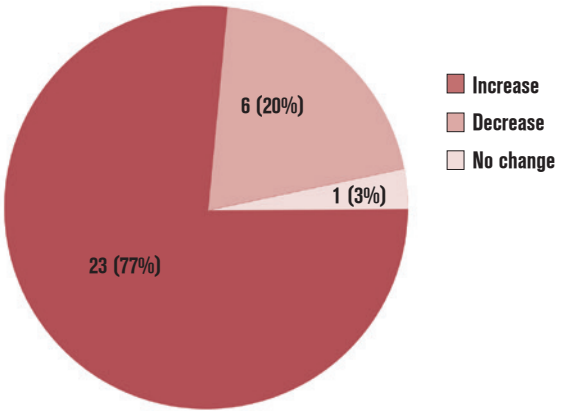

Figure 2: Patient distribution due to changes in the urine NAG levels after treatment

the levels of NAG, a tubular enzyme, start to rise in the first 24 hours, although pathologic changes start 3 days after cisplatin administration. Lieberthal, Lee and Gonzales et al. had shown that high doses of cisplatin causes necrosis in the renal proximal tubule epithelial cells in hours, while low doses results with apoptosis in days.[38-40] The early increase in urinary NAG occurs due to injury of proximal tubules and lysosomes, as well. Lieberthal et al. approved that, in the first 24 hours after cisplatin administration, 50-67% of the drug is excreted in the urine and in the first 4 hours, serum drug concentration decreases by 90% which means that kidneys are exposed to high drug doses causing exposure of proximal renal tubules to high doses of toxic elements resulting in rapid decreases of ATP stores so that in the cells, necrosis occurs rather than apoptosis.[41] As a result, cellular content is excreted in an uncontrolled manner and so molecules such as NAG can be detected in the urine.

In conclusion, the kidney of patients treated with cisplatin is affected but not in all of the cases. Early urinary NAG increase occurs before the increase in serum creatinine levels. Therefore, investigations such as NAG measurement seem to be significant in reaching effective serum drug levels and maintaining efficacy and continuity of the treatment.

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