Original scientific paper
616.831-009.7:616.61-099-085.035.2

EARLY DETECTION OF NEPHROTOXICITY IN PATIENTS WITH MEDICATION-OVERUSE HEADACHE (MOH)

Drita Yzeiri Havziu[1], Biljana Gjorgjeska[2], Dragana Cvetkovska[3], Sefedin Bilalli[4], Igor Nikolov[5]

[1]Faculty of Medical Sciences - University of Tetova, Republic of North Macedonia
[2] Faculty of Medical Sciences -State University Goce Delcev, Shtip, Republic of North Macedonia
[3]Clinic of Neurology, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia
[4] Biochemistry Laboratory, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia
[5]Clinic of Nephrology, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia
Corresponding author: drita.havziu@unite.edu.mk

Abstract

Excessive drug use causes Medication-overuse headache (MOH) which can be manifested of chronic daily headaches, occurring monthly 15 or more days, when the medicament is used redundantly for more than three months. Recent studies concerning the epidemiology of drug-induced disorders suggest that increased risk of nephrotoxicity appears in a group of patients who abuse NSAIDs. The aim is to confirm the early phase of nephrotoxicity in patients with (MHO), divided in two groups: first group users of non-steroidal anti-inflammatory drugs (NSAIDs) and second group that use combinations of various analgesics, compared with 80 healthy individuals.
Besides conventional markers of renal functioning (serum/urine creatinine determined by Jaffe methods, enzymatic assay for urea serum and GFR by Cockcroft Gaunt formula), we will use colorimetric method determining N-acetyl–β-d-glucosaminidase–NAG, Alanin Aminopeptidase-AAP in urine, IFCC for Gamma-glutamyltransferase- YGT. Imunoturbodimetric assay for determination of urinary albumin, microalbuminuria and α1-microglobulinwill be used.
After 12 months treatment oftwo groups of patients, no changes were found in the serum creatinine, serum urea and GFR, but extremely statistical difference ($p < 0.01$)in the values of specific biomarkers, NAG, AAP, GGT, microalbuminuria in all patients, especially with combined therapiein the comparison with healthy patients.
We concluded changes on the glomerular and tubular level, despite the normal values of all the assayed conventional markers for renal function, We can't confirm by nephrotoxicity, but if we follow the elevation of the level of the specific biomarkers, we will have an overview of the real state of functioning of the kidneys in patients with MHO.

Keywords: Biomarkers, Medication-overuse headache, Nephrotoxicity, Nonsteroidalanti inflammatory drugs.

INTRODUCTION

Headache is one of the most common symptoms in the general population, as well as in medical practice in the world, with a prevalence of 8% in males and 12-15% in females. Migraine is the most common cause of headache and contributes to a neurological disorder with a serious socio-economic burden. Migraine affects approximately 13% of adults in the United States, and its prevalence ranges between 12% and 20% in different countries in the world.
A special condition observed in chronic migraine patients, classified as overuse headache (MOH), is characterized by frequent intake of antimigrene drugs, is assumed to increase the frequency and intensity of headache (Negro, 2011).The prevalence rate of chronic migraine (CM) in the general population is 2-4% Stovner LJ, 2010).Each year, approximately 2.5% of patients with migraine episodes (EM) develop a new-onset CM . At this point, CM is the most important challenge for tertiary headaches, where more than 50% of patients are referred to monitoring the chronic process and its possible complication with MOH (Lipton RB, 2009).

Despite the introduction of a new class of migraine-specific drugs with superior efficacy over a 3decade ago, triptans, NSAIDs remain the most commonly used therapy for migraine attack. Some groups are easily accessible and are usually much cheaper than triptans that contribute to their abuse (Rothrock, 2011). NSAID- use in migraine is accompanied by their analgesic, anti-inflammatory and antipyretic effects, supported by indirect evidence that prostaglandins are involved in the pathophysiology of migraine (Tulunay, *et al.* 2000). Schuh et al. emphasize the importance of the cyclooxygenase system in the peripheral arm of the trigeminovascular system TGV and suggest that NSAIDs may be effective in the treatment of migraine by the action of these peripheral nociceptors (Schuh *et al.,* 2006). Despite the many positive effects, however, NSAIDs do not meet the expected results.

Various epidemiological studies have shown that different types of analgesics can cause nephrotoxicity, especially in chronic patients associated with overuse of analgesics, triptans, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids or drug combinations - Medication-overuse drug headache (MOH), where the increased risk of nephrotoxicity belongs in particular to a group of patients who abuse NSAIDs (Bellei *et al.,* 2012). Headache with excessive drug use (MOH) is a cause of chronic daily headache, where headaches occur 15 or more days a month when the therapeutic agent is used overly and regularly for 3 months or more (International Classification of Headache Disorders, 2004). MHO is manifested as an increased frequency and intensity of migraine attacks and as an increased sensitivity to stimuli that cause episodes of migraine (De Felice 2011).

A recent epidemiological study on drug-related disorders has proven that excessive drug use can lead to nephrotoxicity and potential renal impairment (Davies *et al.* 2012; Negro , *et al.* 2011). In particular, drug-related drug nephrotoxicity accounts for 18-27% of all acute kidney disorders in the United States, and can affect all aspects and any part of the kidney structure from various mechanisms of renal dysfunction (Taber, *et al.* 2008). William and co-workers have confirmed that inhibition of prostaglandin-mediated NSAID mediation prevents neurogenically mediated inflammation in the trigeminovascular system and the reduction of pain, but at the same time inhibiting prostaglandin in the kidney may reduce renal blood flow and glomerular filtration rate, thereby promoting sodium and water retention is promoted (William *et al.*, 2003).

NSAIDs are still the most used effective agents in the treatment of mild to moderate migraine attacks, especially taking into account the fact that COX-2 inhibitors minimize serious gastrointestinal adverse effects. However, their nephrotoxicity is still a concern and remains a major subject of research and scientific research in their use (Havziu, 2014; Hyman *et al.,* 1996; Brater, 2002). Many nephrologists report that NSAIDs have been classified into the second group after nephrotoxicity, after aminoglycosides as the cause of AKI (Schrier *et al.,* 1984). Nephrotoxicity caused by NSAID includes the following stages of renal impairment: tubular necrosis, acute tubular nephritis, glomerulonephritis, renal papillary necrosis, chronic renal impairment, electrolyte and water retention, hypertension, hyperkalaemia, and hypoaldosteronism. However, more recent studies have summarized these enumerated phases in the following conditions: acute renal impairment, chronic renal impairment, interstitial nephritis, and subclinical nephrotoxicity (Ejaz *et al.*, 2004, Havziu, 2014).

It is clear that NSAIDs are associated with all forms of renal impairment, but, however, if they are detected early, acute syndromes have a good prognosis. However, this assumption does not apply to chronic renal impairment (Clive *et al.,* 1984; Ejaz *et al.,* 2004; Havziu, 2014).

Traditional laboratory analyzes for the detection of renal impairment, which include creatinine, creatinine clearance, urea, electrolytes, urine sediment, and radiological investigations, are not only sensitive and specific, but do not allow early detection of renal impairment, cannot detect adequate differentiation between the various degrees of AKI and as such cannot be used as a signal for stopping therapy with drugs that exhibit strong nephrotoxicity (Liangos *et al.,* 2007).

In fact, until recently the rise in serum creatinine was widely considered to be the "gold standard" for detecting AKI, it is now clear that serum creatinine changes when 50% of renal function is lost. It is therefore very important, in addition to these parameters, to follow certain biomarkers, which were previously determined in vivo conditions on experimental animals and then used as modeling systems for the human organism (Prasad *et al.,* 2005 ;Havziu, 2014).
Recent literature suggests the possibility of monitoring the activity of certain enzymes in the urine as a bioindicator indicating early detection of nephrotoxicity occurring during NSAIDs (Spasovski *et al.*, 2007, Havziu, 2014). They are actually enzymes that originate from plasma or from the urogenital tract gland (Haschen, 1977; Burchard *et al.*, 1982). The specific urinary enzymes: (NAG), γ-glytamyltransferase (γ-GT), α-glytathione-S-transferase - (α-GST), π-glutathione-S-transferase (π-GST), Alanine aminopeptidase- (AAP), etc., allow precise proving the localization of renal impairment (glomerular or tubular) (Havziu 2014; Maruhd, 1976; Shouk *et al.*,1974).
Apart from enzymes, proteins and albumin that can be of serum or urinary origin are used as biomarkers. Excretion of high molecular weight albumin and protein (> 80000) in urine indicate damage to glomeruli (Davis *et al.*, 1994), and low molecular weight proteins and albumin: ß2M, α1M, indicate changes in the tubules. According to Bellei et al., It has been demonstrated that in the monitoring of certain urinary biomarkers (α1M, Cys-C, etc.) in patients with migraine pain using one-dimensional gel electrophoresis and identified by mass spectrometry, in patients who have been abused by analgesics and NSAIDs, a significantly different expression of the protein profile has been found, therefore there is an increased risk of nephrotoxicity (Bellei *et al.*, 2012: Havziu 2014; Tillyer *et al.*, 1988).
From these findings, it is very important that early detection of nephrotoxicity and the high availability of a large number of NSAIDs without prescription and uncontrolled use by patients with chronic headache contributes to their abuse, especially with a mixture of analgesics.
In general, in the literature on nephrotoxic drugs, there are reports of multiple cases or series of cases of AKI caused by nephrotoxic agents. However, few studies have led to an incidence of AKI in patients with chronic migraine pain caused by NSAIDs in long-term therapy, especially in patients with headache overuse drug use (MHO).As a challenge to address some of the aforementioned issues, we have set the purpose of the work to confirm the early phase of nephrotoxicity in patients with (MHO).

MATERIALS AND METHODS

For realization of the set goals will use urine and venous blood from patients with chronic headache and migraine pains from the University Clinic of Neurology-Skopje who are on chronic therapy with different NSAIDs and combined analgesic therapy in relation to a control group of 80 healthy subjects. Ten of the patients, were treated with Diclofenac with a total dose of up to 200 mg per day, and 10 patients with combination therapy Diclofenac + Caffetin (paracetamol, propyphenazone, caffeine and codeine) after 12 months of therapy. All of the patients have headaches in duration of 15 or more days a month.

The patients included in the examination were informed about the method of implementation and the purpose of the research before giving their written consent. The examination was conducted according to the designed protocol, in accordance with the ethical principles of the Helsinki Declaration on Medical Research on People
and Licenses from the Ethic Committee of the Faculty of Medical Sciences at the University "Goce Delcev" - Stip (WMA, 2000).
The presented results represent the average value of the three measurements, under identical conditions. As a sample for analysis, 5 ml of blood collected in special tubes was used

without anticoagulants, all the material for analysis are measured Clinical Biochemistry in Skopje.
For the determination of creatinine, and specific biomarkers (NAG, AAP и γ–GT, α1M and microalbuminuria), the first morning urine was used. After proper processing the pure supernatate is used for further processing. (Havziu *et al.,* 2017).

METHOD OF WORK

For creatine serum / urine, is used the Jaffe method - during the reaction of the creatinine with the basic reagents (Flex reagent cartridge) a complex of red color is formed which is followed by measuring the change of absorbance at a time interval of 510 nm (DimensionRxl)
Urea serum, the enzymatic-urea hydrolysis under the influence of the urease enzyme, the formed ammonia (NH3) reacts with the catalytic effect of the GLDH (Flex Reagent Cartridge), α-KG (Flex Reagent Cartridge) and NADH (Flex Reagent Cartridge). As a result of the reaction, glutanamic acid and NAD are formed. The decrease in absorbance due to the reduced NADH oxidation is proportional to the release of the urea NH3. Measured at a value of 340, 383nm (Dimension Rxl).
GFR with the Cockcroft Gaunt formula.
Serum electrolytes - Ion Selective Electrodes (ISE) - determine the difference in the electrochemical potential between the glass or liquid membrane electrode (Roche Diagnostics) and the reference electrode (Roche Diagnostics) that is proportional to the concentration of electrolytes in the serum.
For determination of urinary specific enzyme we used the Spectrophotometric Colorimetric Method for NAG and AAP, γ-GT.
For the determination of urinary albumin, microalbuminuria and α1-M, the immunoturbodymmetric method (Cobas Mira Plus) (Havziu *et al.,* 2016).

STATISTICAL DATA PROCESSING

Statistical data processing is done in Microsoft Excel, calculating the average value (M) and the standard deviation (standard deviation, SD). Differences between variance of patients during the course of therapy are recorded by variance analysis (ANOVA) and Student T-test.

RESULTS AND DISCUSSION

10 patients, treated 12 month with Diclofenac a total dose of up to 200 mg per day and 10 patients with combination therapy Diclofenac + Caffetin (paracetamol, propyphenazone, caffeine and codeine) comparison to the control group of healthy individuals according to the protocol, which includes the parameters that are presented in Table 1.

Table 1. It is noted that values of urea serum, creatinine of serum/urine and GFR in 10 patients, who were treated 12 month with Diclofenac a total dose of up to 200 mg per day, and 10 patients with combination therapy Diclofenac compared to the control group, significant statistical difference was found in all measured parameters.

Table 1. Biochemical parameters in patients treated 12 month with Diclofenac

Biohemical parameters	NSAID n=10 M±SD Diclofenac	P value	n=10 M±SD Diclofenac+ caffetin	P value	Control group n=80 M±SD	Referential values
Urea (serum) mmol/L M±SD after12mounth	5.8±1.2	P=0.722	5.60±1.2	P=0.908	5.65±1.3	2,0 – 8,3
Creatinin (serum) µmol/L M±SD after 12mounth	82.0±15.0	P=0.852	87.0±12.1	P=0.388	82.9±14.3	45 – 115
Creatinin (urin) mmol/du M±SD after12mounth	6.9±3.9	P=0.669	7.7±2.0	P=0.883	7.5± 4.2	Man 5,3-22,1 women 5,3-13,3
GFR ml/min M±SD after12mounth	99.0±19.0	P=0.962	94.0±19.5	P=0.055	110.3± 25.6	125 ±15
Natrium mmol/1 M±SD M±SD after12mounth	132.17±1.7	P=0.688	136.48±2.76	P=0.99	132±1.2	132 – 145
Kalium M±SD after12mounth	3.93±0.45	P=0.281	3.85±0.62	P=0.563	3.75±0.5	3.93±0.45
Kloride M±SD after 12 mouth	100.29±3.078	P=0.412	101.19±2.98	P=0.825	101.0±2.5	96 – 108

p <0.01 ** represents a very high statistical difference at values with a 99% confidence interval
p <0.05 * statistically significant difference in values with a safety interval of 95 %

From the results obtained we note that no changes in the values of conventional markers have been observed. According to Liangos , they are termed as traditional biochemical parameters and at the same time thought to be non-specific and non-sensitive for early discovery of glomerular and tubular damage, as well as for adequate detection and differentiation of different stadiums acute renal impairment (Adams *et al.*, 1986, Liangos *et al.,* 2007). In contrast, Ipokratis and colleagues have concluded that the use of NSAIDs can cause changes in renal dysfunction, from a reversible GFR disorder to irreversible renal impairment (Ipokratis *et al.,* 2011). Given the fact that serum creatinine and some of the biochemical parameters according to standard nephrology protocol differ when 50% of renal function is reduced, the most sensitive biomarkers are monitored for early identification of renal impairment in patients. For this reason further studies with more specific urine biomarkers for renal dysfunction in the glomerular and tubular levels are needed. Taking into account the fact that serum creatinine and some of the biochemical parameters according to the standard nephrology protocol change when 50% of renal function is decreased, more sensitive biomarkers are monitored for early identification of changes in the renal state in patients with overuse of drugs. Tested after 12

months with specific urinary enzymes (NAG, γ-GT, AAP) and low molecular weight proteins (α1M and microalbuminuria). They are presented in more detail in Figures 1-3.

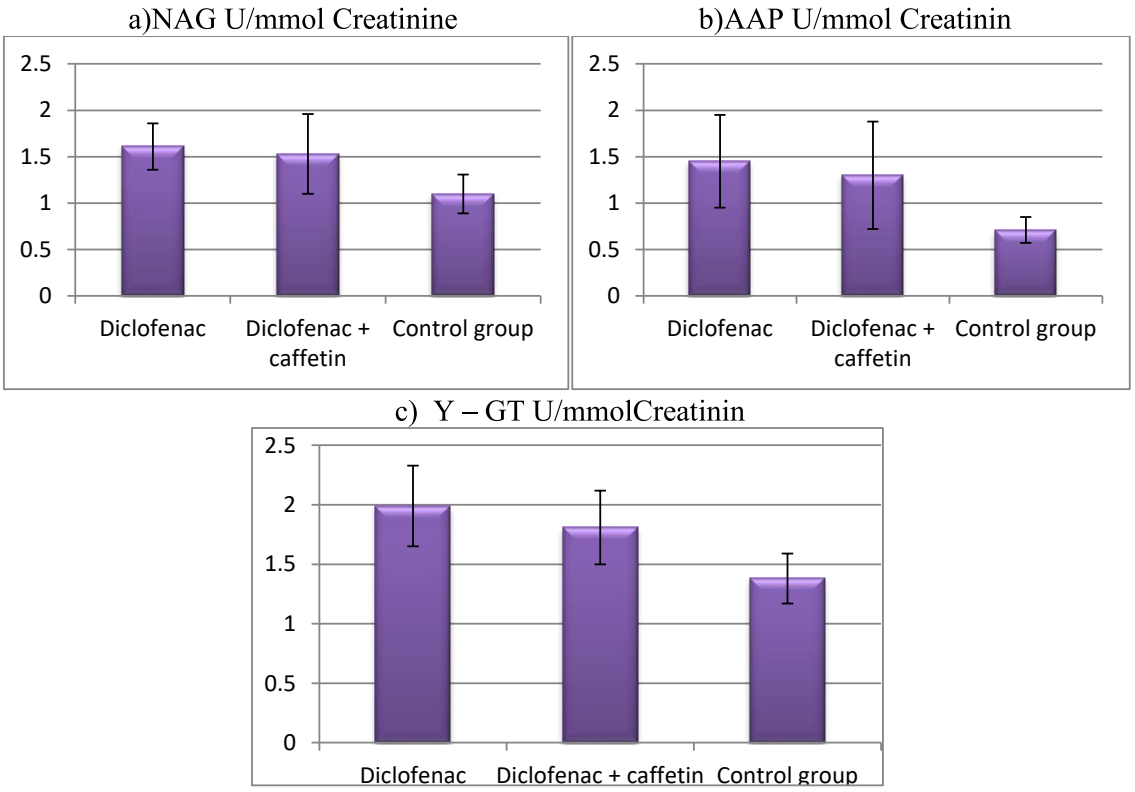

Figure 1. Sensitive biomarkers monitored for early identification of changes in the renal state in patients with overuse of drugs**.**

Extremely statistical difference change in safety intervals of 99% and p <0.01, was found between the patients taking diclofenac and patients taking combination of Diclofenac and Caffetin after12 months for the values of a)NAG and b)AAP c) Y – GT ,compared with the means of the control group (Figure1).

The results obtained correspond with the results obtained by Zafirovska and his co-workers, who in monitoring three specific urinary enzymes for the identification of tubular damage (NAG, AAP and γ-GT) in a group of patients treated with different NSAIDs (Ibuprofen, Naproxen or Indomethacin), abnormal urinary excretion was observed (Zafirovska *et al.,* 1993; Spasovski *et al.*, 2008).

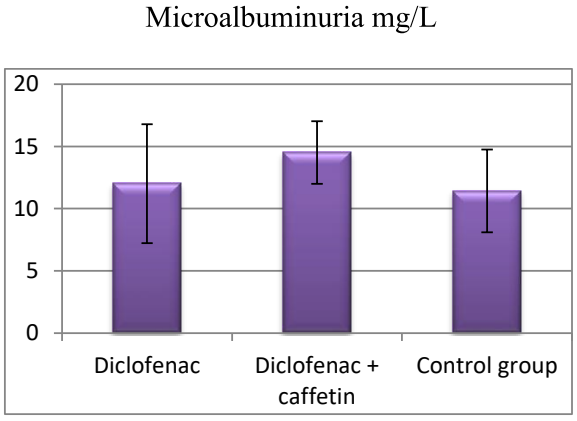

Figure 2. Values of microalbuminuria

For the measured values of microalbuminuria no statistical difference were found in any of the measured values for the group of patients which are on a therapy with only Diclofenac. For the second group of patients that were submitted to the combined therapy of Diclofenac and Caffetin there was big statistical difference on the level of $p<0.01$ compared with the means of the control group of healthy patients (Figure 2)

In this paper, specific urinary low molecular weight albumin, α1M, as well as the specific urinary biomarker for glomerular damage - microalbuminuria are monitored, with significantly higher values compared to the control group of examinees, in all treated patients with different groups of NSAIDs, which means changes are expected at the glomerular level. These results correspond to the claims of (Pedersen *et al.,*1995) That the biomarker microalbuminuria is a more sensitive indicator of renal dysfunction identification, as opposed to monitoring the NAG.

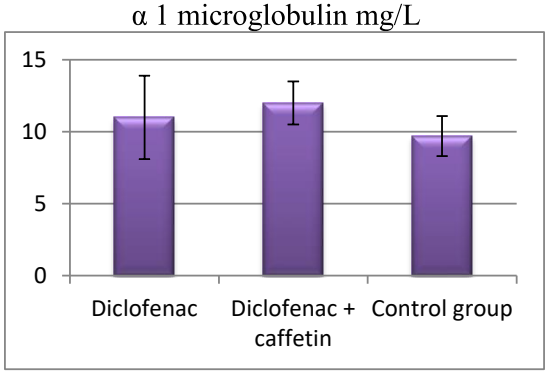

Figure 3. Values of α 1 microglobulin

The values measured on α 1 microglobulin are slightly statistically different with CI of 95% or $p<0,05$ between the patients taking Diclofenac and patients taking combination of Diclofenac and Caffetin after12 months (Figure 3).
The obtained α1M results correlate with the results obtained by Bellei and collaborators who have demonstrated significant α1M expression in the urine in cases of abuse of NSAIDs.
The findings confirm that after 12-month follow-up period with Diclofenac and combined therapy Diclofenac + Caffetin in patients with MHO have been identified changes in glomeruli and proximal tubules, mainly in patients with combined therapies related to the claims of other authors (Bellei *et al.,* 2012; LaFrance *et al.,* 2009; Ipokratis *et al.,* 2011, Havziu, 2017), have

occurred as a result of nephrotoxic agents causing the inhibition of COX -NSAID and combined therapy (paracetamol, propyphenazon) nefrotoxic agent.

CONCLUSION

- Sensitivity of the most sensible bio –indicatorshas been verified to detect nephrotoxicity at an early stage.
- We can not confirm nephrotoxicity for longer-term research based on histopathological data, but if we follow the set up of biomarkers, we can use them as early signals for nephrotoxicity.
- It is recommended that individualized and rational use of NSAIDs be abused, due to increased potential for nephrotoxicity, as well as continuous monitoring of renal function in patients.

REFERENCES

[1]. Ahmad, Sr., Kortepeter, C., Brinker, A., Chen, M., Beitz, J. 2002 *Renal failure associated with the use of celecoxib and rofecoxib.* Drug Saf; 25: 537-544.

[2]. Adams, D.H., Homer, A.J., Michael, J., McKonkey, B., Bacon, P. 1986. *Adu. Nonsteroidal anti-inflammatory drugs and renal failure,*Lancet:1:57-59.

[3]. Abouch V. Krymchantowski1, Jackeline S. Barbosa1, Celia Cheim1, Luiz A. Alves. 2001.*Oral Lysine Clonixinate in the acute treatment of migraine.* Arq Neuropsiquiatr ;59(1):46-49

[4]. Blau J.N., Dexter S.L. 1981*The site of pain origin during migraine attacks. Cephalalgia.* ;1:143–147

[5]. Bellei, E., Cuoghi, A., Monari, E., Bergamini, S., et al. 2012. *Proteomic analysis of urine in medication – overuse headache patients: possible relation with renal damages*. J Headache pain,13:45-52.

[6]. Brater, DC. 2002. *Renal effect of cocloxygenase-2-selectve inhibitors* .J Pain Symptom Manage; 23: S 15-20.

[7]. Brian, W., Reeves, M.D., Richard, J.Foley MD, Edward, J., Weinman, M.D. 2004. *Hostontex Nephrotoxicity From Nonsteroidal Anti-inflammatory Drugs.* Southern Medical Jornal. Vol 78, no, 3

[8]. Burchard, U. et al. 1982. *Ursacheneinerverandertten Enzeime aus scheidung mit dem harn.* Sympos. Harnenzyme-,48, (R75),220

[9]. Clive, Dm., Stoff, JS. 1984. *Renal syndromes associated with nonsteroidal anti-inflammatory drugs.* N.Engl J Med;310:563-572.

[10]. Davies P (2012) Medication overuse headache: a silent pandemic. Pain 153:7–8

[11]. Davis, M.E., Berndt, W.O. 1994. *Renal methods for tocicology. Principles and methods of toxicology. Third edition,* Raven Press Ltd, New York,:871-894.

[12]. De FeliceM, Ossipov MH, Porreca F (2011) Persistent medication-induced neural adaptations, descending facilitation, and medication overuse headache. CurrOpinNeurol 24:193–196

[13]. D.Uzeiri.Havziu.2014. *Нефротоксичност на нестероидни антиинфламаторни лекови*. Фармацевтски факултет -Универзитет „Св. Кирил и Методиј" - Скопје

[14]. D. Yzeiri Havziu M. Hiljadnikova-Bajro, T. Kadifkova Panovska N. Bexheti. 2016. *Renal function of patients with rheumatoid arthritis treated with piroxicam;* Acta Medica Balkanica/International Journal of Medical Sciences; VOL1NO2PP.1-13

[15]. Ejaz, P., Bhojani, E., Joshi. 2004. *NSAIDs and Kidney.*JAPI. Vol. 52; 632-640.

[16]. Evers S, Afra J, Frese A, Goads by PJ, Linde M, May A, Sándor PS; 2009European Federation of Neurological Societies. Eur J Neurol. 2009 Sep;16(9):968-81. doi: 10.1111/j.1468-1331.02748.x.

[17]. Ebersberger A., Averbeck B., Messlinger K., Reeh P.W. Release of substance P, calcitonin gene-related peptide and prostaglandin E2 from rat dura mater encephali, following electrical and chemical stimulation *in vitro*. Neuroscience. 1999;89:901–907. doi: 10.1016/S0306-4522(98)00366-2

[18]. Gruder, W. and Roos, B. 1984. *Enzyme distribution along the nephron.* Kidney Int., 26; 101-111.

[19]. Haschen, R. J. 1977. *Diagnostic Significance of proteoliticenzymes. Z. med.*Lab.diag.-; 18, 147-158Acta-1976;(55);179-197.

[20]. Huerto, C., Castellsague, J. et al. 2005. *Nonsteroidal anti-inflammatory drugs and risk of ARF.* Am J kidney; Dis 45;531-539

[21]. Headache Classification Subcommittee of the International Headache Society, authors. The international classification of headache disorders. Cephalalgia. 2004;24(Suppl. 1):9–160. doi: 10.1111/j.1468-2982.2003.00824.x.

[22]. Hyman T,. et al. 1996. *An Evidence-Based Approach to Prescribing NSAIDs in Musculoskeletal Disease: A Canadian Consensus.* CAN MED ASSOC . JULU1,111-996;155(1).
[23]. International Classification of Headache Disorders, 2nd Edition (2004).Headache Classification Subcommittee of the International Headache-Society. Cephalalgia 24(Suppl 1):8–152
[24]. Ippokratis P., Theodora G, Howard B, Peter V. 2011. *Giannoudis. Nonsteroidal anti-inflammatory drugs: prostaglandins, indications, and side effects.* International Journal of Interferon, Cytokine and Mediator Research:3 19–27.
[25]. JohnF. Rothrock, MD.2011Editor-in-Chief, Headache. Director, Headache Treatment and Research Center. University of Alabama. Birmingham, AL, USA.
[26]. Zafirovska,G., Bogdanovska K,V., Nada,M., Todor G. et al. 1993.*Urinary Excretion of Three Specific Renal Tubular Enzymes in Patients Treated with Nonsteroidal Anti-Inflammatory Drugs (NSAID).*Issue TOC., Vol. 15, No. 1 , Pages 51-54.
[27]. Kamal Hassan, Khalid Khazim, Fadi Hassan &Shadi Hassan (2011) *Acute Kidney Injury Associated with Metamizole Sodium Ingestion,* Renal Failure, 33:5, 544-547, DOI: 10.3109/0886022X.2011.569107
[28]. Koppert, W., Frotsch, K. et al. 2006. *The Effects of Paracetamol and Parecoxib on Kidney Function in elderly Patients Undergoing Orthopedic Surgery.* Anesth Analg;103:1170-6.
[29]. Lafrance, M.D., Donald, R., Miller, Sc.D. et al.2009. *Selective and nonselective non-steroidal anti-inflammatory drugs and the risk of acute kidney injury.* Pharmacoepidemiology and Drug Safety; 18 (100:923-931).
[30]. Le Hir, M., Dubach, U. et al. 1979. *Quantitative distribution of lysosomal hydrolases in the rat nephron.* Histochemistry; 63; 245-251.
[31]. Liangos, O., Perianayagam, M.C., Vaidya, V.S., Han, W.K., Wald, R. et al. 2007. *Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure.* J. Am. Soc. Nephrol;18:904–12.
[32]. Lipton RB, Stewart WF, Scher AI. 2001.Epidemiology and economic impact of migraine. Curr Med Res Opin. 2001;17
[33]. Lipton RB (2009) Tracing transformation: chronic migraine classification, progression, and epidemiology. Neurology 72:S3–S7
[34]. Maruhd, D. 1976. *Rapid colorimetrical assay of beta- galactosidase and –N-acetil-beta-D-glucozaminidase in human urine.* Clin. Chim. Acta., (73); 453-461.
[35]. Negro A, Martelletti P (2011) Chronic migraine plus medication overuse headache: two entities or not? J Headache Pain 12:593–601

[36]. Pardutz A, Schoenen J. NSAIDs in the Acute Treatment of Migraine: A Review of Clinical and Experimental Data. *Pharmaceuticals*. 2010;3(6):1966-1987. doi:10.3390/ph3061966.
[37]. Prasad, D., Jonthan, M. 2005. *Barasch Method for early detection of renal injuries.* New York, NY (US) ;116/096,113.
[38]. Pedersen, L.M., Nordin, H., Svensson, B., Bliddal, H. 1995. *Microalbuminuria in patients with rheumatoid arthritis.* Annals of the Rheumatic Diseases; 54: 189-192.
[39]. Raab, W. 1972. *Diganostic value of urinary enzyme determination*. Clin. Invest.;(18), 5-19.
[40]. Rossi, S. 2006. *Australian medicines handbook.* ISBN 0-9757919-2-34, 2006.
[41]. Rozen TD, Swanson JW, Stang PE, et al. 1999 Increasing incidence of medically recognized migraine headache in a United States population. Neurology. ;53(7)
[42]. Rozalski, S. B. and Wilkinson, J. H. 1959. *Urinary lactic dehudrogenas in renal diseases*. Lancet 2, 237-241.
[43]. Stewart WF, Lipton RB, Celentano DD, et al. Prevalence of migraine headache in the United States. JAMA. 1992;267(1):64–69.
[44]. Stovner LJ, Andree C (2010) Prevalence of headache in Europe: a review of the Eurolight project. J Headache Pain 11:289–299]
[45]. Schrier, R.W., Henrich, W.L. 1984. *Nonsteroidal anti-inflammatory drugs: caution still indicated.* Jama;251:1301-1302.
[46]. Shouk, C. E. et al. 1974. *Enzyme patterns in human tissue. Glucolitic enzyme patters innormalygant human tissue.*Cnacer Res. (24);722-727.
[47]. Spasovski D. Gruev T. et al. 2007b. *Vrednost N-acetil-ß-D-glukozaminidaze i mikrolabuminurije kod reumatoidnog artritisa.* Journal fo Medical Biochemistry, Vol. 26,Br.4, str.300-308.
[48]. Spasovski D., Gruev T., Marina N., Calovski J., Rajcevska L., et al., 2008a. *Alanine Aminopeptidase, g-Glutamil Transferase and b2-microglobulin as diagnostic markers in patients with rheumatoid arthritis*.JMB 27: 59–63.
[49]. Schuh-Hofer S., Tayefeh M., Reuter U., Dirnagl U., Arnold G.2006. Effects of parecoxib on plasma protein extravasation and c-fos expression in the rat. Headache;6:276–285

[50]. A. M. Strassman S. A. Raymond, R. Burstein. 1996. Sensitization of meningeal sensory neurons and the origin of headaches. *Nature* volume384, pages560–564 doi:10.1038/384560a0
[51]. Tillyer, C.R., Rawal, Y. 1988. *An Immunoturbidimetric Method for the Measurement of Beta-2 Microglobulin in Serum and Plasma on a Centrifugal.* Analyser Annals of clinical biochemistry, ncbi.nlm.nih.gov.
[52]. Tulunay F.C. *NSAIDs: Behind the mechanisms of action*. Funct. Neurol. 2000;15(Suppl. 3):202–207.
[53]. Taber SS, Pasko DA (2008) The epidemiology of drug-induced disorders: the kidney. Expert Opin Drug Saf 7(6):679–690
[54]. Unsworth, S. Struman, J. Lunec and Blake. 1987. *Renal imparmentassocited with non-steroidal anti-iflamatori drugs.* Annals of the Reumatic Disease;46,233-236.
[55]. YzeiriHavziuDrita, Hiljadnikova-BajroMarija, KadifkovaPanovskaTanja, Zylbehari, Ljutvi and Gjorgjeska, Biljana (2017) *Nephrotoxicity of NSAIDs and MTX.* In: 2nd International Scientific Conference of the Faculty of Medical Sciences, 13-14 Oct 2017, Tetovo, Republic of North Macedonia.
[56]. Vaidya Vishal S., Michael A.Ferguson and Joseph V. 2008: *Bonventre Biomarkers of Acute Kidney Injury.* Annu Rev Pharmacol Toxicol :48:463-493:doi:10.1146/annurev .pharmtox.48113006.094615.
[57]. Whelton, A. 1999. *Nephrotoxicity of nonsteroidal anti-inflammatory drugs: Physiologic foundations and clinical implications.* Am J Med;106:13s-24s.
[58]. Williamson D.J., Hargreaves R.J. Neurogenic inflammation in the context of migraine. Microsc. Res. Tech. 2001;53:167–178
[59]. William L. Baker, PharmD, Jon Folstad, PharmD. 2003; *Cardiovascular Effects of Migraine Therapy.* ProgCardiovasc Nurs;18(3)
[60]. WMA-World Medical Association Declaration of Helsinki Ethical principles for medical research involving human subjects. JAMA 2000;284:3043-3045.