Eosinophil Cationic Protein in Uremic Dialyzed Patients with Intractable Pruritus

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Abstract: We have analyzed 6 patients(pts) with chronic uremic pruritus(UPG) treated with intermittent hemodialysis more than 5 years,and 7 uremic pts treated under the same conditions but without pruritus(UG).In all pts and in appropriate control group(CG)of healthy subjects(N^0 = 5)we have measured the serum concentration of s.c.*eosinophil cationic protein*(ECP) known as a protein of allergic states(bronchial asthma as a classic example).We have found elevated serum concentrations of ECP in uremic pts($X_{ECP\ cg}$ = 2.03±0.02 μg/L vs $X_{ECP\ ug}$ = 2.63 ±0.29 μg/L),but especially at the uremics with intractable itching($X_{ECP\ upg}$ = 8.37±1.67 μg/L). **The aim pf the paper**:The dialyzed pts with transitory uremic pruritus(u.p)or itching related to the other additioned conditions (like hepatocellular or obstructive jaundice)not differ significantly from the uremics without itching bearing in consi-deration the serum concentration of ECP. ***Material and methods:***1.*patients and control group:* we have analyzed 6 patients(pts)with chronic imperative u.p(duration more than 6 months!)treated with intermittent hemodialysis more than 5 years(Department of nephrology,Clinical Centre,Skopje,R.of Macedonia) and 7 uremics treated under the same conditions but without up.In all pts(age and sex matched,N^0= 13)and in the appropriate control group of healthy subjects(c.g,N^0 = 5),we have measured the serum concentrations of s.c.eosinophil cationic protein(ECP)known as a protein of allergic states(allergic rhinitis and conjunctivitis,atopic dermatitis and bronchial asthma as a classic example).The blood was picked up at 8 a clock for investigated persons and before hemodialysis for uremic pts to.**Results:** From the precedent tables is evident almost the same frequency in men and women(males = 10,females=8),with the mean age(all groups included) of about 44 years.The serum concentration of ECP is lowest(≈ 2 μg/l) in the CG with coefficient of variation(CV) under 2%!The UG is not so compact regarding sECP with CV ≈ 30%,and finally the uremic pts suffering from intractable itching(UPG) are heterogenous group with more elevated level of sECP(at $p < 0.05$),and a very high CV(156%).The statistical significance of differences between mean values for sECP in investigated groups is sufficient($Z > 1.96$ SEM) but especially evident comparing the CG and UPG(Z= 3.8 SEM),respectively UG and UPG($Z > 3.3$ SEM).We have concluded that in the cases of very promounted u.p,the allergic component is higly included.The further investigations are needed to detect indoubtely the presence of ECP,EPX or eosinophils in the selected planes of the skin tissue and to develop more efficacious antipruritus drugs.

Index trems*:* uremic intractable pruritus eosinophil cationic protein(ECP),Hemodialysis

1 Introduction:

Among the various multiorganic abnormalities associated with advanced chronic uremia and dialysis therapy,pruritus(u.p)is certainly the most clinically disturbing symptom. Itching is an imperative,unpleasant and vexing sensation that provokes an intense desire to scratch[1].Contrary of the old consideration that the pruritus is only a submodality of pain from the neurophysiologic point of view,the more recent researchs[2,3] showed that the pruritus and pain are different primary sensations which are transmitted through specialized sensory neurons[4].

The causes of pruritus related to chronic renal failure are still unsatisfactory known. Never-theless, many "pathogenetic" factors may be incriminated and tacked in consi-deration like intradermic microprecipitation of divalent ions,disturbed metabolism of vitamine A, hyperparathyroidism, periferal neuropathy, epidermal hyperxerosis(icht-hyosis) and others (mainly allergic ,hypersensitivity) reactions with or without histamine liberation[5].The uncertainty of the causes is partially responsible for the different therapeutical approach and non-sufficiently satisfactory results in many cases.

The aim of this paper is to present the possible role of hypersensitivity in the genesis of intractable uremic itching measuring the serum ECP concentration and to propose a more accurate measures for more successful therapy and prevention.

2 Material and methods:

1.*patients and control group:* we have analyzed 6 patients(pts)with chronic imperative u.p(duration more than 6 months!)treated with intermittent hemodialysis more than 5 years(Department of nephrology,Clinical Centre,Skopje,R.of Macedonia) and 7 uremics treated under the same conditions but without up.In all pts(age and sex matched,N^0= 13)and in the appropriate control group of healthy subjects(c.g,N^0 = 5),we have measured the serum concentrations of s.c.eosinophil cationic protein(ECP)known as a protein of allergic states(allergic rhinitis and conjunctivitis,atopic dermatitis and bronchial asthma as a classic example).The blood was picked up at 8 a clock for investigated persons and before hemodialysis for uremic pts to.

2.*method:* the ECP is primary determinated as a marker of activity and therapeutic efficacy in the pts with bronchial asthma and other inflammatory diseases.The serum concentration of ECP is precisely measured with the immunoCAP system (UniCAP^{TM} ECP)introduced by Pharmacia CAP technology in 1989 using the method of specific and unique assay for IgE determination based on human recombinant autoantigens following the principle of fluoroenzyme immunoassay (Pharmacia CAP system ECP FEIA,1997)[6]. Using the proposed immunoche-mical method the serum concentration of ECP is presented in μg/l. PharmaciaCAP system ECP FEIA is a vitro test for the quantitative measurement of ECP in human serum.ECP is secreted only by activated eosinophils.High concentration of serum ECP reflect the protracted inflammation in the target organ(lung,skin..)during the acute stage or exacerbation of anyone atopic disease.By following the ECP levels during the anti-inflammatory therapy,the treatment efficacy may be also monitored.

Warning: blood collection, coagulation time and working temperature must be kept under control,since this affect the release of ECP in serum samples!Plasma and hemolyzed serum cannot be used.

The laboratory procedure of Pharmacia CAP system ECP FEIA is as follows:

- blood is collected by venipuncture filling the tube(Becton-Dickinson vacutainer SST) completely(2.5-4 ml)and after that gently invert the tube 5 times
- clot for 60-120 minutes(min) at room temperature(RT)between 20-24^{0}C.The clotting procedure is important step and requires special handling. It represents the first incubation in the procedure.The ECP is to be *reproducibly* released from the activated eosinophils.Special blood collection procedures may be therefore required.
- Centrifuge at 1000-1300 g for 10 minutes at RT
- Transferring serum into a new tube
- Test principle,incubation time (inc) and temperature:pre-wash $\Rightarrow$ reaction step 1: antigen (Ag)-antibody(Ab)reaction in pts' samples stndards and controls+ 1st inc - 30 min at RT; $\rightarrow$ sample wash with remove of excess sample $\Rightarrow$ reaction step 2:conjugate reaction in pts' samples, standards and controls + 2nd inc - 150 min. at RT;$\rightarrow$conju gate wash with remove of excess conjugate $\Rightarrow$ reaction step 3:development reaction in pts' samples,standards and controls + 3rd inc - 10 min.at RT $\rightarrow$ elution and measure ment(FEIA:total IgE/specifi cIgE). Total process time-2.5 hours.
- reading of results : from a constructed curve of calibration using 5 calibrators with a known concentration (2,5,15,100,200 μg/l)of ECP.The introduced curve is memorized by the computer for about one month.Using the described curve of calibration and one control serum,we are able to measure the ECP serum concentration for all used pts' serums with unknown ECP quantity. The controls of the curve(specific IgE,total IgE or ECP)estimate whether the introduced curve of calibration is valid for a reagents utilized in the procedure.
- Specification:UniCAP-ECP FEIA as a test for "in vitro"ECP quantification in human serum; measuring range:2-200 μg/L; detection limit:< 0.5 μg/L.The range may be extended by sample dilution with ECP diluent
- Precision:total coefficient of variatuion is smaller than 10%(within and between assay).In our own experience: between 0.2 and 2.6%;Recovery:between 87%and 103%;Specificity:cross reaction with eosino-phil protein X(EPX) is smaller than 0.02%;Expected values:for healthy subjects(CG,N^0 = 99):geometric mean=4.4 μg/L;90 and 95 upper percentile:10.1 and 11.3 μg/L >;Values for our own CG:X_{CGECP} = 2.03± 0.02 μg/L(as a arithmetic mean).
- Storage: RT-maximum 24 hours;+(2-8)^{0}C-maximum 5 days;(- 20)^{0}C-if analyzed later
- Needed volumes of reactants: sample/stan-dard - 50 μl;enzyme-anti ECP - 50

μL;development - 50 μl;stop solution - 400 ml. Each original package contains reagents for 96 determinations, sufficient for 43 samples and one standard curve in duplicate. All the reagents are ready for use[7,8].

Results:

The results for serum ECP concentration for uremic pts(with/UPG/ or without pruritus/UG/)and appropriate control group/CG/(age and sex matched) are presented in the next table 1:

*Table 1:***The investigated subjects(N^0 =18)**

NUMBER	*SEX*	*AGE(years,Mean±SEM)*	*ECP(μg/l,Mean±SEM)*
Control group			
1.	M	26	≤ 2.00
2.	M	36	≤ 2.00
3.	M	54	2.07
4.	F	49	2.06
5.	F	61	≤ 2.00
total: 5 subjects	(M=3;F=2)	(45.20±6.30)	(2.03±0.02)
Uremics(without pruritus)			
6.	M	22	2.74
7.	F	16	2.37
8.	F	59	4.10
9.	M	30	3.15
10.	F	65	≤ 2.00
11.	F	59	2.08
12.	M	70	≤ 2.00
total:7 subjects	(M=3;F=4)	(45.86±8.43)	(2.63±0.29)
Uremics(with pruritus)			
13.			
14.	F	32	4.73
15.	M	44	6.10
16.	M	58	6.72
17.	M	29	10.40
18.	F	40	13.90
total:6 subjects	M	39	95.60
	(M=4;F=2)	(40.33±4.19)	(22.91±14.60)
Total(all groups)	(M = 10;F = 8)	43.83 ± 3.81	9.22 ± 5.14

The *table 2* presents the sECP concentration in investigated subjects with the statistical significance of Means difference(Z) between the analyzed groups.

*Table 2:***Serum ECP concentration in control group and our uremic patients with or without pruritus**

GROUPS	*Serum ECP concentration (X ± SEM,in μg/l)*	"p"(CV)** / Significance of Means difference(Z)
Control group/CG/(N^0 = 5)	2.03 ± 0.02	**p < 0.001(2.00%)**
Uremic pruritus group* /UPG/(N^0 = 5)	8.37 ± 1.67	**p < 0.05(156.14%)**
Uremics without pruritus /UG/(N^0 = 7)	2.63 ± 0.29	**P < 0,005(29.43%)**
$\mathbf{Z}_{CG \leftrightarrow UPG}$		**3.80** SEM
$\mathbf{Z}_{CG \leftrightarrow UG}$		2.02 SEM

$Z_{CG \leftrightarrow UG+UPG}$		2.63 SEM
$Z_{UG \leftrightarrow UPG}$		**3.38** SEM

*****-one patient(case $N^0$18) is not taked in consideration because an extremely high value for serum ECP(95.6 μg/L)
**- CV = coefficient of variation

From the precedent tables is evident almost the same frequency in men and women(males = 10,females=8),with the mean age(all groups included) of about 44 years.The serum concentration of ECP is lowest(≈ 2 μg/l) in the CG with coefficient of variation(CV) under 2%!The UG is not so compact regarding sECP with CV ≈ 30%,and finally the uremic pts suffering from intractable itching(UPG) are heterogenous group with more elevated level of sECP(at $p < 0.05$),and a very high CV(156%). The statistical significance of differences between mean values for sECP in investigated groups is sufficient(Z >1.96 SEM) but especially evident comparing the CG and UPG(Z= 3.8 SEM),respectively UG and UPG(Z >3.3 SEM).
Bearing in mind the data from the literature, values for sECV over 15 μg/l should be considered elevated.However,there are a significant difference in distribution and reproducibility for sECP between geographically different control groups of healthy subjects for normal sECP values(25 years old subjects;sECP ≈15-20μg in Sweden,20-25 μg/l in Norfolk,UK and 2 μg/l in our own experience!).Reference equation for sECP(healthy men) is as follows:$lg_{10}ECP=1.3966-[(age-20)x0.0057]$[9]

4 Discussion:

It is very good known the evolution of skin allergy: begenning in the first years of life(1-2)with the allergy prevalence of about 15% and that stabilise at the value of about 10% in the next decade. After the 12 years of life the skin atopie diminishes with the tendency to be near a healthy state[10].The total and specific amount of IgE in human serum(tIgE,sIgE)are the measure of total and specific allergenic stimulation and sensitization using the uniCAP Pharmacy system.tIgE represents a total amount of IgE antibodies(abs)that are produced as a result of repeated allergenic(multiple) stimu-lation.The titre correlates with the level of allergy load and the patient's atopic state maintained during adult life.Measuring sIgE levels helps to identify provoking allergens(like ECP in our case),predicting the risk of developing allergy and guide clinical decision.For some(food,endogenous..) allergens the $KU_{A/l}$(kilounits allergen per liter)value of sIgE can be an useful "optimal decision point"when deciding whether to proceed with double blind placebo controlled challenges in pts with atopic or uremic allergodermatitis[11].Nevertheless,the healthy individuals have a very low level of sIgE(< 35 kU_A/l,uniCAP sIgE detects IgE abs in the range:0.35 to 100 kU_A/l).ECP is quantitatively detected using a titer of sIgE(ECP FFIA)in serum also ensuring the correct antigen confirmation corresponding to native counterpart in human tissue(tissue ECP)[12].ECP measures the level of eosinophil cationic protein released in serum from activated eosinophils(Eo)during the inflammatory process.ECP is a higly cytotoxic protein found in Eo granules.As is known ,Eo are the cells chiefly responsible for produ-cing the infammation caracteristic of atopic states,especially of bronchial asthma[13].When Eo in the skin are activated,they undergo degranulation,ECP release causing deep skin cells damage,skin inflammation/desquamation and thereby irritation of the sensitive skin organs(like *Paccini's corpuscula*) and/or the beginning fibres of spino-thalamic way clinically manifested as a protracted pruritus.This one may increase hypersensitivity and lead to chronic inflammatory disease of the skin("uremic skin").Pts with atopic states and Eo induced inflammation have elevated levels of sECP and probably other body fluids such as bronchial,alveolar fluid,sputum or fluid from skin atopic lesions.The amount of sECP is a an objective indicator of Eo involvement in chronic atopic inflam-mation..Thus,from the clinical utility point of view,it is extremely important to analyze the severity of the disease in atopic pts and to determine the effectiveness of treatment.Measuring ECP in a serum sample may be a direct way of estimating the severity of the skin inflammation(atopic aspects of chronic uremic pruritus)and following the course of the disease,for guiding medicaments treatment in atopic states and to find non-compliant pts.
Eosinophils are inflammatory cells produced in the bone marrow that travel through the blood and that are attracted to the target organs(lung,nasal,conjunctival mucosa or skin)in pts with atopic disease .IL-9(whose serum level is higly elevated in allergic states) accentuates Eo function by increasing their survival and by inducing IL-5R expression on Eo,that encances Eo differentiation and precipitates Eo induced infammation.IL-5 inha-lation may increase degranulation of Eo granules containing ECP,EPX,major basic protein(MBP) and Eo

peroxidase having an important role in host defense mecha-nisms.The activated Eo,through the degranulation process,release their toxic granule proteins on adjoining tissues or tumor cells,and often are engaged in the tissue or tumor cells apoptosis. During skin allergic reaction ICAM-1 is locally upregulated(under the influence of IL-4,sCD-14 ad sSelectin)mediating also Eo activation and migration to skin epithelium with or without activation of circulating Eo or their involvement in skin allergy process.[14]In the skin attracted Eo release substances that damage the deep skin structures or contribute to the perpetuation of skin inflammation process.The skin infection may be also involved in the genesis of pruritus probably by local tissue impregnation of microbial antigens into to skin(mainly streptococcal or staphylococcal allergens).The bacteria,viruses,fungi endo/exoallergens or environmental factors provoke different cells activation (macropgages,basophyles,mast cells,lymphocytes)and mediators release (Eo,neutrophils,plasmacells..) with pathological skin responses like oede-ma,glandular hypersecretion, vascular lesions with plasma leakage,nervs activa-tion,augmented epithelial permeabilty with Eo infiltration in deeper skin epithelia ,mus-cle contrction(mm.erectores pylorum),connective tissue neoformation and clinical sym-ptoms like pruritus and pain[15].Serum ECP may be transitory but extremely elevated in non-atopic pts with acute or exacerbated pruritus(in our serie the case N^0 18,sECP= 95.6 μg/L),but some authors can not find relation between sECP,symptoms score and medi-cation efficacy.[16,17].In this cases cortiosteroid therapy restores the skin normal state,removes prurirtus,and in addition to this,sECP levels decreases.

Hypereosinophilia is frequently observed in hemodialysis pts without apparent rea-sons.Following one study,the pts may be divided in three groups:non-Eo,intermitent Eo and permanent Eo pts(16.5%),but without difference in sECP levels within the groups.The cause of Eosinophilia is not merely due to the uremic state or dialysis procedure per se.[18].

Theoretically,the allergic component of uremic pruritus may be associated with skin deposition of many medicaments,contact or nutritive allergens(epidermal,plants and animal proteins) bearing in mind the allergens accu-mulation in the body after injection,ingestion or direct contact due to kidney failure("skin implanted aller-gens").Mainly is a case for food's plant or animal antigens and especially gelatine, ethylene oxide, protamine sulfate/in pts with protracted bleeding related to heparinisation/, formaldehid formation or dialysis membranes related allergens.The cited antigens may be *in situ* detected using a specific reagents like a monoclonal antibodies:anti human ECP(EG_1) and antihuman ECP/EPX(EG_2)[19].

Severity of allergic symptoms may be classified following the sIgE abs titer from :0-I(allergen not detected);to II-IV(moderate, increased or severe sensitivity where the allergen is not the major cause of the symptoms or he is the main cause.In food allergy pruritus may be present in few pts but the circulating IgE abs may remain undetectable despite a clinical symptoms, because these abs may be directed towards allergens that are revealed or altered during industrial processing, cooking or digestion and therefore do not exist against the original(tested) food(false-negative test)[20].The egzo/endotoxins accumulation in uremic body in relation to chronic excretory kidney failure(elevated level of denaturated proteins, advanced glycated or oxidated end peptides with or without high cells/nuclear/damages) may complicate the situation and provoke pruritus with extremely unpredictable and hardly revealing mechanisms.

A part of classical and many other therapeutic issues(UV skin exposure, systema-tically used xylocain , locally applied lubricants and ointments,parathyroidectomy, inorganic phosphorous bowls helators),the treatment of chronic uremic itching is still difficult and controversial.The systemic or local antihistamines may be useful or without effect.Topical application of immunosuppressive drugs(especially tacrolimus/FK-506/ and ascomycine-derivatives) is available.The inhibition of T-cells activation is proposed as a mechanism of action.Topical ointment(0.03-0.1%) of FK-506 is today acceptable,but the side effect of drug(burning of the skin) is not so popular. The interferon γ,applicated subcoutanely,is a potent Th-1 like cytokine inhibitor which diminishs the number and activity of circulating Eo[21].Corticoids are,still in our days,very often used drugs(NF_k blocking agents mediated activity).They considerably reduce the sECP concentration nearly the normal values,restore the normal skin state(pruritus removed)but their effect diminish with the time(hysteresis phenomenon).

5 CONCLUSION

Because theirs systemic adverse effects and/or local skin atrophy, there are not recommended for a long way therapy. Uremic pruritus(UP) may be scored as a mild(40-50%),moderate(30-40%)or severe (about 10%).Serotonin and histamine (especially histamine x serotonin product)as a possible mediators for UP are pretty recognized many years ago.This is an explanation for good acceptance of antihistaminic therapy and known effect of 5-HT_3-receptors selective inhibitors like ondansetron(2x4 mg/day)[22].The intensity of UP correlates with the higher serum Al concentration and higher (Ca x Pi)-product as an additional factors predisposing to pruritus[23].CsA and etretinate do not decrease

the sECP(after ten weeks of therapy the sECP may increase in 50% of pts!) but the number of circulating Eo diminish with pruritus relief[24].

Finally, the optimal dialysis improves UP.S ignificantly higher values of BUN and plasma β_2 microglobulin were observed just before the dialysis session in pruritic pts with lower dialysis efficacy estimated by eKt/V score and bad protein catabolic rate($\leq$1 g/kg/bw/ day).The higher dialysis efficacy(eKt/V score increased for more than 25-30%) with good controlled nutritional and iron state,reduces the prevalence and degree of UP in dialyzed pts[25].

Literature:

1. Torazza MC,Salomone M,Albanesi T,Deabate MC,Zina A,Triolo G. Il prurito uremico.Minerva Urol Nephrol 1997;49(3):125-132
2. Greaves MW. Itching-research has barely scratched the surface.New E J Med 1992;326:1016-1017
3. Fantini F,Baraldi A,Sevigliani C,Spattini A,Pincelli C,Gianneti A. Cutaneous innervation in chronic renal failure patients.Acta Dermatovenereologica(Stockholm)1992; 72:102-105
4. Steensen N. Nervous tissue. In Textbook of histology,Ed.by Geneser F,1986, Munksgaard,Copen-haagen,pp 285-332
5. Dimkovic N,Djukanovic L,Radmilovic A,Bojic P,Juloski T. Uremic pruritus and skin mast cells. Nephron 1992;61:5-9
6. Liappis N,Starke A. Evaluation des immunoCAP systems in der *in vitro*-allergiediagnostic,Aller-gologie 1997;20(6):291-295
7. Paganelli R,Ansotegni IJ,Sastre J,Lange CF,Roovers MH,de Groot H,Lindholm NB,Ewan PW. Specific IgE antibodies in the diagnosis of atopic disease.clinical evaluation of a new *in vitro* test system,UNI CAP^{TM}.in six European allergy clinics.Allergy 1998;53:763-768
8. Johanseen SG,Yman L. *In vitro* assay for immunoglobulin E.Methods,indications and interpretation.Clin Rev Allergy 1988;6:93-139
9. Marks GB,Kjellerby J,Luczynska CM,Burney PG. Serum eosinophil cationic protein:distribution and reproducibility in a randomly selected sample of men living in rural Norfolk,UK. Clin Exp Allergy 1998;28(11):1345-1350
10. Zimmerman B,Forsyth S. Diagnosis of allergy in different age groups of children:use of mixed allergen RAST discs,Phadiatop and Pediatric mix. Clin Allergy 1988;18:581-587
11. Sampson HA,Ho DG. Clinical aspects of allergic disease.Relationship between food-specific IgE concentrations and the risk of positive food challenge in children and adolescents. J Allergy Clin Imminol 1997;100:444-451
12. Ahlstedt S. Mediators in allergy diagnosis. ACI International 1998;10(2):37-44
13. Kristiansson S,Strannegard IL,Wennergren G. Inflammatory markers in childhood asthma. Ann Med 1996;28(5):395-399
14. Furue M,Koga T,Yamashita N. Soluble E-selectin and eosinophil cationic protein are distinct serum markers that differentially represent clinical features of atopic dermatitis. Br J Dermatol 1999;140(1):67-72
15. Zimmerman B. Clinical experience with measurement of eosinophil cationic protein. Clin Exp Allergy 1993;23,Suppl 2:8-12
16. Oh JW,Shin JC,Jang SJ,Lee HB. Expression of ICAM- on conjunctival epithelium and ECP in tears and serum from children with allergic conjunctivitis. Ann Allergy Asthma Immunol 1999;82(6):579-585
17. Winter L,Moseholm L,Reimert CM,Stahl Skov P,Kaergaard Poulsen L. Basophil histamine release,IgE,eosinophil counts,ECP and EPX are related to the severity of symptoms in seasonal allergic rhinitis. Allergy 1999;54(5):436-445
18. Lee CC,Leu ML,Kuo HP,Huang CC. Hypereosinophila in hemodialysis patients. Chang King I Hsueh Tsa Chih 1995;18(1):20-26(English)
19. Pharmacia &Upjohn Diagnostics AB.Specific research reagents:monoclonal antibodies;Diagnos-tic product catalogue “Millenium”. Internal basic product documentation 1999;Uppsala,Sweden
20. Sigurs N,Hattevig G,Kjelman B,Kjelman N-IM,Nilsson L,Bjroksten B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. J Allergy Clin Immunol 1994;94:757-763

21. Reinhold U,Kukel S,Brzovka J,Kreysel HW. Systemic interferon gamma treatment in severe atopic dermatitis. J Am Acad Dermatol 1993;29(1):58-63
22. Balaskas EV,Bamihas GI,Karamouzis M,Voyiatzis G,Tourkantonis A. Histamine and serotonin in uremic pruritus:effect of ondansetron in CAPD-pruritic patients. Nephron 1998;78(4):395-402
23. Friga V,Linos A,Linos DA. Is Aluminium toxicity responsible for uremic pruritus in chronic hemodialysis patients? Nephron 1997;75(1):48-53
24. Schopf RE,Hultsch T,Lotz J,Brautigam M. Eosinophils,pruritus and psoriasis:effect of treatment with etretinate or cyclosporin A. J Eur Acad Dermatol Venereol 1998;11(3):234-239
25. Hiroshige K,Kabashima N,Takasugi M,Kuroiwa A.Optimal dialysis improves uremic prurirtus. Am J Kidney Dis 1995;25(3):413-419

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