Annotation
thesis for the PhD doctor's degree
on the theme "Molecular-cellular predictors of chronic kidney diseases"
Author: Assel Nurgaliyeva

Relevance

Nowaday, more than 10% of the population in the world suffer from Chronic Kidney Disease (CKD). Such scale of spread of this disease allows to consider CKD as an epidemic (Тугушева Ф.А., Зубина И. В., 2009).

Development of CKD is followed by membranodestructive processes and as a result leads to the structurally functional disorganization of kidney membranes. One of the reasons of development of a membranodestruction is the oxidizing stress. One of important conclusions of researches of the last years is that processes of a lipopreroxidation developes at the earliest stages of CKD, increasing the risk of formation of disfunction of an endoteliy, cardiovascular diseases and other complications and associated diseases. Processes of lipoperoxidation are also considered as one of factors of progressing of CKD and development of a terminal renal failure (Тугушева Ф.А., Зубина И. В., 2009; Gall J., Seibold S., 2003). Процессы липопероксидации также рассматриваются как один из факторов прогрессирования ХБП и развития терминальной почечной недостаточности (Spimizu M.N.M., Coimbra T.M., 2005).

Active forms of oxygen and toxic products of a lipoperoksidation are capable to interact with proteins and nucleinic acids. In the first case various oxidized proteins, in – the second the modified nucleinic acids are formed. An intensive influence of pro-oxidizers can lead to fragmentation of proteins and nucleinic acids (Gutowski M., Kowalczyk S., 2013). The relevance of a research in this direction is documented by the fact that the oxidized proteins are capable to render both the direct detrimal effect and mediated, influencing redoks-regulation or by stimulation of formation of jet metabolites of oxygen. From these positions it is offered to consider, in particular, AOPP as a new class of kidney pathogenic mediators (Плешкова Е.М., 2014). The ratio of various types of the oxidized proteins, as well as an orientation of their change in plasma and cells at patients with CKD of various stage are almost not studied.

Oxidizing damage of nucleinic acids is considered as one of the reasons of increase in extracellular nucleinic acids. To other reasons of an exit of nucleinic acids in a blood-groove is an various forms of cellular death, including the creation of the neutrophyl of extracellular traps.

Research neutrophyl extracellular traps at a chronic disease of kidneys wasn't established.

Increase in maintenance of fragments of extracellular DNA in circulation is positioned as a factor of a stress an alarm system at an oxidizing stress now (Ermakov, A.V., Konkova M.S., Kostyuk S.V. et al., 2013). Besides, it is shown the role of extracellular nucleinic traps as modifiers of the immune answer, and also their influence for the state of endoteliy condition and hemostasis system (Муравлева Л.Е., Молотов-Лучанский В.Б., Клюев Д.А. и соавт., 2010; Туаева Н.О., Абрамова З.И., Мустафина Д.М., 2008.; Gahana P B., Swaminathan R., 2008 ; Pisetsky D. S. , 2007).

The ambiguous orientation of change of the extracllular traps at patients with glomerulopatiya and tubulopatiya is established by earlier conducted researches (Муравлева Л.Е., Молотов-Лучанский В.Б., Телеуов М.К. и соавт., 2010). At patients with the CKD of various stage the research of extracellular nucleinic acid wasn't conducted.

It is prespective to research of the extracellular purines nucleotides and products of their catabolism in blood of patients with CKD at various stage. Need of a research of extracellular purin and products of their catabolism is caused by their participation in regulation of kidneys function and a vascular tone, and also to act as molecules - alarmin.

Change of a ratio of extracellular purin and products of their catabolism at patients with tubulopatiya and glomerulopatiya is shown by earlier conducted researches and the working

hypothesis of participation of extracellular purin in development of the pathology of kidneys associated with an arterial hypertenzion is formulated (Muravlyova L.E., Molotov-Luchanskiy V.B., Teleuov M.K. et al., 2010; Muravlyova L.E., Molotov-Luchanskiy V.B., Kulmagambetov I.R. et al., 2011).

It is not almost studied the metabolism of neutrophils of blood of patients during the progressing of CKD. Researches in this direction are of unconditional interest as possible metabolic changes in neutrophils, including development of an intracellular oxidizing stress, can't but have significant effect on behavior of these cages during the progressing of CKD.

The analysis of structure-forming properties of plasma of blood of patients at CKD of various stage wasn't carried out. Identification of various patterns of structurization, comparison to biochemical parameters of plasma of blood at patients with CKD represents an unconditional value both for understanding of mechanisms of progressing of CKD, and from search of new diagnostic and predictive markers.

In general, it should be noted that researches in the declared directions will allow to deepen theoretical ideas of features of metabolic changes at various stages of CKD that is a necessary basis for development of new therapeutic approaches to delay of rates of progressing of CKD.

Research objective

To define the molecular and cellular predictors of development and progressing of a Chronic Kidney Disease.

Research problems

1. To determine the level of the circulating of nucleinic acids, extracellular intermediat of the catabolism of purin in plasma of blood of patients with CKD at the various stages.
2. To define a possibility of creation an extracellular neutrophiles traps in blood of patients with CKD at the various stages.
3. To study an indicators of oxidizing modification of proteins and peroxidation of lipids in plasma of blood and lysates of neutrophils of patients with CKD at the various stages.
4. To study patterns at the morthotypes of thesiogramms of blood's plasma with CKD at the various stages.
5. To reveal the molecular predictors of development and progressing of Chronic Kidney Disease.

Scientific novelty and theoretical significance of the research:

At the first time it was revealed the consequence increasing of the extracellular purine bases (adenine, a guanine) and intermediates of purin's catabolism (xanthene and hypoxanthene) in plasma of blood of patients depending of the stage of CKD.

At the first time it was established the reliable growth of an Extracellular Ribonucleic Acids in plasma of blood at patients with the CKD stage 1 and 2.

Development of a chronic renal failure is followed by sharp decrease of the Extracellular Ribonucleic Acids in plasma of blood of patients.

It was for the first time established that the level of Extracellular Deoxyribonucleic Acid authentically increases in plasma of blood of patients with CKD, but at patients with CKD stage 5 there was sharply decreasing with an antibate growth of acid-soluble predecessors.

It was revealed that at patients with CKD stage 3 at the debut of a Chronic Renal Failure, the content of jet carbonyl derivative proteins authentically increases in plasma of blood and a lysate of neutrophils.

It was revealed the creation of the spontaneous extracellular neutrophiles traps in blood at 33% of patients with CKD stage 1, at 5% with CKD stage 2, at 25% of patients with CKD stage 3, at 29% of patients with CKD stage 4 and at 35% of patients with CKD stage 5.

It was revealed the differences for both - as for the quantity of morthotypes of tesiogrammes in the blood's plasma and for the patterns of their structure-construction at patients with the various stage of CKD.

For the first time it is offered to consider as predictors of development of the CKD such indicators as extracellular nucleonic acids.

For the prognostication of a Chronic Renal Failure it is offered to define in plasma of blood of patients an intermediates of purin's catabolism - oxypurine and xanthene, and also jet carbonyl derivatives.

Practical importance

It is offered for introduction in practice definition of morphotypes of thesiogrammes in blood plasmas for the receiving a dynamic picture of change of the biochemical status of plasma of blood, during the progressing of CKD, and also for assessment of efficiency of a hemodialysis at patients with CKD 5 stages.

Introductions of results into the practice

The obtained data are introduced and "Molecular bases of pathology" are used in educational process of department of biological chemistry when training on elective disciplines (bachelor degree) and "Biochemistry of pathological processes" (magistracy).Materials and methods.

The main provisions for the defense

In plasma of blood of patients with the CKD the contents of the extracellular purine bases (adenine, a guanine) and intermediates of a catabolism of purin (xanthene and oxypurine) is increase, at the same time extent of increase depends on the stage of the disease.

In plasma of blood of patients with CKD stage 1 and 2 it is increasing the contents from Extracellular Ribonucleic Acid .

During the accession and progressing of Chronic renal failure the contents of the Extracellular Ribonucleic Acid in blood's plasma is sharply decreasing.

At patienst with the CKD in blood's plasma the level of Extracellular Deoxyribonucleic Acid is increasing. During the development of the terminal Chronic renal failure the level of Extracellular Deoxyribonucleic Acid sharply decreases, but the level of acid-soluble predecessors increases.

At patients with CKD stage 3 in plasma of blood and a lysate of neutrophils it is authentically increasing the content of jet carbonyl derivative proteins.

At the part of patients with CKD it was revealed the creation of the spontaneous extracellular neutrophiles traps.

Patients with various stage of CKD have the changing of structure-forming properties of blood's plasma with the formation of several dynamic types at different forecasts of an outcome of a disease.

Extracellular nucleinic acids can be considered as predictors of development of CKD.

The determination of the concentration of oxypurine, xanthene and jet carbonyl derivative proteins in plasma of blood at patients with the CKD can be used for the prognostication of development and progressing of the Chronic Renal Failure.

Connection of the thesis with other research works

The thesis was carried out at department of biological chemistry within the research project of MES RK "Molecular and cellular mechanisms of development of a chronic renal failure" (2013-2015), GR No. 0113PK00410.

Work approbation

Results of a research are reported at the International conference of young scientists "The world of science and youth: traditions and innovations" (on February 19, 2014, Karaganda, Kazakhstan); at the scientific session of the Perm state medical university of a name of the academician E. A. Wagner "Towards to the 100 anniversary of the higher medical education in the Urals" Perm, 2015; at the international conference "Young Scientists of Orenburg Oblast — Science of 21 Centuries", Orenburg, 2015), on 40 FEBS Congress "The Biochemical basis of life" (Berlin Germany, 4-9 July 2015); on the VI All-Russian school conference with the

international participation "Blood circulation physiology" (Moscow, Russia, on February 2-5, 2016).

Data on publications

By results of a research it was published : the Monograph "Structure-forming Properties of Blood at Patients with a Chronic Kidney Disease" (approved by the Republican center of development of health care), 12 publications, including 2 articles and 1 thesis in the editions indexed in Scopus and Thomson-Reuter; 3 articles in the editions entering to the list of KKSON; was received 3 certificates on registration of a subject of copyright.

Materials and methods

On the basis of branch of the City hospital No. 1 of the Karaganda city, clinic of LLP "Hyppocrate", policlinic of KSMU ,Scientific and biochemical laboratory at department of Biological chemistry of KSMU it was leaded the clinical examination of the patients having the Chronic Kidney Disease (CDK) in 5 groups (control group; groups of patients with CKD 1, CKD2, CKD3, CKD4 and CKD5 respectively).In the clinical observation took part 117 people. To all participants of a research the following methods examinations have been conducted: the main (inquiry, an objective research, including a palpation, percussion, an auscultation of internals) and additional (measurement of arterial pressure, the ECG, special tests of urine across Nechiporenko and Zimnitsky, ultrasound examinations of kidneys and abdominal organs). Survey at the oculist and the neuropathologist. Obligatory methods of laboratory researches were the general test of blood and urine, biochemical tests of determination of the creatinine level and urea in blood. Assessment of the speed of a glomerular filtration and an indicator of a channel reabsorption which was carried out by Reberga-Tareev's method and using the Kokroft and Gault's formula (SKF Cockroft-Gaul formula).

In plasma of blood it was defined the maintenance of two types it is oxidized the modified proteins: advanced oxidation protein products (AOPP) which was determined by the Witko-Sarsat et al method. Content of the jet carbonyl derivative proteins was determined by the method of by the R.L. Levine et al.

In plasma of blood it was registered the level of malondialdehyde in reaction with thiobarbituric acid by method of Goncharenko M. S., Latipova A. M. Level of a guanine (G), adenine (A), oxypurine (GKS), xanthene (Kc) and the uric acid (UA) determined spectrofotometric method by the E.V Oreshnikova and coauthors. In blood plasma it was also registered contents of the extracellular desoxiribonucleic acid , rebonucleis acid and acidsoluble fraction of predecessors of nucleinic acids by the method of L.I. Markusheva and coauthors.

For determination of quantity of the spontaneous extracellular neutrophiles trips in blood of the examined persons was used I. I. Dolgushin and Yu. S. Andreyeva's methodical method.

Patterns of structurization of plasma of blood was determined by the method of wedge-shaped dehydration of Shatokhina S. N.

For the assessment of the degree of an interrelation at the studied indicators was used the nonparametric correlation analysis of Spirmen.

Conclusions

1. Content of extracellular nucleonic acids, and also the purine bases, xanthene and oxypurine depending on a stage of a chronic kidney disease increases in plasma of blood of patients.

2. In plasma of blood of patients with CKD are recorded the ambiguous trends of change of Extracellular Nucleonic Acids . Reliable increased the contents of Extracellular Ribonucleic Acid in plasma of blood of patients with CKD stage 1 and 2 . At accession of a chronic renal failure this indicator sharply decreased. The Level of Extracellular deoxyribonucleic acid at patients with the CKD stage 2 and 4 authentically exceeded the level at the control group in plasma of blood.

In plasma of blood of patients with CKD stage 5 the contents of growth of Extracellular deoxyribonucleic acid decreased lower than at control group with the authentic increasing the acid-soluble predecessors.

3. In lysates of neutrophils and plasma of blood of patients with CKD it was observed the tendency to increase the content of jet carbonyl derivative proteins, but reliable differences from control group were founded at patients with the CKD stage 3. The AOPP level authentically increased in plasma of blood of patients with CKD stage 5.

4. At 33% of patients CKD stage1 , at 5% of patients with CKD stages 2, at 25% of patients with CKD stage 3, at 29% of patients with CKD stage 4 and at 35% of patients with CKD stage 5 in blood there were observed the creation of spontaneous extracellular neutrophiles traps. The quantity of the extracellular neutrophiles traps varied from 1 to 14.

5. At patients with CKD of 1 stage there were revealed 6 morphotypes of thesiogrammes of blood's plasma, whereas at patients with CKD stage 2 the number of morphotypes was reduced to 2 . At patients with CKD stage 3 and 4 it was revealed only one type of thesiogrammes, whereas at patients with HBP 5 was revealed 2 options of morphotypes.

6. It is offered to include Extracellular Ribonucleic Acid to the list of DAMPs at patients with CKD stage 1. At the CKD stage 2 it is optimum to add to the list of DAMPs an extracellular deoxyribonucleic acid, extracellular ribonucleic acid, the purine bases, xaintine and hypoxantine. In a debut of a chronic renal failure it is expedient to include to the list of DAMPs the oxypurine, xanthene, jet carbonyl derivatives of proteins.

7. It is offered to use the thesiogrammes as additional criterion of progressing of Chronic Renal Failure and an assessment of the efficiency of the carried-out treatment.