ANALYTICAL METHOD — INTO PRACTICE

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DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF DETERMINATION OF NATURAL AUTOANTIBODIES TO RENAL ANTIGENS IN DEVELOPMENT OF PYELONEPHRITIS IN CHILDREN

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It is well-known, that healthy people's body produces natural autoantibodies (NAAB) to antigens of its own organs and tissues in the course of the whole life. It is anticipated that such NAAB take part in body clearance from natural catabolism products and participate in regulation of many physiological functions [1, 2]. Serum content of NAAB of the same specificity in healthy people is relatively stable and levels of their synthesis and secretion normally vary individually very little and very little depend on sex and age [1]. It has been established that, normal content of serum NAAB of various specificity in young children ranges from -40 to -10 conventional units (c. u.) [3]. At the same time development of pathological processes is accompanied by significant changes of production and serum content of NAAB of corresponding organ specificity. In some cases these changes may be primary (and form the basis of the disorder in question) and in other cases they may be secondary with respect to the pathochemical changes of organs originating from some other (non-immune) etiology [1, 4, 5]. Development of any disease on the molecular level is accompanied with disrupted synthesis and degradation of organ cell components, leading to changes in production of organ-specific NAAB, which may be regarded as marker of the onset (presence) of pathological changes in the organ [6]. Changes of content of these disease-predictors may occur many months or even years prior to clinical manifestation of the disease [3, 5].

Natural history of pyelonephritis (PN) comprises nonspecific (inflammatory) and specific (immunological) stages that are mutually conjugated and constitute an integrated mechanism of pathogenesis. At the stage of specific immune inflammation the basis of the pathological process consists of infiltration of the kidney interstitium with lymphocytes and plasmocytes, intensive synthesis of the NAAB to renal antigens, formation of immune complexes and their deposition on basal membranes of the kidney tubules with release of biologically active lymphokines enhancing destruction processes [7]. As PN becomes chronic interstitial fibrosis and tubules dilation occur; their generalized atrophy also may occur along with flattening and dystrophy of epithelium and thickening of basal membranes of the tubules [8]. Pathological process progression in renal parenchyma most probably should lead to changes of production of nephrotropic NAAB that can be determined by registering changes of their serum content.
The aim of this study is to establish diagnostic and prognostic significance of measuring serum level of NAAB to renal antigens in healthy newborns, in children of the group of high risk of development of urinary system pathology and in patients with clinically manifested PN.

Materials and methods of the study

The study comprised 106 children: two observed groups (n=96) and one reference group (n=10).

The 1st observed group (high risk group) comprised 56 newborns. The majority of their mothers (82.1%) had suffered from gestation PN, and the others (17.9%) were diagnosed with inflammatory diseases of pelvic organs (PID) of chlamydia (Chl. trachomatis) or mycoplasma (M. hominis) etiology, without signs of PN. The newborns were examined on the 5th-6th day of their life, then at the age of 6 months and afterwards they were under 2.5-year observation, in the course of which the children underwent clinical examination and urinalysis and, if necessary, ultrasonography of the urinary system and renal function tests once every three month.

The 2nd observed group comprised 40 children aged from 3 months to 16 years with verified diagnosis of PN (from 3 months to 3 years - 12 patients; from 3 to 7 years - 10; from 7 to 10 years - 6; and from 10 to 16 years - 12 children). 8 children had acute and 32 – chronic PN (recurrent course of the disease in 16

and latent course in the other 16). Obstructive PN was found in 12 children and 6 of them had ureterohydronephrosis. Duration of PN in 20 children (50%) was more than 5 years and in 8 (20%) - from 1 to 5 years, in 4 (10%) – less than 1 year, and 8 (20%) patients had newly diagnosed PN. Children with PN were under observation in the nephrology department, where they underwent regular clinical and laboratory examination (analyses of blood and urine, urine culture, assessment of partial renal functions, etc.) and various instrumental diagnostic procedures (kidney ultrasonography and, upon education, excretory urography, miction cystography cystourethrography, cystoscopy, uroflowmetry, etc.).

The reference group comprised 10 healthy mature newborns from somatically healthy mothers without INPO and/or signs of other infectious inflammatory diseases.

Serum content of NAAB in all children of all groups was measured using certified test systems ELI-Test (Immunculus Medical Research Center, Moscow). Content of nephrotropic NAAB was measured by means of immunoenzyme analysis (IEA) using ELI-Nephro-Test method [1]. We measured the content of the IgG class NAAB to the proteins of renal parenchyma: KiM-05-40 (anionic protein of the membrane fraction of the kidney with molecular weight about 40 kD), KiM-05-300 (anionic protein of the membrane fraction of the kidney with molecular weight about 300 kD), KiS-07-120 (protein with pronounced anionic properties relating to the cytoplasmic fraction of the kidney with molecular weight about 120 kD) [1]. With the help of IEA (ELI-AIM-Test) we also measured the serum content of NAAB to the double-helix DNA and to Fc-fragments of the immunoglobulins (Ig), better known as rheumatoid factor (RF) [1]. Changes of the RF content reflect the overall level of non-specific polyclonal activation of the immunity system (during inflammatory process of whatever location). Simultaneous measuring of the level of nephrotropic NAAB and of the RF (NAAB to the double-helix DNA and Fc-fragment of the immunoglobulin Ig), makes it possible to distinguish between isolated (specific) lesions of renal parenchyma and the overall (nonspecific) enhancement of body immunoreactivity [1].
Immediately prior to testing the serum of the subjects under study was diluted to 1:200 by 0,15 M NaCI, the samples were put into the wells of the plate (Nunk-Maxisorb, Denmark) with preliminary absorbed antigens and incubated at $+2...+8^0$ C for 14-16 hours, after which the standard procedure of IEA was carried out. The solution of tetramethylbenzidine with H_2O_2 was used as chromogen. Registration was performed at wavelength 450 nm using IEA-analyzer (EFOS, Russia). Results of assessment of serum immonoreactivity (IR) of the samples under analysis, measured in the units of optical density, were recalculated with respect to the IR in the “inner standard” serum with the same antigens and expressed in c. u. [4]. Thus, if the IR of the serum under analysis with some antigen was higher than that of the “inner standard”, it was expressed with the sign “+”, if lower – with the sign “-“ (for instance, 120% of the IR value of the reaction with the “inner standard” were expressed as +20 c. u., and 80% – as -20 c. u.).

Results and discussion

In the 1st group under observation (n=56) in the neonatal period elevated level of nephrotropic NAAB was found in 21children (37.5%). In particular, 15 children (26.8%) showed elevated level of all the three classes of nephrotropic NAAB; 4 children (7.1%) – of the two classes of NAAB (to one of the membrane antigens KiM-05-40 or KiM-05-300 and to cytoplasmic antigen KiS-07-120) and 2 children (3.6%) had elevated level of NAAB only to cytoplasmic antigen KiS-07-120 (see the table). Isolated elevated level of NAAB, aimed selectively at membrane antigens, was not found. Among the newborns with elevated level of nephrotropic NAAB 16 children were born from mothers with gestation PN, 3 – from mothers with pelvic inflammatory disease (PID) and in 3 from among them antenatal diagnosis of kidney malformations was established (by ultrasonography). Correlation analysis revealed direct reliable correlation between elevated level of nephrotropic NAAB and exacerbation of gestation PN in the mother ($r=0.68$; $p<0.05$). By the age of 6 months the number of children with elevated level of nephrotropic NAAB increased and reached 30 people (53.6%) (fig. 1). At the same time, in 94.7% of the children who had had elevated level of three or two nephrotropic NAAB at birth (n=19), enhanced production of the same NAAB persisted (n=18). We have found direct correlation between the content of the corresponding nephrotropic NAAB, found in the same children in the neonatal period and at the age of 6 months ($r=0.91$; $p<0.05$).

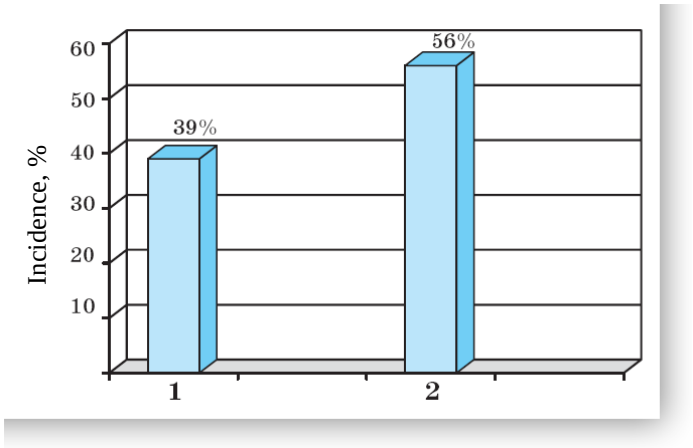

Fig. 1. Number of children of the 1st group with elevated level of nephrotropic autoantibodies in the neonatal period and at the age of 6 months

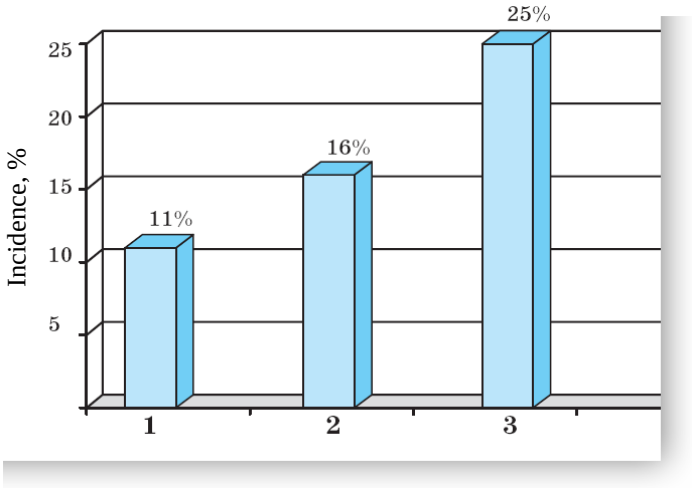

Fig. 2. Number of children of the 1st group having developed pyelonephritis during the period of observation

Таблица

Structure of elevated serum level of natural antibodies to renal antigens in newborn children of the 1st group

Elevated level of NAAB	Number of children (%)
to three renal antigens (KiM-05-40, KiM-05-300 and KiS-07-120)	15 (26.8%)
to two renal antigens (KiM-05-40 or KiM-05-300 + KiS-07-120)	4 (7.1%)
to one renal antigen (KiS-07-120)	2 (3.6%)
Total	21 (37.5%)

In the course of further monitoring of the children of the 1st group increased morbidity with PN was found (fig. 2): at the age of 1 year PN was registered in 6 (10.7%) patients, by the age of 1.5 years - in 9 (16.1%), and by the age of 2 years - in 14 (25%). All the children having developed PN had elevated level of three or two nephrotropic NAAB both at birth and at the age of 6 months.

Depressed level of NAAB to the double-helix DNA and Fc-fragment of Ig (RF) was found in 8 (14.3%) newborns of 1st group and elevated level – in 3 children (5.4%). The discovered changes were interpreted as signs either of immunosuppression or of enhanced nonspecific immunoreactivity respectively. 2 more children (3.6%) had combined increase in the level of nephrotropic NAAB and RF, which we did not regard as a marker of kidney pathology.

It is worth mentioning that in children of the 1st observed group (risk group) the values of nephrotropic NAAB were higher in the neonatal period than at the age of 6 months, at the same time, as a rule, it was associated with low values of RF (NAAB to DNA and Fc-fragments of Ig). From our point of view,

elevated level of nephrotropic NAAB in newborn children from the high risk group may be due to transplacental transport of NAAB of the same specificity, produced in the body of their mothers suffering from gestation PN and PID. It is possible that in such situations maternal immune imprinting occurs [1], via which sui generis antenatal programming (tuning) of peculiarities of the child's immune response is mediated. It is not excluded that this phenomenon forms the basis of a number of congenital disorders (in all probability, not only kidney pathology).

In the 2nd observed group (patients with PN) elevated level of at least one of the three classes of renal NAAB was found in 24 children (60%), in 18 (75%) of which chronic PN was found and in 6 (25%) - acute PN was detected. In 9 (37.5%) out of 24 patients with elevated level of nephrotropic NAAB elevated content of all the three types of nephrotropic NAAB was found; 5 (20.8%) from among these children had ureterohydronephrosis and in 4 (16,7%) of them the duration of PN was more than 5 years. Elevated content of two classes of NAAB (KiM-05-40 and KiM-05-300) was found in 15 (37.5%) patients. Isolated elevation of NAAB level to cytosol antigen in this group was not found.

In the majority of the children (82.5%) of the 2nd group (with active PN) no changes of the RF (NAAB to DNA or Fc-fragments of Ig) content were found. However, one child (2.5%) had minor depression of the level of RF (apparently, transitory); 6 children (15%) had elevated level of the RF, probably, due to intercurrent acute respiratory disease at the moment of examination.

Dynamic observation of the children of the 2nd group (patients with PN) in the course of one year revealed a fact, rather interesting in our opinion: in children with elevated level of all the three classes of NAAB recurrence of the disease was registered in 80% of cases, whereas in children with elevated level of NAAB to two renal antigens – only in 44% of cases. Thus, combined increase in production of NAAB to membrane and cytosol renal antigens in the majority of cases preceded (or accompanied) recurrence of PN.

It is worth mentioning, that the results of our studies completely coincide with suppositions and conclusions of A. Notkins [4], substantiating his statement that NAAB may be significantly informative predictors of the onset of various diseases and syndromes (not necessarily autoimmune). Moreover, changes in the level of organ-specific NAAB in the majority of cases precede clinical manifestation of the corresponding forms of pathology by months and years.

In the reference group in 9 of 10 newborns the concentration of NAAB to renal antigens did not exceed the normal levels. One child had slightly elevated level of all the three classes of nephrotropic NAAB, and of NAAB to DNA and Fc-fragments of Ig, which we interpreted as manifestation of general nonspecific enhancement of the activity of the immune system. Most probably, this increase was associated with excessive antigen load against the background of food-borne allergy; it was of transitory nature and was already not detectable at the age of 6 months. Detailed examination of this child using various diagnostic methods did not reveal any pathology of the urinary system or other systems.

Thus, detection of elevated level of nephrotropic NAAB can make it possible to reveal pathological changes in the urinary system at the preclinical phase, which is especially important for the children with high risk of development of PN, and to predict the probability of recurrence in patients with already manifested PN. It is quite probable that further studies aimed at measuring the level of organ-specific NAAB to renal proteins will make it possible to predict the course and the outcome of the inflammatory process in the urinary system and to monitor the adequacy and efficiency of treatment. However, this requires further studies aimed at determination of NAAB to different classes of renal antigens in various urinary system diseases.

Conclusions

1. Elevated level in serum of two or three NAAB to renal antigens (KiM-05-40, KiM-05-300 and KiS-07-120) in newborn babies may be a prognostic (preclinical) sign of development of PN.
2. Elevated level in serum of nephrotropic NAAB, detected during neonatal period in children whose mothers had suffered from gestation PN or had PID, persisted in subsequent months. This indicates persistent (prolonged) disorders in production of nephrotropic NAAB, which confirms our opinion that assigning these children to the group of high risk of development of the urinary system disorders is both justified and expedient
3. Among the patients with active PN the number of recurrent attacks was significantly higher in children with combined increase in the level of NAAB to cytosol and membrane renal antigens (80%), than in children with high level of NAAB to only membrane antigens (44%). In our opinion, it is a serious argument in favor of introducing the method of measuring the content of NAAB to membrane (KiM-05-

40, KiM-05-300) and cytoplasmic (KiS-07-120) antigens into nephrological (especially pediatric) practice, as it can be a valuable prognostic criterion with respect to the risk of PN recurrence.
4. In all probability, there exists a necessity to perform parallel assessment of the content (especially pediatric nephrologists) of NAAB to renal antigens and of NAAB to double-helical DNA and Fc-fragments of Ig, which makes it possible to distinguish between specific kidney lesions and non-specific polyclonal immunity system activation and to avoid incorrect interpretation of the results (i. e. to reduce the risk of diagnostic error).

REFERENCES