Chronic tubulointerstitial nephritis in children: new approaches to diagnosis and treatment

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Abstract. A survey of 120 children with HTIN, aged from 4 to 15 years, was conducted. Taking into account the clinical variant of HTIN, all patients were divided into 2 groups: group 1 – 52 (43%) children with recurrent form of HTIN and group 2 - 68 (57%) patients with latent HTIN. Among them, there were 65 boys (54%), 55 girls (46%). The conducted studies have shown that with the development of rHTIN and lHTIN, an important mechanism of damage to interstitial kidney tissue, the development of clinical symptoms and the course of the disease is both a metabolic disorder leading to structural shifts at the level of various elements of the nephron and changes in the functional state of the kidneys, and instability of the cytomembranes of tubular cells. The analysis of the results of the study showed that the method of treatment proposed by the authors is the most effective way of treating HTIN, due to accelerated recovery, both clinical and laboratory parameters of the disease and indicators of protein metabolism, as well as in relation to the restoration of the functional state of the kidneys, which leads to a reduction in the length of hospital stay, a reduction in the number of relapses of exacerbation, prevention complications of the chronic process. All this contributes to preventing the development of disability and reducing the number of child deaths from CRF.

1 Relevance

Advances in the diagnosis and treatment of nephrological diseases in children are colossal, but nevertheless, in about 23% of patients, the disease has a progressive course, which significantly affects the quality of life.

The inflammatory process in the tubulointerstitial tissue (TIT) of the kidneys progresses against the background of specific and nonspecific etiological factors. The interstitial tissue of the kidneys is the focus of pathology in TIPP, which further covers the blood, lymphatic vessels and tubules of the renal stroma.

The microscopic findings of TIN are: infiltration (lymphoid or macrophage) of the interstitial tissue with a transition to loose or coarse fibrous sclerosis, dystrophy and/or atrophy of the tubular epithelium.

Research in recent decades has proven an important role in the origin of TIPP kidney damage molecules. They can simultaneously participate in many processes of the formation of pathological changes in the kidney. The results obtained in this study will be used to develop new methods of treatment and prevention of TIPP.
2 Target

To develop a method for the complex correction of CTIN in children, taking into account the identified pathogenetic significance of the parameters of endogenous intoxication and disorders of tubular functions.

3 Materials and research methods

This study presents the results of examination and treatment of 120 children with CTIN, in the phase of an active inflammatory process, who were in the pediatric nephrology department of the Children's Regional Multidisciplinary Scientific Center in Samarkand, in the period from 2019 to 2021.

Taking into account the clinical variant of CTI, all patients were divided into 2 groups: group 1 - 52 (43%) children with recurrent CTI and group 2 - 68 (57%) patients with latent CTI. Among them, there were 65 (54%) boys and 55 (46%) girls. Patients underwent general clinical, laboratory and instrumental examinations.

The clinical diagnosis of CTIN was made according to the diagnostic criteria proposed in the classification by N.A. Korovina (2003), where special attention was paid to the characteristics of the pedigree anamnesis: the definition of UTI, TIN, KSD, metabolic disorders at an early age, which were symptoms of exudative-catarrhal diathesis, dysuric disorders against the background of crystalluria.

"Urinary syndrome" was characterized by: abacterial leukocyturia, high osmotic density of urine, microproteinuria, microhematuria, crystalluria.

The control group consisted of 30 practically healthy children who did not suffer from chronic diseases, had not been sick for the last 6 months, with a favorable family history of nephrology, aged 4 to 15 years.

Renal parameters were assessed during the period of exacerbation of the disease, during the formation of clinical and laboratory remission, 1 year, 2 and 3 years after the period of exacerbation. The study did not reveal children with CTIN on the background of severe congenital pathology in combination with a violation of the functional state of the kidneys.

The state of renal functions was assessed on the basis of two groups of functional methods:

Group I - methods indicating the quantitative state of the renal functions of various parts of the nephron.

a) The state of the filtration function of the kidneys (endogenous creatinine clearance) was assessed using the Van Slyke formula:

Using immunotubidimetry on the Cobas apparatus Integra 400 plus (Roche, Switzerland) determined cystatin C:

- is a protein that is formed in the nucleus of cells at a constant rate;
- has the property of free filtration in the glomeruli;
- inversely correlates with GFR and is highly sensitive to its changes compared to its changes in creatinine [9].
- metabolized in the proximal tubules during reabsorption
- is formed regardless of gender, body weight or tumors, the presence of inflammatory processes;
-—
To determine the concentration ability of the kidneys, the Zimnitsky test was used. In addition, the magnitude of ammonioacidogenesis was determined (titratable acids and ammonia were determined in daily urine).

In all examined patients, protein metabolism indicators were determined (protein fractions, total serum protein, total and effective albumin concentration, toxicity index in blood serum, albumin binding capacity). Serum urea and creatinine levels were also determined.

The results obtained and their discussion

The clinical group (group 1: 52 patients) with rCTIN was identified based on the presence of typical signs of the disease, such as dysuria (32.7%), neurogenic bladder (10%), pastosity of the soft tissues of the eyelids in the morning hours (46.5%), back pain (30.8%) due to physical activity (26.9%).

Whereas, the clinical group (group 2: 68 patients) with lCTIN was identified on the basis of a more permanent symptom of “salt-wasting kidney”, which leads to the development of muscle hypotension - 41.2% (28) and arterial hypotension - 27.9% (19), dysuria - polyuria in 54.4% (37) patients, the presence of abacterial lesions of the renal tissue against the background of hyperoxaluria - 100% (68), the abundance of epithelium in 92.6% (63), lympho-monocytic cells - 88.2% (60), brown cylinders - 100% (68). Urine culture is sterile.

Diagnostic criteria for the latent course of CTIN: were detected against the background of respiratory diseases, they did not receive due attention due to their short duration, hereditary history was not taken into account.

In our studies, a high percentage of morbidity with a continuously relapsing form of CTIN occurred in children aged 10-14 years, which amounted to 43.2% of the total number of patients with a continuously recurrent form of CTIN.

We associate the recurrent course of the disease with the presence of a secondary immunodeficiency state, indirect signs of which are: frequent recurrence (more than 2 times a year) and a protracted course (preservation of clinical and laboratory signs for more than 6 months), a short-term effect of antibiotic therapy, multiple foci chronic infectious pathology, susceptibility to SARS.

In the clinical status of patients with chronic recurrent course of TIN, the frequency of exacerbation of the disease over the past period was determined and revealed that in 20 (38.7%) children the frequency of exacerbation was 1 time per year, in 19 (36.5%) children 2 times per year and 12 (23.1%) children more than twice a year.

In all examined patients, the parameters of protein metabolism were determined (total serum protein, TCA, ECA, protein fractions, CSA, altered albumin concentration and toxicity index, MPP in urine and blood, globulin fractions, cystatin C concentration, indicators of the functional state of albumin, urea level, creatinine).

At present, it has been established that with the development of multiple organ and polysystemic insufficiency, products of impaired metabolism, endotoxins, accumulate in the body. Endotoxins include products of natural metabolism that accumulate in the body in high concentrations, MPP are intermediate products of proteolysis, variable products, ingredients of non-viable tissues that are heterogeneous in composition and accumulate in the body due to inhibition of natural detoxification mechanisms and metabolic disorders.

There is a direct relationship between the degree of EI and the volume of UTI in the urine, depending on the severity of CTI.

Studies of kidney function and EI parameters are necessary to predict the course of CTIN. The degree of damage to the membrane structures of kidney cells was assessed by the level of MPP and TCA in the urine, in the blood by the total concentration of albumin, ECA, CSA, IT and CIA.
The data obtained showed that the concentration of MPP in the urine in patients with rCTIN in the acute phase was 16.3 times higher than in the control group (Fig. 1), while in children with rCTIN it was 8 times higher. More pronounced disorders of cellular structures were noted in patients with rCTIN compared with patients with LCTI.

An increase in the level of MPP in the urine in CTIN is apparently due to the fact that during the inflammatory-destructive processes of the tubulointerstitial system, the reabsorption of MPP in the proximal tubules is disturbed, since they are reabsorbed there by 99.9%, as a result of which their excretion with urine is observed. The accumulation of MPP in the urine contributes to impaired renal excretory function, leading to tubular atrophy and organic structural disorders.

Fig.1. EI parameters for CTIN in children on admission (M ± m)

Both in the active stage and in remission in rCTIN, the state of protein metabolism was the same as in the acute course of the process. A significant decrease in the concentration of total serum protein in this pathology was uncharacteristic (67.6±0.25 g/l) and TCA (49.23±0.28 g/l). The protein-synthetic function of the liver compensated for the slight loss of protein associated with a febrile state.
In recent years, febrile conditions have been rarely observed in patients, and exacerbations of CTI have been asymptomatic. The normal level of protein synthesis was maintained due to the absence of thermal inactivation of liver enzymes.

The active phase of CTIN was characterized by a decrease in ECA, as in the acute process, but more pronounced (32.04±0.26 g/l). The decrease in ECA was combined with a decrease in CC to 64.8±0.65% (Fig. 4).

In our opinion, the identified changes are associated with more active and prolonged intoxication, which is the cause of excessive accumulation of toxic substances that contribute to the formation of endotoxicosis and homeostasis disturbance. The nature of intoxication, its severity in one form or another of the disease affects the rate of decay of protein structures.
A high level of the toxicity index indicates the presence of intoxication, which is determined in all periods of the disease (Fig. 5.).

Less pronounced, but persistent changes in protein metabolism are characteristic of the latent course of CTIN. Children are characterized by a decrease not only in the ECA, but also in the general on.

We found in patients with a sluggish process in the kidneys the presence of violations of the protein-synthetic function of the liver.

Against the background of intoxication, immune disorders and sluggish inflammation in the body, the liver loses its ability to provide compensation for protein metabolism disorders. The level of ECA in lCTIN changes to a lesser extent compared to rCTIN, which is associated with compensatory mechanisms in the liver.

An adaptive reaction against the background of a long pathological process is that albumin is synthesized in a smaller amount, but more complete. High SSA contributes to a decrease in the level of intoxication, unlike other variants of TIN, which indicates such an indicator as IT (Fig. 6.). Such changes in albumin lead to the formation of chronic TIN, which indicates that the nonspecific effector system of the body is functioning [4;7].
Fig. 6. Indicators of SSA in the active stage in various forms of CTIN in children. In CTIN, there is despiralization of the protein molecule. Conformational disturbances lead to the formation of discrete forms of albumin, as indicated by a decrease in the level of albumin binding capacity. The limited ability of albumin to bind drugs, this applies to antibiotics, which significantly affects the formation of a chronic process.

In recent years, more attention has been paid to the compensatory capabilities of the kidneys in CTIN in children, which directly depend on the effectiveness of the treatment. An analysis of the literature shows that the compensatory capacity of the kidneys and the effectiveness of the treatment of CKD are significantly increased with the complex use of medicaments and physiotherapeutic procedures.

For a comparative analysis of various methods of treatment of chronic tubulointerstitial nephritis, sick children were divided into 2 groups depending on the method of therapy. The traditional method of therapy was carried out in 42 (35%) children (Group 1). The main contingent of children 30 (71.4%) were admitted to the clinic on the 3-10th day from the onset of the disease, and in 12 (28.5%) cases at a later date.

The second group consisted of 41 (34.1%) patients with CTIN, aged 4 to 15 years (22 (53.7%) girls, 19 (46.3%) boys), who received Rutin against the background of traditional therapy.

In patients with CTIN of group 2, both extrarenal and renal symptoms of the disease disappeared significantly earlier than in group 1. The complex of therapeutic measures included the drug "Rutin" (Group 2). The choice of this drug was made taking into account its numerous positive pharmacological effects: it improves metabolic, immunological, regenerative processes, has an antioxidant effect and improves the permeability of the walls of blood vessels and capillaries. One of the important advantages of the drug is its high safety.

As a result of the use of the drug "Rutin" in patients with CTIN on 11.2±0.21 (p=0.001) days, we observed the transition of the active phase of the disease to the stage of remission, and in the compared group, clinical recovery occurred only by 12.3±0.6 days from the start of treatment, which is 1.1±0.39 days later than in group 2.

Thus, in the group of patients after treatment supplemented with the drug "Rutin" for an average course of treatment of 10-11 days, the clinical signs of the inflammatory process in the urinary tract decreased and completely disappeared.
The results of studies of the EI index after treatment by the proposed method showed that in patients of the 2nd group, the level of MPP in the urine had a relatively high tendency to decrease, but still remained 4 times higher than the level in healthy children and amounted to $0.605 \pm 0.023$ units. opt. sq. ($p_1 = 0.001$). Recovery of the studied parameter occurred on the 10-11th day of treatment. The elimination of the elevated level of MPP in the urine in patients of the 1st group was observed only on the 12-13th day of treatment and amounted to $1.2 \pm 0.034$ units. opt. sq. ($p_1 > 0.1$), which was not significant in comparison with the indicators in children of the 2nd group.

That is, the dynamics of the elimination of MPP in the urine occurred faster in group 2 compared to group 1, but did not reach the control standards, both in dynamics and at discharge.

The use of this drug significantly increased the level of ECA, SSA and reduced the level of IT in blood plasma, as well as MPP in the urine in children of the 2nd group. An increase in the level of ECA in children of group 2 is probably associated with the antioxidant and nephroprotective properties of the drug. We noted a more pronounced positive dynamics of the ECA indicator after the use of the drug “Rutin” in patients of group 2, which amounted to $33.04 \pm 0.5$ g/l ($p_1 = 0.001$) compared with group 1, where the level of ECA was $30.74 \pm 0.58$ g/l at discharge from the hospital ($p_1 > 0.1$) (Fig. 7).

Fig. 7. Dynamics of indicators of protein metabolism (SCA, ECA, IT, CIA in blood plasma) depending on the use of therapy methods in children with CTI.

We determined that the level of SSA and IT in children of the 2nd group on the background of therapy had more positive dynamics and amounted to $79.4 \pm 1.2$% and $0.25 \pm 0.005$ arb. units ($p_1 = 0.05$ and $p_1 = 0.05$), while in children of group 1 these figures were $72.2 \pm 0.6$% and $0.36 \pm 0.01$ arb. units ($p_1 = 0.05$ and $p_1 > 0.1$). In healthy children, these parameters were $93 \pm 0.9$% and $0.12 \pm 0.01$ arb. units, respectively (Fig. 7).

Analyzing the results of the study, it was found that these indicators, such as ECA, CSA, IT, CIA in blood plasma, and MPP in the urine after the use of the drug “Rutin” had a high positive trend, but still did not reach the level in healthy children. Apparently, “Rutin” in an insufficiently effective degree affects the processes of endotoxicosis, which occurs in aseptic inflammatory diseases of the renal tissue, which requires the development of new methods for its correction.

There was a positive trend in the study of the functional state of the kidneys in patients of the 2nd group.
The effectiveness of the drug "Rutin" is confirmed by a decrease in leukocyturia. Thus, leukocyturia statistically significantly decreased in patients of the 2nd group to 5-6 cells in p. z. compared with group 1 (7-8 cells in p. z.). Against the background of the ongoing therapy, more pronounced changes were noted in the 2nd group of children.

Analysis of the results of the study of daily proteinuria in the compared groups showed that after treatment in group 2, this indicator of urinary syndrome significantly decreased and was found only in 4 (11.4%) patients, and in children of group 1, this indicator remained elevated in 8 (25%) of patients.

In the compared groups, the hematuria index changed unidirectionally. There was a decrease in erythrocytes in the urine, but in group 2 these changes were more pronounced.

After the treatment, a significant increase in GFR was noted only in children of the 2nd group 78.0±0.9 ml/min/1.73 m^2 (p=0.01), while in patients of the 1st group this figure was 73.8±1.56 ml/min/1.73 m^2 (p>0.1). The GFR indicator had significant values only in children of the 2nd group, where it increased by 6%, and in children of the 1st group only by 1%.

Fig. 8. Dynamics of renal functions of the kidneys with CTIN after therapy (M ± m). After the treatment, GFR (according to cystatin) increased: in patients of the 2nd group - from 67.4±10.3 to 83.2±9.6 ml/min/1.73 m^2 (p=0.001); in children of the 1st group - from 67.4±10.3 to 74.9±9.7 ml/min/1.73 m^2 (p=0.02).

A predominant improvement in GFR (according to cystatin) was found, first of all, in children of group 2 (p=0.001), in whom the growth rates of GFR were the highest - by 19%.
which is associated with the positive properties of the proposed method of therapy, which is characterized by a pronounced nephroprotective, anti-inflammatory, antispasmodic, membrane-stabilizing effect of the drug "Rutin".

When assessing the functional state of the kidneys according to the dynamics of the level of creatinine and cystatin, undoubtedly, the indicators of the level of cystatin are more reliable. In patients, an increase in GFR by cystatin is noted earlier than that determined by creatinine (p=0.02).

Against the background of the treatment, an increase in daily diuresis was noted in both groups. We determined that in group 1 the daily diuresis slightly increased to 4%, while in the comparison group this figure increased to 8% (p=0.05), which corresponds to the nature of changes in FSP in TIN.

Thus, the dynamics of indicators of partial functions of the kidneys and protein metabolism indicates that "Rutin", having an indirect effect on the tendency to normalization, is characterized by a significant advantage over the traditional method, to a greater extent in terms of eliminating clinical symptoms and in terms of the level of the tendency to normalize protein parameters in blood plasma and urine, but to a lesser extent in the dynamics of recovery of the "urinary syndrome": leukocyturia, proteinuria, hematuria and restoration of partial kidney functions.

Taking into account the results of the use of the drug "Rutin" in the treatment of children with CTIN obtained by us in previous studies (groups 1 and 2), as well as in order to optimize the positive effects and eliminate the shortcomings of treatment, we monitored group 3 patients who, in the complex of traditional therapy, an additional application of electrophoresis with 0.5% aminofillin was carried out.

Electrophoresis was performed with aminophylline 0.5% solution in warm water on the lumbar region, during urine sanitation (on the 3rd-4th day of treatment), contraindication: high activity of the pathological process and impaired urodynamics.

The study of the effectiveness of the modified method of therapy was carried out in 37 sick children. Among them, 19 (51.4%) girls, 16 (48.6%) boys aged 4 to 15 years. As a result of the use of complex therapy in patients with CTIN, on day 9.1±0.3 (p=0.001) we observed a clinical recovery in all extrarenal and renal signs of the disease, and in group 2, bed-days amounted to 11.2±0.21 days, which is significantly shorter by 2.1 days and 5 days compared with the group of children who received traditional therapy.

Thus, complex treatment turned out to be significantly effective in all analyzed clinical symptoms of the disease in comparison with other groups of patients.

As a result of studying the effectiveness of complex treatment in terms of protein metabolism: MPP in urine, ECA, CCA, IT, CIA in blood plasma in patients of group 3, we determined a significant decrease in MPP in urine to 0.207 ± 0.012 units. opt. sq. (p<0.001), regardless of the initial level of EI and the form of the disease. Whereas, in patients of group 2, the level of MPP in the urine during therapy was 0.605 ± 0.023 units. opt. sq. (p<0.05).

Restoration of the studied parameter in children of the 3rd group occurred on the 8th-9th day, and in most patients of the 2nd group, even on the 11th-12th day of treatment, it remained unchanged.

In addition, this modified method of treatment contributed to a significant increase in the level of ECA, SSA and a decrease in IT, CIA in blood plasma in children of the 3rd group.

We determined that complex treatment has a positive effect on the level of ECA, which in children of group 3 was 37.5 ± 0.3 g/l (p<0.001, p=0.05), while in patients of group 2, this indicator was 33.04±0.5 g/l (p<0.05) (Fig. 3).

The dynamics of the CIA indicator in patients receiving complex modified treatment was positive in relation to that in children of the 2nd group (Fig. 9).
Fig. 9. Dynamics of indicators of protein metabolism (SSA, ECA, IT, CIA in blood plasma) in CTIN in children, depending on the method of treatment.

The use of complex therapy contributed to a significant increase in the level of SSA in children of group 3, which amounted to 87.9 ± 0.3% (p₁=0.001; p₂=0.001), while in children of group 2 this figure was 79.4 ± 1.2% (p₁=0.001), respectively (Table 2).

We observed a similar picture in relation to IT, its decrease in group 3 was 44% and was significant 0.16 ± 0.004 arb. units (p₁=0.001; p₂=0.05) compared with the 2nd group, where this indicator was 0.25 ± 0.005 units (p₁=0.05) (Fig. 9).

Thus, the complex treatment was combined with a statistically significant decrease in the MPP in the urine, as well as an increase in ECA, SSA and a decrease in IT, CIA in the blood plasma. This effect is associated with the positive effect of complex treatment on the stability of renal cytomembranes.

Thus, the analysis of the dynamics of urinary MPP, ECA, SSA, IT, CIA in the blood plasma of sick children of the 3rd group indicates a pronounced "antitoxic" effect of complex treatment, which allows it to be used to correct impaired protein metabolism in CTIN in children.

The results of the studies indicate that the complex treatment of patients in group 3 leads to a more stable correction of shifts in partial kidney functions already in the dynamics of treatment.

At the same time, the level of hematuria in children reached the control values in 98%, while in children of the 2nd group this indicator was increased in 12% of children and amounted to 2-3 cells per p. (p₁=0.001; p₂=0.001), while in patients of group 2 this parameter was 6-7 cells per p. (p₁=0.01), respectively, which is 2 times more than in group 3.

The level of daily proteinuria remained above the control value in 4 (11.4%) patients of group 2, while in patients of group 3 clinically significant proteinuria was not found in any patient, which we attribute to the anti-inflammatory and reparative effect of the author's rehabilitation treatment regimen.
After treatment, GFR increased in both groups (78.0±0.9 ml/min/1.73 m² and 84.75±1.72 ml/min/1.73 m², respectively. The degree of increase in GFR was: in group 2 by 7.5% and in group 3 by 15%.

GFR (according to cystatin) increased: in children of the 3rd group from 67.4±10.3 to 93.8±1.61 ml/min/1.73 m² (p=0.0003); in patients of group 2 from 67.4±10.3 to 83.2±9.6 ml/min/1.73 m² (p=0.02) (Fig. 9).

The most positive dynamics was revealed in children of group 3 (p = 0.001), while this indicator in group 1 by 7.5% and in group 2 by 17%.

We attribute this to the nephroprotective, anti-inflammatory, antispasmodic, reparative properties of electrophoresis with 0.5% aminofillin which lead to an improvement in microcirculation in the renal tissue.

When comparing GFR in children of the 3rd group after therapy, calculated by the level of cystatin and creatinine in the blood, a higher reliability of the calculation of GFR by cystatin (p=0.01) was noted, which makes it possible to more accurately assess the severity and prognosis of complications.

The results of the study of daily diuresis made it possible to determine that this indicator in patients of group 2 was 1.15 ± 0.037 l / day (p = 0.05), and in group 3 1.42 ± 0.046 l / day (p = 0.05; p = 0.05), respectively, which undoubtedly indicates a "partial" advantage of the modified method of treatment.

Fig. 10. Dynamics of renal functions of the kidneys with CTIN after therapy (M ± m)
These research results showed that the author's method of correction of CTIN, along with a pronounced clinical effect on the elimination of extrarenal and renal pathological symptom complexes, positively affects not only the indicators of protein metabolism and "urinary syndrome", but also the partial functions of the kidneys, and contributes to their rapid reaching levels in healthy children.

In addition, the modified method of treatment proposed by us contributes to a more stable correction, not only of the aseptic inflammatory process, but also of metabolic and partial disorders. The positive effect of electrophoresis was due to the improvement of renal hemodynamics, tissue oxygenation, cellular metabolism, reducing the increased tone of the smooth muscles of the upper urinary tract and activating the secretory function of the kidneys, electrophoresis promotes the excretion of sand and small stones from the renal tissue into the urine. Under the influence of heat, electrical stimulation and 0.5% aminofillin, blood circulation, metabolic and reparative processes are activated, and kidney function improves.

5 Conclusions

Thus, the conducted studies have shown that in the development of rCTIN and lCTIN, an important mechanism of damage to the interstitial tissue of the kidneys, the development of clinical symptoms and the course of the disease is a metabolic disorder, leading to structural changes at the level of various elements of the nephron and to changes in the functional state of the kidneys. and instability of tubular cell cytomembranes. This justifies the need for combination therapy in patients with CTIN, which will contribute to the elimination of the inflammatory process, the excretion of endotoxins from the renal tissue, the stabilization of cell cytomembranes and kidney functions.

Analysis of the results of the study showed that the proposed method of treatment is the most effective way to treat CTIN, due to accelerated recovery, both clinical and laboratory parameters of the disease, and indicators of protein metabolism, as well as in relation to the restoration of the functional state of the kidneys, which leads to a reduction in the time stay in the hospital, reduce the number of relapses of exacerbation, prevent complications of the chronic process. All this helps to prevent the development of disability and reduce the number of child deaths from CRF.

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