Annotation of PhD thesis of Zhuzzhasarova Aygerim Aymahanbetovna entitled « Optimization of clinical and laboratory diagnostics of complicated measles in children» presented as an application for the PhD degree on the speciality 6D110100-Medicine

Relevance of the study

Measles is an acute viral infection characterized by high contagiousness and widespread spread. It is the leading cause of morbidity and mortality among young children worldwide (Schoini P. et al., 2019., Imdad A., 2017., Ilyas M., et al., 2020)

In about 30% of children under the age of 5, this disease is characterized by the development of complications, especially in patients with impaired nutrition and immunodeficiency conditions.

The problem of measles elimination at the present stage remains one of the main health issues, despite significant success in reducing morbidity and mortality due to vaccination. The World Health Organization (WHO) has set a goal of reducing measles mortality by 95% and morbidity by 90% compared to levels that were observed before the measles vaccination program (WHO/EPI/GEN/92.2). An increase in the incidence of measles, which takes on the character of epidemic outbreaks, is periodically registered throughout the world.

Planned measles vaccination, carried out in the Republic of Kazakhstan since 1967, contributed to a steady decrease in morbidity by more than 150 times (Amireev S.A., Esmagambetova A.S., Kuatbaeva A.M. et al., 2014). Monitoring of the incidence of measles over the past 18 years in the Republic of Kazakhstan has shown that vaccination carried out for several years provided a steady decrease in the incidence in the country with the registration of only isolated cases (statistical data of the Consumer Protection Agency). However, at the same time, despite such achievements, periodic outbreaks of measles are observed every 6-7 years, which were observed in 1993, 1998 and 2005, which may have been due to the accumulation of a non-immune stratum of the population. According to the statistical data of the "Agency for Consumer Protection", since October 2013, an increase in the incidence of measles has been registered in the republic again. So, for 4 months of 2014, the indicator was 0.82 per 100,000 population. Among people with measles, the adult population prevails in the republic as a whole, amounting to 63.18%, of which 36.81% are children.

Studies have shown that there are certain problems in the diagnosis and registration of measles, which leads to unreliable statistics on the incidence and prevalence of the disease. That, in turn, all this entails a number of inadequate therapeutic and diagnostic, anti-epidemic and preventive measures, as it can lead to hypo- or overdiagnosis.

According to the "Agency for Consumer Protection" of the Ministry of Health of the Republic of Kazakhstan, the number of unvaccinated persons in 2013 increased 1.1 times more than in 2012. The main reason for the lack of vaccination of the population is often unjustified medical withdrawals, often unjustified, out of the total number of unvaccinated, they amounted to 68.2% in

2012, 65.7% in 2013, 47% in 4 months of 2014 (Children of Kazakhstan: stat. sat. / Committee on Statistics 2017).

Again, the rise in the incidence of measles in the Republic of Kazakhstan is noted in 2018-2020. According to statistics, the incidence of measles in the Republic of Kazakhstan in 2018 amounted to 3.15 per 100 thousand population. The peak incidence of measles in the country occurred in 2019, where the incidence rate increased 22 times and amounted to 71.97 per 100 thousand population, and only in 2020 in the month of May there was a tendency to decrease this indicator by 4.2 times - 17.21 per 100 thousand population. Taking into account the increase in the incidence of measles, an additional program of immunization of the population with the CCP vaccine was launched in the Republic of Kazakhstan, which was carried out from April 1, 2019. The vaccination program includes children who have reached the age of nine months, persons under the age of 30 with an unknown vaccination status who have not been vaccinated before and have not had measles, those who were vaccinated five or more years ago with one measles vaccine in the foci of infection, as well as medical workers (On the approval of Sanitary Rules "Sanitary-epidemiological requirements for preventive vaccinations to the population": approved on June 13, 2018, No. 361).

At the present stage, despite the existence of a safe and effective vaccine, measles is registered in all climatic zones. A significant percentage of complications and deaths in measles causes high economic and material damage to countries. Thus, in order to eliminate measles among the population, universal immunization of children and vaccination of all susceptible people with measles vaccine is recommended. According to the literature review, we have not found information on a comprehensive clinical, laboratory, instrumental and pathomorphological study of the course of measles in children with a burdened premorbid background.

As part of the research work, we systematized comprehensive clinical, laboratory, instrumental and morphological information on the study of the epidemiology and course of measles in children at the present stage, and also presented the main predictors of complications and cases of mortality in children with unfavorable premorbid background.

The purpose of the study.

To study the prevalence of measles in children in the Republic of Kazakhstan, as well as to improve the differential diagnostic criteria for the complicated form of measles in children.

The object of the study

Patients aged from 1 month to 18 years with a clinical diagnosis of Measles. The patients included in the study were recruited in the intensive care and intensive care unit, in the infectious diseases Department № 1 and 2 of the SCCP of the Multidisciplinary City Children's Hospital № 3 of the Akimat of Nur-Sultan.

Research objectives

1. To study the prevalence of measles in the Republic of Kazakhstan among children and determine the genotypes of circulating strains of the virus in 2018-2019;
2. To analyze the age structure, gender characteristics, vaccination status and clinical course of measles at the present stage;
3. To investigate the features of the course of measles in children with an unfavorable premorbid background and to develop an algorithm for managing patients;
4. To identify predictors of complicated course and fatal outcome in measles in children.

Research methods

Clinical studies were conducted according to the clinical protocol "Measles in children" dated June 9, 2016 Protocol № 4; "Pneumonia in children" dated October 5, 2017 Protocol № 29; "Myocarditis in children" dated June 23, 2016 Protocol № 5.

1. Clinical: anamnesis, complaints, data from an objective research method and analysis of medical records of patients undergoing inpatient treatment in the form 003/y, 003-2/y.
2. Laboratory test indicators: general blood analysis and biochemical blood tests, general urine analysis, coprogram.
3. Blood gas composition
4. ELISA: to detect virus-specific AT IgM and IgG
5. Chest X-ray (in order to detect pneumonia);
6. ECG, EchoCG, NSG.
7. Consultation of a cardiologist, neurologist.
8. Molecular genetic research measles viruses.
9. Histological examination of autopsy materials

For genotyping, a standardized technique was used based on the analysis of the nucleotide sequence of the C–terminal fragment of the N–gene with a length of 450 nucleotides – the most variable part of the viral genome. Nucleotide sequences were analyzed using BioEdit Sequence, Mega 7 programs. The material for virus and viral RNA isolation was urine samples collected during the first three days of the disease and in accordance with the protocol recommended by WHO, in patients with a clinical diagnosis of measles. The clinical diagnosis was confirmed in the laboratory by detecting specific IgM against measles in the enzyme immunoassay test system manufactured by Vector–Best, Russia. Viral RNA was isolated using a commercial Pure Link RNA Mini kit (Life Technologies, USA) in accordance with the manufacturer's instructions. Reverse transcription PCR (RT/PCR) was performed using primers MeV216, MeV214, MeV217, a commercial one–stage set system for RT-PCR Superscript ® III with Platinum® Taq DNA polymerase (Life Technologies, USA). DNA electrophoresis was performed using a commercial set of reagents for electrophoretic determination of amplification products in agarose gel "EF" – 200 (AmpliSens, Russia) in 1.7% agarose gel with a trisborate buffer of concentrated ethidium bromide. To extract DNA from the gel, a commercial gel

extraction kit was used (Life Technologies, USA). The concentration of purified DNA was measured using a commercial Qubit dsDNA HS (Life) kit (technology, USA). The genome of the measles virus was sequenced on an automatic genetic analyzer Genetic Analyzer 3500 (Applied Biosystems, USA) using a commercial kit Big Dye Terminator Kit v3.1 (Applied Biosystems, USA). Sequencing products were purified using the Big Dye X Terminator kit (Applied Biosystems, USA) in accordance with the manufacturer's instructions.

Autopsy material for histological examination was subjected to traditional histological treatment in several stages: fixation in a 10% solution of neutralized formalin; dehydration and degreasing in solutions of ethyl alcohol of ascending concentration (70%, 80% and 96%); impregnation with paraffin and pouring into paraffin blocks. Sections 5-7 microns thick were made of paraffin blocks and stained with hematoxylin and eosin. Microscopic examination and microphotography of histological preparations were carried out using a CX41 microscope ("Olympus") equipped with a C5050Z digital camera and software for processing and archiving photographic material.

Scientific novelty

The registration of new genetic lines of measles virus genotypes D8, B3 was determined for the first time ("MVs/Frankfurt Main.DEU/17.11/[D8]", "MVs/Osaka.JPN/29.15/[D8]", "MVs/Gir Somnath.IND/42.16/[D8]" and "MVs/Dublin.IRL/8.6/[B3]", "MVs/Kabul.AFG/20.14/[B3]") and its territorial distribution in the Republic of Kazakhstan;

The circulation of strain B3 in Nur-Sultan during the measles epidemic in 2018-2019 was characterized by a high level of morbidity, aggressiveness of the course and deaths (0.5 per 100 thousand population), mainly in patients with an unfavorable premorbid background;

On the basis of multifactorial mathematical analysis, the factors contributing to the realization of complications of measles infection in children are systematized and determined, as well as predictors of the fatal outcome of patients with a burdened premorbid background are identified

For the first time, a systematic, comprehensive assessment of the clinic, laboratory – instrumental and pathomorphological data for complicated forms of measles infection in children was carried out

Practical significance

– high rates of measles incidence in the regions of the Republic of Kazakhstan, including in Nur-Sultan – 250.36, in Shymkent – up to 302.26, in Mangistau – 210.7 and Turkestan regions – 54.47 cases per 100,000 population indicate the need for vaccination and revaccination of the population in order to avoid repeated outbreaks of the disease, in order to increase alertness and early diagnosis diagnosis it is important to increase the literacy of medical workers and the population;

Clinical and laboratory criteria for the diagnosis of severe forms of measles, determined by the method of multifactorial mathematical analysis for patients with a burdened premorbid background, allowed us to develop an algorithm for patients with a complicated form of measles, which will eliminate the prolongation of the infectious process, reduce drug polypragmasia and prevent deaths;

When assessing the condition of children with measles in the presence of an unfavorable premorbid background, lack of vaccination and late admission to the hospital, a wide differential of the disease clinic from competing and concomitant diseases should be maintained, it is always necessary, along with the leading pathological syndrome, to identify aggravating factors with their subsequent correction.

Provisions submitted for protection:

During the measles epidemic in 2018-2019, genetic lines of virus strains "MVs/Frankfurt Main.DEU/17.11/[D8]", "MVs/Osaka.JPN/29.15/[D8]", "MVs/Gir Somnath.IND/42.16/[D8]", "MVs/Dublin.IRL/8.6/[B3]" ,"MVs/Kabul.AFG/20.14/[B3]".

During the period of measles incidence from November 2018 to December 30, 2019, 13,873 cases were registered, 72.6% of them children. More than half were patients under the age of 5 years, in a number of patients with an unfavorable premorbid background, an atypical course was revealed: the lengthening of the catarrhal period - 13.3%, in 8.2% - the absence of a specific rash, along with the development of complications in the form of pneumonia – 32.9%, myocarditis - 5.7%, ARDS - 5%, encephalitis -1.3%, SSE - 1.3%, mortality was – 15.8% in the group of children with a burdened background.

Predictors of severe complications in measles that lead to death are the presence of a burdened background: CMVI, IDC, cerebral palsy, malignant neoplasm of the brain in various combinations, young age, lack of vaccination, late treatment.

Conclusions

Measles incidence rates among children in the Republic of Kazakhstan in 2018-2019 are characterized by the spread of the virus in all regions of the country. The highest figures are recorded in Nur-Sultan – 250.36, in Shymkent – up to 302.26, in Mangistau – 210.7 and Turkestan regions – 54.47 cases per 100,000 population, and new genetic lines of circulating genotypes of B3 and D8 viruses were registered for the first time ("MVs/Frankfurt Main.DEU/17.11/[D8]", "MVs/Osaka.JPN/29.15/[D8]", "MVs/Gir Somnath.IND/42.16/[D8]" and "MVs/Dublin.IRL/8.6/[B3]", "MVs/Kabul.AFG/20.14/[B3]").

The high incidence of measles in the republic was found in children aged 12 to 47 months - 55.3%, males prevail - 56.8%, unfavorable premorbid background is recorded in 46.7%, low vaccination coverage in the age group from 12 to 23 months (31.4%-not vaccinated), circulation of strains B3 and D8 it was characterized by an aggressive course, mortality - 0.15 per 100 thousand population.

In children with an unfavorable premorbid background, a twofold prolongation of the catarrhal period was noted in 13.3% ($p<0.001$) of cases, in 9.5% of patients the duration of fever increased by 8.7 times ($p<0.05$) compared with the control group, in 8.2% there was no specific rash during the peak period and in

10.1% of patients the prolongation of hypoxemia in in the form of desaturation up to 11-14 days with the development of grade 3 RF.

In the group of patients with a burdened premorbid background, formidable complications leading to death were revealed: pneumonia – 32.9% ($p<0.001$), myocarditis – 5.7% ($p<0.001$), ARDS - 5% ($p<0.05$), encephalitis – 1.3% ($p<0.05$), SSPE – 1.3% ($p<0.05$), predictors of an unfavorable outcome: IDC – 2.5% ($p<0.01$), BEN – 10.8% ($p<0.001$), cerebral palsy - 8.7% ($p<0.05$), CMVI – 4.4% ($p<0.05$) in various combinations, early and persistent DN on the background of pneumonia confirmed by laboratory, radiological and pathomorphological data, the increase of the AST marker on day 8 by 2.7 ($p<0.05$) times and on day 11 by 4 times ($p<0.001$), as a manifestation of myocarditis, proven histologically: cardiomyocytes in a state of granular dystrophy, some in a state of necrosis, necrobiosis.

Publications on the topic of the dissertation

Based on the research materials, 10 printed works have been published in periodicals, including: 3 publications in periodicals of Kazakhstan recommended by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 2 articles in English indexed in the Scopus Citoscore information base with percentile 2020 =49, 2021=71 (Great Britain, Macedonia). 5 publications in the conference materials (Kazakhstan and Russia); 1 textbook has been prepared for publication.

The results obtained have been implemented in practical healthcare: "Algorithm for the management of patients with a complicated form of measles in children" on the basis of the SCCP MGDB No. 3 of Nur-Sultan (dated 06/25/2021); 3 certificates of registration of rights to the copyright object (Appendix A): 1) on the topic of the dissertation "Optimization of clinical and laboratory diagnostics of complicated measles in children" No. 21753g dated 11/16/2021, 2) "Algorithm for the introduction of patients with complicated measles in children" No. 23039 dated 01/25/2022, 3) JV "Measles". "Infectious exanthemums in children. Measles, rubella, chickenpox, scarlet fever, meningococcal infection" No. 23606 dated 15.01.2022

Approbation of the dissertation

1. Scientific and practical conference "Topical issues of vaccination of infectious diseases". Almaty, 2019 The topic of the oral report: "The course of measles in children with a premorbid background"

2. Republican scientific and practical conference "Infectious diseases in the practice of a doctor", Karaganda, 2019 The topic of the oral report: "The course of measles in children with a premorbid background"

3. Interdisciplinary scientific and practical conference "No. 1 Club of young scientists and infectious diseases" Nur-Sultan, 2020 The topic of the oral report: "Molecular genetic characteristics of the measles virus in the Republic of Kazakhstan for 2018".

4. The first Kazakhstan Congress "Infectious diseases in the context of globalization: challenges and solutions" Nur-Sultan, 2020 The topic of the oral report: "Epidemiological characteristics of measles in the Republic of Kazakhstan".

5. Russian scientific and practical online conference "Controlled and other socially significant infections: diagnosis, treatment and prevention". St. Petersburg, 2021

6. The second Kazakhstan Congress "Infectious diseases in the context of globalization: challenges and solutions" Nur-Sultan, 2021 The topic of the oral report: "Epidemiological characteristics of measles and phylogenetic analysis of the virus circulating in the Republic of Kazakhstan in 2018".

7. I scientific and practical conference of students and young scientists "Chronic inflammatory processes of the skin. Interdisciplinary problems" Nur-Sultan, 2022. The topic of the oral report: "The course of measles at the present stage in children with an unfavorable premorbid background".

8. International scientific and practical conference "COVID - 19 and other topical infections of Central Asia" Shymkent 2022. The topic of the oral report: "Clinical and morphological characteristics of measles in patients with fatal outcome of MGDB No. 3 Nur-Sultan".

9. At the expanded meeting of the Department of Pediatric Infectious Diseases of the NAO "Nur-Sultan Medical University", June 20, 2022.

Personal contribution of the dissertation

The work was carried out in accordance with the direction of development of science in the field of "Life and health Sciences" approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan. The dissertation independently conducted the collection of material, carried out a clinical examination of patients after surgical treatment of colon cancer, questioning patients to determine the quality of life. The dissertation independently analyzed and summarized the results of the study, conducted statistical data processing.

The scope and structure of the dissertation

The dissertation work is presented on 117 pages of a computer set, consists of an introduction, a literature review, 4 sections of own research, where materials and methods, research results, comparative characteristics of the obtained data of two groups, discussion of own results, conclusion and practical recommendations are presented. The list of sources used is presented by 181 sources, of which 12 are in Russian, 169 are in foreign languages.