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Congenital Anomalies of Circulatory System among the Pediatric Population in Kazakhstan: Space and Time Assessment of Incidence

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Abstract: The purpose of study is to investigate the epidemiological features of the incidence of congenital anomalies of circulatory system (CACS) among children in the whole country and its regions. The epidemiological characteristics of the disease incidence are described in the research. This is a retrospective study for the years 2003-2011. The main source of study materials was summary report from the Ministry of Public Health of the Republic of Kazakhstan (Form 12), about children, patients with CACS (International Classification of Diseases-Q20-Q28), in general, established for the first time in life. We used descriptive and analytical methods of modern epidemiology. For space-time assessment of the under study pathology in children, the method of compiling cartograms was used, based on the definition of a standard deviation (σ) from an average of (x). It is found that 73.3% of CACS fall on the child population under the age of 15. The CACS average annual incidence among children in the whole country amounted $152.0\pm14.7^{0}/_{0000}$. The adjusted incidence rate trends tended to increase (T=+12.0%). A space and time incidence was assessed according to the administrative division with the following morbidity levels: low-up to $99.3^{0}/_{0000}$, average – from 93.3 to $208.9^{0}/_{0000}$ and high – from $208.9^{0}/_{0000}$ and above. The incidence trends of all the studied regions tended to increase and the rates of increase were most registered in South Kazakhstan (T=+29.6%) and Pavlodar (T=+36.5%) regions. The results of the CACS incidence indicate intensive growth of this disease among the pediatric population of Kazakhstan. Perhaps, this is due to the inefficacy of undertaken prevention, or, it could be explained by the improved diagnostic measures. Also, on the cartogram, a high incidence rate was revealed in the regions with adverse environmental conditions.

Key words: Congenital anomalies of circulatory system・Incidence・Pediatric population・Kazakhstan

INTRODUCTION

The need and relevance for studying congenital anomalies of circulatory system (CACS) is primarily determined by the increased share of this disease in the causes of infant mortality, morbidity and disability, as well as the growth in the absolute number of CACS cases among children [1-4]. In the classification of congenital anomalies of circulatory system, congenital heart diseases (CHD) occupy the leading position. Thus, according to the American Heart Association, the frequency of CHD in the United States is accounted as 4-10 cases per 1,000 and, on the average, about 8 cases per 1,000 live births. In Europe, the prevalence is 6.9 cases per 1,000 live births and up to 9.3 cases per 1,000 live births are registered in Asia [5]. As per statistical data of Russian Federation, CACS form at least 30% of all congenital malformations (CM) and its prevalence is increasing in the course of time. Based on the majority of domestic and foreign researchers, 8 out of 1,000 born children have congenital heart diseases [6-8].

Despite the intensive researches, the mechanisms of congenital malformations are not studied enough.

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There is no doubt that the basis for CACS is formed by both genetic factors [9-11] and adverse effects of harmful environmental factors on the embryo and fetus, which classify this pathology as an environmental-related disease [10, 12-14].

At present, particular attention is paid to the issues of the regional prevalence, incidence, mortality and medical and social aspects of disability in children with cardiovascular diseases, which should underlie the planning of priority actions on improving the pediatric population health. In this regard, there is a need for the epidemiological studies of the CACS incidence and the improvement of prevention, especially in the areas that are concerned as extreme for the climatic and geographical features; the degree of industrial development and; the population development method.

It is certain that the medical and genetic consequences of such rapid urbanization, outbreeding and environmental changes are of a considerable scientific and practical interest. Thereby, the epidemiological studies are conducted to study the causes and assessment of the efficacy of prevention; the important part is the rate of transition of the under study population from the state "healthy" to the state "patient", i.e. studying the incidence.

The aim of this research is to investigate the epidemiological features of CACS incidence in the whole country and in separate regions and to set changes in the frequency of CACS in time and space.

MATERIALS AND METHODS

The main sources of information, when preparing the topic, were the materials of state record of patients with CACS (International Classification of Diseases-Q20-Q28). Summary report form ¹ 12 of the Ministry of Public Health of the Republic of Kazakhstan for the years 2003-2011 and data about the quantity of child population for the years 2003-2011 from the Agency of Statistics of the Republic of Kazakhstan [15] were used in the research.

According to the law of the Republic of Kazakhstan "About State Statistics" [16], the information in the summary report is confidential and may only be used for statistical purposes. The information may be shared for research purposes only if a requesting organization provides the data security and undertakes all the necessary actions in making unable the identity of respondents, in concordance with the Principles of the World Medical Association (WMA) Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly in Helsinki, Finland, in June 1964.

The incidence of CACS in children under the age of 15 was analyzed retrospectively (2003-2011). The materials were collected and sorted by administrative-territorial divisions of the country (14 regions and 2 cities: Astana and Almaty).

Different methods of modern biomedical statistics were used: extensive indicator (EI) analysis, incidence rate (IR) analysis (annual, average annual, errors) and analysis of dynamic series (least squares fitting, average compound rate-average annual Growth / Loss rates, T, %), 95% confidence interval (95%CI=M±1.96×m).

The main method, used in the study of CACS incidence, is a retrospective study with descriptive and analytical methods of epidemiology. EI and II of the incidence are defined by the standard technique used in modern biomedical statistics. The justification of basic calculation formulas have not been performed, as they are shown in details in the methodological guidelines and textbooks on health statistics [17-19], yet some are presented below:

$$Extensive\,rate = \frac{n \times 100\%}{N}$$

with n being number of cases from the total population of N.

$$Incidence\,rate = \frac{n \times 100000}{N}$$

with n being number of diseases, from the average population of N.

The average compound rate, equal to the n-th root of the product of annual indices, is used to calculate the average annual growth rates and / or increase of dynamic series:

$$T_{\Pi p} = \sqrt[n]{T_1 \times T_2 \times T_3 \times T_n}$$

where T is annual growth/loss rate; n is number of indices.

In compiling the cartograms were used the CACS incidence rates in children for 9-years period (2003-2011). We used a method of compiling cartograms, proposed by S.I. Igissinov [20] in 1974, based on the definition of a standard deviation (σ) from an average of (x). The scale levels are calculated as follows: taking σ as an interval, we defined the maximum and minimum levels of the disease according to this formula: $x \pm 1.5\sigma$, with the minimum level of $x-1.5\sigma$ and a maximum equal to $x+1.5\sigma$. Then, we defined the scale levels of the cartogram: *1)* $(x-1.5\sigma)+\sigma$; *2)* $(x-1.5\sigma)+2\sigma$; *3)* $(x-1.5\sigma)+3\sigma$, etc.; a clustering of indices is derived from the formula $x \pm 0.5\sigma$, corresponding with the average level ($x-0.5\sigma$ and $x+0.5\sigma$); the values that are distant from the average incidence by σ, show lower $((x-0.5\sigma)-\sigma)$ and higher $((x-0.5\sigma)+\sigma)$ values.

When grouping the parameters for construction of equal intervals (σ), a formula proposed by A.Ya. Boyarsky was used [21]:

$$\gamma = \frac{X_{\max} - X_{\min}}{1 + 3{,}22 \lg n}$$

where $\times_{max}$ is a maximum index; X_{min} – a minimum index; and n is a number of population, i.e. amount of areas and cities Viewing and processing materials was made on the computer (using software package Microsoft Office: Excel, Word, Access; BIOSTAT, EpiInfo 7).

RESULTS AND DISCUSSION

General Numeric Patterns and the Dynamics of the Incidence in the Entire Country and in Separate Regions: In the whole country of Kazakhstan, the total number of patients registered for the first time over the given period amounted 71,102, of whom 52,123 (73.3%) are children. Herein, low extensive indices of CACS among children were shown in Atyrau (1.5%) and North-Kazakhstan (2.1%) regions and the highest proportion of patients was traced in the southern capital of the republic, Almaty, (19.5%) and in South Kazakhstan (22.6%) (Table 1).

Table 1: The number of the CACS incidence among children in the Republic of Kazakhstan, provided by regions for the years 2003-2011

Region / City	abs.	%
Atyrau	791	1.5
North Kazakhstan	1,102	2.1
Mangystau	1,296	2.5
West Kazakhstan	1,406	2.7
Aktobe	1,437	2.8
Kyzyl-Orda	1,463	2.8
Astana city	1,682	3.2
Karaganda	2,094	4.0
East Kazakhstan	2,320	4.5
Kostanai	2,388	4.6
Zhambyl	2,767	5.3
Almaty	3,142	6.0
Pavlodar	3,296	6.3
Akmola	5,022	9.6
Almaty city	10,141	19.5
South Kazakhstan	11,776	22.6
Total in Republic	52,123	100.0

The average annual incidence rate of CACS in the republic among children is accounted for $152.0 \pm 14.7^0/_{0000}$ (95%CI=123.2-$180.9^0/_{0000}$). Dynamically, the CACS incidence rate in children in the whole country increased from $92.3 \pm 1.6^0/_{0000}$ (95%CI=89.3-$95.4^0/_{0000}$) in 2003 to $188.5 \pm 2.2^0/_{0000}$ (95%CI=184.2-$192.7^0/_{0000}$) in 2011. 95%CI of rates did not superimpose on one another, meaning the difference was statistically significant ($p<0.05$). The Trends of aligned incidence rates of congenital anomalies of circulatory system among children in dynamics also grew with an average annual growth rate of T=+12.0% (Fig. 1).

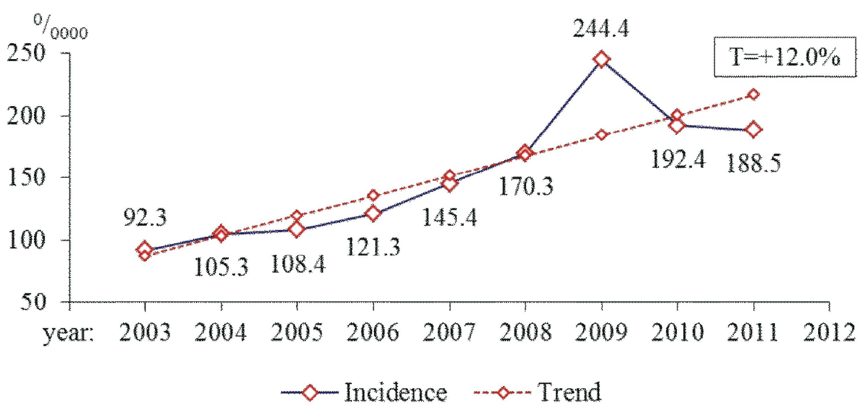

Fig. 1: The dynamics of incidence rates of congenital anomalies of circulatory system among children in the Republic of Kazakhstan for the years 2003-2011

Table 2: The average annual incidence of CACS among children by regions of Kazakhstan for the years 2003-2011

	Incidence, $^0/_{0000}$		
Region / city	P±m	95%CI	T, %
Atyrau	62.5±6.0	50.8-74.1	+9.9
Kyzylorda	81.5±2.6	76.4-86.6	+1.3
Karaganda	82.8±3.6	75.8-89.9	+3.4
Almaty	83.1±4.6	74.0-92.1	+2.6
East Kazakhstan	90.7±3.2	84.5-96.8	+3.6
Aktobe	90.8±5.4	80.3-101.4	+6.3
North Kazakhstan	96.5±9.1	78.6-114.4	+7.5
Zhambyl	106.1±9.7	87.0-125.1	+12.1
Mangystau	113.1±14.3	85.1-141.1	+5.4
West Kazakhstan	113.5±12.5	89.0-137.9	+13.8
Republic	152.0±14.7	123.2-180.9	+12.0
Kostanai	154.1±21.9	111.1-197.1	+18.3
South Kazakhstan	167.0±32.2	103.9-230.1	+29.6
Astana city	170.8±9.6	151.9-189.6	+0.3
Pavlodar	249.6±49.2	153.2-346.0	+36.5
Akmola	342.7±51.7	241.3-444.1	+19.8
Almaty city	461.1±13.5	434.7-487.6	+3.1

Table 3: The scale levels of the cartogram of the CACS incidence among the pediatric population in Kazakhstan for 2003-2011

Incidence levels	Children
Low	Up to 99.3$^0/_{0000}$
Average	from 93.3 to 208.9$^0/_{0000}$
High	from 208.9$^0/_{0000}$ and above

The CACS incidence in children by the regions of the republic had regional features. The lowest rate was also defined in Atyrau region-62.5±6.0$^0/_{0000}$ (95%CI=50.8-74.1$^0/_{0000}$) (Table 2).

In Kyzylorda (81.5±2.6$^0/_{0000}$, 95%CI=76.4-86.6$^0/_{0000}$) and Karaganda (82.8±3.6$^0/_{0000}$, 95%CI=75.8-89.9$^0/_{0000}$) regions, low CACS incidence rates among children were registered. (Table 2). The analysis of 95%CI for the abovementioned regions showed that they superimposed on one another overlap, i.e. the differences were not statistically significant (p>0.05).

The highest average annual incidence rates of CACS were registered in Akmola region-342.7±51.7$^0/_{0000}$ (95%CI=241.3-444.1$^0/_{0000}$) and in Almaty city- 461.1±13.5$^0/_{0000}$ (95%CI=434.7-487.6$^0/_{0000}$). In these regions, the same factors have influenced to the formation of incidence rates, as their 95%CI superimposed on one another, so, there was no statistically significant difference (p>0.05) (Table 2). At the same time, there is a statistically significant difference (p<0.05) in comparison to the incidence of CACS in children of other regions.

In the dynamics, the incidence rates of CACS in children grew in all studied regions and the average annual growth rate of adjusted parameters were registered most in the South Kazakhstan (T=+29.6%) and Pavlodar (T=+36.5%) regions (Table 2).

Thus, the analysis of the CACS incidence among the pediatric population of the country showed that there are regional differences and trends of CACS incidence are growing. At the same time, there are regions where the average annual growth rates were noticed highest.

So, the established features of CACS incidence across the country show that the pathology becomes epidemic, which requires the development of targeted measures to counteract CACS. The incidence rates could be used in assessing the need for medical care or in planning health services.

Allocation of the Incidence among Regions: One of the types of space evaluation is providing a medical-geographic cartogram, which is an advanced technique that enables the health authorities to conduct targeted therapeutic and preventive arrangements. The levels, which are presented in Table 3, were determined to produce the cartogram of the CACS incidence in the Republic of Kazakhstan.

Based on the above scale we have compiled a cartogram of the CACS incidence among pediatric population in a variety of medical and geographic areas of the republic and have identified the following groups of regions (Figure 2):

Regions: 1. Akmola, 2. Aktobe, 3. Almaty, 4. Atyrau, 5. East Kazakhstan, 6. Zhambyl, 7. West Kazakhstan, 8. Karaganda, 9. Kostanai, 10. Kyzylorda, 11. Mangystau, 12. Pavlodar, 13. North Kazakhstan, 14. South Kazakhstan

1. Regions with low level (up to 99.3$^0/_{0000}$)-Atyrau (62.5$^0/_{0000}$), Kyzylorda (81.5$^0/_{0000}$), Karaganda (82.8$^0/_{0000}$), Almaty (83.1$^0/_{0000}$), East Kazakhstan (90.7$^0/_{0000}$), Aktobe (90.8$^0/_{0000}$) and North Kazakhstan (96.5$^0/_{0000}$) regions.
2. Regions with average level (from 93.3 to 208.9$^0/_{0000}$)-Zhambyl (106.1$^0/_{0000}$), Mangystau (113.1$^0/_{0000}$), West Kazakhstan (113.5$^0/_{0000}$), Kostanai (154.1$^0/_{0000}$) and South Kazakhstan (167.0$^0/_{0000}$) regions and Astana city (170.8$^0/_{0000}$).
3. Regions with high level (from 208.9$^0/_{0000}$ and above)-Pavlodar (249.6$^0/_{0000}$) and Akmola (342.7$^0/_{0000}$) regions and Almaty city (461.1$^0/_{0000}$).

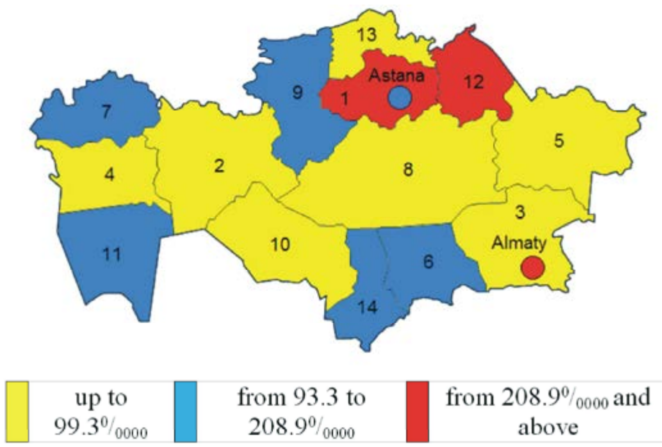

Fig. 2: The cartogram of the CACS incidence among children in the Republic of Kazakhstan for 2003-2011

Table 4: The calculation and assessment of the "normalcy" of allocation of the CACS incidence among children, by Westergaard

		Number of districts		
Interval	Obtained data	Abs.	%	% by Westergaard
$x\pm0.3\sigma$	121.2-187.0	3	19	25
$x\pm0.7\sigma$	77.4-230.8	12	75	50
$x\pm1.1\sigma$	33.6-274.7	14	88	75
$x\pm3.0\sigma$	0.0-482.9	16	100	99.8

Table 5: The calculation scheme for determining normal and theoretical allocation of the CACS incidence among children and Pearson criterion (x^2)

N	GR*(γ=0,23)	CM(V)	NoD(p)	$V\times p$	$d=V-x$	d^2	$d^2\times p$	$t==(V-x)/\sigma$	$F(t)$	TF($(\gamma\times\Sigma p)/\sigma)\times\times F(t)$	RTF, p'	$p-p'$	$(p-p')^2/p'$
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	0.0-81.7	40.9	1	40.9	-113.3	12829.3	12829.3	1.03	0.2347	2.800	3	-2	1.16
2.	81.7-163.4	122.6	10	1225.5	-31.6	996.5	9964.5	0.29	0.3825	4.564	5	5	6.48
3.	163.4-245.1	204.3	2	408.5	50.1	2513.4	5026.7	-0.46	0.3589	4.282	5	-2	1.22
4.	245.1-326.8	286.0	1	286.0	131.8	17380.0	17380.0	-1.20	0.1942	2.317	2	-1	0.75
5.	326.8-408.5	367.7	1	367.7	213.5	45596.5	45596.5	1.95	0.0595	0.710	1	0	0.12
6.	408.5-490.2	449.4	1	449.4	295.2	87162.7	87162.7	2.69	0.0107	0.128	0	1	5.96
		$\Sigma p=n=16$		$\Sigma V*p=2777.8$		$\Sigma d^2*p=177959.8$		-	-	-	16	x^2	=15.7

*GR - grouping regions, CM – class mark, NoD – the number of districts, TF – theoretical frequency, RTF – refined theoretical frequency, 10th column is determined in a special table [22].

The cartogram of the CACS incidence among children repeats the patterns that were presented in this paper and more clearly reflects the spatial allocation of CACS in separate territories.

To assess the "normalcy" of the allocation, the number of Westergaard was determined (Table 4).

The findings prove that the actual incidence of CACS in children by regions and cities, more precisely complies with the normal allocation law. Thus, the number of regions that exceed the arithmetic mean (x) at 3σ compose 6 regions, i.e. exceeding the average ($154.1^{0}/_{0000}$) or 38% and the asymmetry of the normal curve was AS=p%-50%=38%-50%=-12%, i.e. a negative left-sided asymmetry occurred. The CACS incidence among adolescents in most regions of the country should be less than average.

The cartogram of the CACS incidence among the pediatric population repeats the patterns described in this study and more clearly reflects the spatial allocation of CACS in the country. The discrepancy between theoretical and actual allocations of the CACS incidence on selected areas and cities is insignificant, the Pearson criterion (x^2) is 15.7 (calculations are shown in Table 5), which is higher than the tabulated value 0.4232 (with k=6). Therefore, the actual allocation of the frequency of CACS on areas of Kazakhstan is close to the normal allocation.

CONCLUSIONS

Thereby, it was found that a high proportion of patients with congenital anomalies of circulatory system (CACS) were diagnosed in children under the age of 15 (73.3%), which means, other malformations are diagnosed at an older age. Why does this happen? Apparently, it is directly connected with the type of CACS. In the classification of the current pathology, congenital heart defects dominate in the country (about 62.5-65.9%, the data for 2011-2012). In this article we do not consider this aspect. Hence, different (later) clinic manifestations occur and, respectively, the diagnostic issues arise too. Unfortunately, in registration and reporting documents, only from the year 2011 the heart defects have been accounted separately from the total number of CACS. As for the age groups, the division is only for children (under the age of 15), adolescents (15-17 years) and adults (18 and older), which, in our opinion, is wrongs. Naturally, this implies the establishment of a national population-based register for monitoring patients with congenital malformations, especially with CACS. High extensive indices of CACS were noticed in Almaty city and South Kazakhstan region, which occupy a leading position in a number of demographic indicators-high population, high birth rates, etc. In order to make an assessment, it is necessary to study prevalence and incidence, since the absolute numbers and proportion, in spite of their value, do not represent the true epidemiology of CACS. So, the highest incidence rates were remarked in Pavlodar ($249.6^{0}/_{0000}$) and Akmola ($342.7^{0}/_{0000}$) regions and in Almaty city ($461.1^{0}/_{0000}$). Perhaps, this is due to the environmental factors, for these regions, because of a number of factors, are not considered as environmentally prosperous areas of the country.

Certainly alarming is the fact that in all the studied regions, the rising trends of the CACS incidence among children are noted: especially in South-Kazakhstan (T=+29.6%) and Pavlodar (T=+36.5%) regions. The increase of incidence rates evidence that the cause-and-effect relationships are not stated, the preventive measures are inefficient, or whether it is connected with the improved diagnostics.

Considering the received results of our research, which is generally of a descriptive nature, further on, studying the disease in different age and sex groups of the population, taking into account the effect of various exogenous and endogenous risk factors, including the environmental factors and the degree of contamination, will be of the main priorities of our future research.

REFERENCES

1. Morton, W.E. and L.A. Huhn, 1966. Epidemiology of Congenital Heart Disease. Journal of the American Medical Association, 195: 1107.
2. Hoffman, J.L. and S. Kaplan, 2002. The Incidence of Congenital Heart Disease. Journal of the American College of Cardiology, 39(12): 1890-1900.
3. Bernier, P.L., A. Stefanescu, G. Samoukovic, *et al.*, 2010. The Challenge of Congenital Heart Disease Worldwide: Epidemiologic and Demographic facts. Seminars in Thoracic Cardiovascular Surgery: Pediatric Cardiac Surgery Annual, 13(1): 26-34.
4. www.heart.org/idc/groups/heart-public/@wcm/@sop/@smd/documents/downloadable/ucm_319830.pdf
5. Belokon', N.A. and V.P. Podzolkov, 1991. Congenital Heart Defects. Moscow: Meditsina (in Russian).
6. Bokeriya, L.A., I.N. Stupakov and R.G. Gudkova, 2006: Morbidity and Congenital Malformations of Circulatory System in Children (Prevalence and Correction). Childhood Diseases of Heart and Blood Vessels, 1: 1-3 (in Russian).
7. Gracheva, O.N. and O.I. Lineva, 2009. Prevention of Congenital Heart Defects in the Fetus and the Optimization of the Follow-up of Pregnant Women with CHD. Medical Alphabet. Clinics, 3.1: 19-24.
8. Bentham, J. and S. Bhattacharya, 2008. Genetic Mechanisms Controlling Cardiovascular Development. Annals of the New York Academy of Sciences, 1123: 10-19.
9. Lage, K., S.C. Greenway, J.A. Rosenfeld, *et al.*, 2012. Genetic and Environmental Risk Factors in Congenital Heart Disease Functionally Converge in Protein Networks Driving Heart Development. Proceedings of the National Academy of Science of the United States of America, 109(35): 14035-14040.
10. Fahed, A.C., B.D. Gelb, J.G. Seidman, *et al.*, 2013. Genetics of Congenital Heart Disease: the Glass Half Empty. Circulation Research, 112(4): 707-720.
11. Stoll, C., Y. Alembik, M.P. Roth, *et al.*, 1989. Risk Factors in Congenital Heart Disease. European Journal of Epidemiology, 5(3): 382-391.
12. Tikkanen, J. and O.P. Heinonen, 1990. Risk Factors for Cardiovascular Malformations in Finland. European Journal of Epidemiology, 6(4): 348-356.

13. Brent, L., 2004. Environmental Causes of Human Congenital Malformations: The Pediatrician's Role in Dealing With These Complex Clinical Problems Caused by a Multiplicity of Environmental and Genetic Factors. Pediatrics, 113: 957-968.
14. Rankin, J., T. Chadwick, M. Natarajan, *et al.*, 2009. Maternal Exposure to Ambient Air Pollutants and Risk of Congenital Anomalies. Environmental Research, 109(2): 181-187.
15. www.stat.kz – The official site of the Statistics Agency of the Republic of Kazakhstan.
16. The official site of the Ministry of Justice of the Republic of Kazakhstan. The Law of the Republic of Kazakhstan "On State Statistics". Date Views 07.07.2013. www.adilet.gov.kz/ru/node/846.
17. Merkov, A.M. and L.E. Polyakov, 1974. Sanitary Statistics. Leningrad: Meditsina (in Russian).
18. The Use of Statistical Analysis Methods to Study Public Health and Healthcare Service, 2004. Moscow (in Russian).
19. Tango, T., 2010. Statistical Methods for Disease Clustering (Statistics for Biology and Health), New York, Springer.
20. Igissinov, S.I., 1974. The Method for Constructing and Using Cartograms in Oncology Practice. Health Care in Kazakhstan, 2: 69-71 (in Russian).
21. Bojarsky, A.Y., 1977. General Theory of Statistics. Moscow (in Russian).
22. Sepetliev, D., 1968. Statistical Methods in Medical Research. Moscow: Meditsina, Annex, (in Russian), 31: 410.