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DYNAMICS OF FREQUENCY AND PECULIARITIES OF THE STRUCTURE OF CONGENITAL MALFORMATIONS IN SOUTH UKRAINE (MONITORING STUDY)

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Background. *In Ukraine, the unfavorable demographic situation makes monitoring of the birth rate of children with congenital malformations urgent issue to identify regional features of epidemiology and develop methods for prenatal diagnosis and prognosis.*

Objective. *Objective of this study is to characterize the frequency dynamics, to identify structural features of congenital malformations of newborns in Kherson region over a 20-year period (2000-2019) and to compare the prevalence of various nosological forms of malformations in the region, in Ukraine and in European countries.*

Methods. *Research methods: epidemiological, medical-statistical.*

Results. *In Kherson region, the average frequency of congenital malformations over the past 20 years is: for newborns – 31.57±1.25‰; for live births – 31.38±1.11‰; for stillborns – 197.7±0.65 per 10,000. In the structure of defects, cardiovascular malformations are leading (31.77%), musculoskeletal malformations (25.14%), genital malformations (17.5%). Increased prevalence of developmental anomalies in the region is mainly associated with an increase in the frequency of model malformations recorded by EUROCAT ($r=0.69$, $p<0.05$). The increase in the total frequency of congenital malformations is caused by increased number of births of children with cardiovascular defects (by 4.67‰), genital defects (by 1.21‰), other congenital malformations (by 1.55‰), multiple malformations (by 0.37‰).*

Conclusion. *Monitoring results showed an increase in congenital malformations incidence in Kherson region over a 20-year period by 7.94‰ possibly caused by population decline due to negative natural and mechanical growth. The prevalence of hereditary defects is at the same level. The frequency of some nosological forms significantly exceeds in the region compare to that in Ukraine and Europe: cardiovascular defects – in 1.5 times, genital malformations – in nearly 3 times, musculoskeletal defects – almost twice.*

KEYWORDS: **congenital malformations; chromosomal pathology; population; newborns.**

Introduction

An unfavorable demographic situation in Ukraine (decrease in birth rate, high mortality and disability, negative natural population growth) necessitates monitoring of the genetic load of human populations, which averages 50-70 per 1000 newborns (5-7%): congenital malformations account for 2-5%, hereditary diseases – 1.5% (chromosomal – 0.5%, genetic – 1%), diseases with a significant hereditary predisposition – 3-3.5% [1]. Monitoring the birth rate of children with congenital malformations is necessary for identifying the epidemiology of its various nosological forms and for development of methods for prenatal diagnosis and prognosis [2]. According to current concepts, congenital malformations are defects in morphogenesis in the early period of fetal life of genetic and/or epigenetic etiology [3].

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The spectrum of mutations in genes that control the form-developing processes in embryogenesis may be ethno specific. Therefore, during monitoring of the prevalence and structure of malformations, the influence of ethnic factors should be taken into account, as well as not only epidemiological, but also genetic and demographic aspects [4,5].

Thus, the aim of the study was to define the dynamics of frequency, to identify the structural features of congenital malformations of newborns in Kherson region over a 20-year period (2000-2019) and to compare the prevalence of various nosological forms of congenital malformations in the region, Ukraine and European countries.

Methods

The study was conducted in Kherson region, covering all 18 territorial-administrative regions. For the analysis, the 20-year observation period (2000-2019) was divided into five-year

periods (2000-2004, 2005-2009, 2010-2014, 2015-2019).

The objects of the study were newborns (live and stillborn) with isolated and multiple congenital malformations, which were born to residents of Kherson region during the study period. The obtained material was analyzed using the registration of all forms of malformations included in the list of ICD-10 of section Q in order to assess their frequency and structure.

When conducting a comparative analysis of the incidence of malformations in populations, it is necessary to take into account standard markers – those forms of them that are often encountered and are unambiguously diagnosed by doctors of all specialties in one age cohort. Such forms have a well-defined phenotype and are well diagnosed at birth. These are: multiple congenital malformations and compulsory model defects (Q00-Q99). Long-term monitoring of populations for such markers makes it possible to assess the prevalence and dynamics of malformations and conduct their comparative analysis in populations, which is one of the tasks of the European international register EUROCAT (European surveillance of congenital anomalies).

Since the number of abortions is not large enough to have a significant impact on the prevalence of defects, calculations for this indicator were carried out in a cohort of newborn children. Most cases of malformations among all pregnancies occur in children born alive; therefore, it is the assessment of the dynamics of their prevalence in this cohort, and not all births, that is the most acceptable form of analysis of epidemiological data [6].

The prevalence of malformations was calculated as the ratio of the number of newborns with congenital malformations to the total number of newborns multiplied by 1000 (‰). Similar calculations were carried out in cohorts of live and stillborn children. The data for a comparative analysis of the frequencies of congenital malformations were taken from the open official website of the European register of the international organization EUROCAT [http://www.eurocat-network.eu].

The theoretical indicator of the number of hereditary congenital malformations was calculated as the sum of cases of chromosomal pathology of living children and half of the remaining part of congenital malformations (without chromosomal diseases), since it is established that about half of cases of congenital malformations (regardless of clinical manifestation) are caused by genetic factors [7].

Statistical processing of the research results was carried out using MS Excel: determining of the average value, error of the average value, growth rate. Using the Statistica 6.0 software package, the statistical connection of features was determined by the Spearman rank correlation method.

Results

In total, in 2000-2019, 222,191 newborn babies were born in Kherson region; 220,067 of them were live-born and 2,124 still-born. Congenital malformations were found in 7015 newborns, including 6973 live births and 42 stillborns. The obtained data allowed estimating the population frequency of congenital malformations in children of Kherson region, which is: for newborns – 31.57±1.25‰; for live births – 31.38±1.11‰; for stillbirths – 197.7±0.65 per 10,000. Based on the data of the Regional Department of Statistics in Kherson region, the average annual number of live births in the region for the last 20 years has been calculated, which is 11,003 children (with an average statistical deviation of up to 5%).

Theoretical calculations of the prevalence of congenital malformations in the region show that approximately 556 newborns (5%) may be born with signs of congenital pathology. Since the contribution of the genetic component to the structure of malformations is on average about 50% (the other half is a consequence of teratogenesis) [8], thus for Kherson region the hereditary component of malformations is expected at the level of 278 people per year (2.5% of the number of newborns). Actual calculations of the prevalence of congenital malformations are presented in Table 1.

According to Table 1, with the decrease in the birth rate in the region, the prevalence of congenital malformations is rising (from 20.6‰ in 2004 to 37.6‰ in 2017) and on average over a 20-year period was 31.4‰. Among all congenital malformations, the actual number of cases of hereditary forms (181 people per year) turned out to be lower than the theoretically calculated indicator (278 people per year); it did not undergo significant changes in recent years and averaged within 52% of all anomalies; this testifies the stable level of mutation process in the region. But in a long-time tendency, the share of hereditary forms is gradually increasing: in 2000 it was 1.29%, in 2019 it was 1.62%. There is a gradual increase in the

frequency of multiple malformations: from 0.29 to 0.66‰.

Since the incidence of neonatal malformations and spontaneous miscarriages correlates with their prevalence among live births, we analyzed the dynamics of nosological forms of congenital anomalies in the last cohort (Table 2).

According to Table 2 in the structure of congenital malformations malformations of the cardiovascular system are leading (31.77% of all cases, an average annual increase of 9.1%), the frequency of which is constantly increasing from 5.1 to 9.93‰ on average over the past 20 years. Congenital malformations of the musculoskeletal system (25.14% of all cases, the prevalence slightly decreases from 7.93 to 6.13 and averages 7.35) are the second. Stably high frequency of malformations of the genital organs (on average 5.23‰; in the structure – 17.5% of the total number of cases).

There is a gradual increase in the frequency of multiple malformations – from 0.29 to 0.66‰, and a slight increase in their share in the overall structure of anomalies (by 0.8%). The prevalence of chromosomal abnormalities (1.0-1.17‰) is characterized by relative stability, which confirms the previous assumption about the constancy of the level of the mutation process in the region.

The greatest lethality is caused by defects of the central nervous system (Q00-Q07), in particular anencephaly, multiple congenital malformations (Q89.7) and chromosomal abnormalities (Q90-Q99).

Then a comparative analysis of the prevalence of congenital malformations in Kherson region was carried out regarding Ukraine and European countries (according to the international register EUROCAT) (Table 3). It has been noted that there is a significant increase in the frequency of malformations of the car-

Table 1. Dynamics of congenital malformations prevalence (Kherson region, 2000-2019)

Years	Number of live births	Congenital malformations									
		Total	Frequency, ‰	Genetically determined congenital malformations:							
				cases of chromosomal pathology					total genetically determined	part, %	
				Q90-Q99	of them:					from congenital malformations	from live births
					Ds	Ps	Es	Ts			
2000	10 184	255	25.0	8	8	-	-	-	131.5	51.6	1.29
2001	9 757	243	24.9	9	9	-	-	-	126.0	51.9	1.29
2002	9 972	280	28.1	11	8	-	1	-	145.5	52.0	1.46
2003	10 502	216	20.9	15	14	-	-	-	115.5	53.5	1.10
2004	10 363	201	20.6	8	5	-	-	-	104.5	52.0	1.00
2005	10 150	307	30.2	11	7	1	-	1	159.0	51.8	1.57
2006	11 475	397	34.6	13	13	-	-	-	205.0	51.6	1.83
2007	11 570	444	38.4	21	18	3	-	-	232.5	52.4	1.79
2008	12 473	556	44.6	18	16	1	-	1	287.0	51.6	2.30
2009	12 323	384	31.2	21	18	1	1	-	202.5	52.7	1.64
2010	12 388	391	31.6	18	13	-	1	-	204.5	52.3	1.65
2011	12 085	418	34.6	15	9	1	-	-	216.5	51.8	1.79
2012	12 643	428	33.8	16	14	-	-	-	222.0	51.9	1.76
2013	12 300	388	31.5	15	15	-	-	-	201.5	51.9	1.64
2014	12 308	433	35.2	11	10	-	-	-	222.0	51.3	1.80
2015	11 372	352	31.0	17	13	-	2	-	184.5	52.4	1.62
2016	10 769	350	32.5	11	9	-	2	-	180.5	51.6	1.68
2017	9 964	375	37.6	4	3	-	-	-	189.5	50.5	1.90
2018	9 095	293	32.2	13	12	-	1	-	153.0	52.2	1.68
2019	8 374	262	31.3	9	9	-	-	-	135.5	51.7	1.62
M	**11003.4**	**348.65**	**31.4**	**13.2**	**11.2**	**0.4**	**0.4**	**0.1**	**180.9**	**51.9**	**1.62**
m	281.48	20.15	1.28	1.02	0.9	-	-	-	10.36	0.13	0.065

Note: Ds – Down syndrome; Ps – Patau syndrome; Es – Edwards syndrome; Ts - Turner syndrome.

Table 2. Dynamics of the frequency and structure of congenital malformations of compulsory registration (Q00-Q99) among live births in Kherson region

Form CM (patient's medical record-10)	Units	Monitoring periods (years)				On average over 20 years	Rank	Average increase rate, %
		2000-2004	2005-2009	2010-2014	2015-2019			
Congenital malformations:								
Nervous system (Q00-Q07)	abs. ‰ %	7.8 0.77 3.40	10,6 0,93 2,62	7,8 0,64 2.01	7.2 0.73 2.34	8.35 0.76 2.59	8	+0.55 + 0.05 - 0.81
Ear. face and neck (Q10-Q18)	abs. ‰ %	1.8 0.18 0.78	5.0 0.44 1.24	5.6 0.46 1.44	4.4 0.44 1.43	4.2 0.38 1.22	11	+ 2.4 + 0.2 + 0.44
Congenital heart defects (Q20-Q28)	abs. ‰ %	52.0 5.10 22.67	130.0 11.35 32.13	150.6 12.27 38.83	102.8 10.37 33.46	108.9 9.93 31.77	1	+ 56.9 + 4.67 + 9.1
Respiratory (Q30-Q34)	abs. ‰ %	4.0 0.39 1.74	5.2 0.45 1.29	5.4 0.44 1.39	3.0 0.30 0.98	4.4 0.40 1.35	10	+ 0.4 + 0.01 - 0.39
Cleft lip with or without palate (Q35-Q37)	abs. ‰ %	10.0 0.98 4.36	11.8 1.03 2.92	10.2 0.83 2.63	9.2 0.93 3.0	10.3 0.94 3.23	7	+ 0.3 - 0.04 - 1.13
Other congenital malformations of the digestive system (Q38-Q45)	abs. ‰ %	10.0 0.98 4.36	19.0 1.66 4.70	12.4 1.01 3.20	10.0 1.0 3.26	12.85 1.17 3.88	6	+ 2.85 + 0.18 - 0.48
Genital (Q50-Q56)	abs. ‰ %	41.0 4.02 17.87	61.4 5.36 15.18	64.6 5.26 16.66	62.2 6.27 20.25	57.3 5.23 17.50	3	+ 16.3 + 1.21 - 0.37
Limb (Q65-Q79)	abs. ‰ %	80.8 7.93 35.22	99.6 8.70 24.62	81.2 6.62 20.94	60.8 6.13 19.79	80.6 7.35 25.14	2	- 0.2 - 0.58 - 10.08
Other congenital malformations (Q80-Q89)	abs. ‰ %	8.8 0.86 3.84	34.8 3.04 8.60	33.8 2.75 8.72	29.4 2.97 9.57	26.7 2.41 7.68	4	+ 17.9 + 1.55 + 3.84
Multiple malformations (Q89.7)	abs. ‰ %	3.0 0.29 1.31	10.6 0.93 2.62	8.2 0.67 2.11	7.4 0.75 2.41	7.3 0.66 2.11	9	+ 4.3 + 0.37 + 0.80
Chromosomal abnormalities (Q90-Q99)	abs. ‰ %	10.2 1.0 4.45	16.6 1.43 4.10	14.0 1.18 3.61	10.8 1.08 3.52	12.9 1.17 3.92	5	+ 2.7 + 0.17 - 0.53
of which Down syndrome (Q90)	abs. ‰ % from chrom.	8.8 0.90 90.2	14.4 1.26 86.7	12.2 0.99 72.7	9.2 0.93 85.2	11.15 1.02 83.7	-	+ 2.35 + 0.12 - 6.5
on average per year (Q00-Q99)	abs. ‰ %	229.4 22.51 100.0	404.6 35.33 100.0	393.8 31.60 100.0	307.2 31.0 100.0	333.8 30.11 100.0	-	+ 104.4 + 7.6
Other congenital malformations	abs. ‰ %	9.0 0.88 3.67	17.2 1.50 4.08	25.8 2.10 6.24	23.6 2.38 7.13	18.9 1.72 5.28	4	+ 9.9 + 0.84 + 1.61
In total all congenital malformations	abs. ‰	238.4 24.06	421.8 36.83	419.6 33.70	330.8 33.36	352.7 32.0	-	+ 114.3 + 7.94

Note: abs. – the number of cases of birth of a child with congenital malformation of this nosology over 5 years (monitoring period) on average

Table 3. Dynamics of the frequency of various nosological forms of congenital malformations among live births [https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence_en]

Nosological form of congenital malformations	Kherson region. ‰		Ukraine. ‰		EUROCAT. ‰	
	Years:					
	2001-2005	2013-2017	2001-2005	2013-2017	2001-2005	2013-2017
nervous system	0.87	0.93	1.006 (0.657-1.475)	1.85 (1.615-2.111)	1.274 (1.232-1.317)	1.162 (1.126-1.199)
ear, face and neck	0.24	0.41	1.277 (0.879-1.794)	0.126 (0.07-0.207)	0.458 (0.433-0.484)	0. 157 (0.144-0.171)
congenital heart defects	5.86	10.48	7.20 (6.203-8.311)	7.878 (7.383-8.398)	7.473 (7.371-7.576)	6.96 (6.872-7.050)
respiratory	0.38	0.35	0.039 (0.00-0.219)	0.117 (0.064-0.197)	0.261 (0.243-0.281)	0.334 (0.315-0.354)
cleft lip with or without palate	1.00	0.97	1.161 (0.783-1.658)	0.804 (0.651-0.981)	0.854 (0.820-0.889)	0.726 (0.697-0.755)
digestive system	1.00	0.85	1.084 (0.720-1.566)	1.348 (1.148-1.573)	1.627 (1.579-1.675)	1.558 (1.516-1.601)
genital	3.81	6.12	0.735 (0.443-1.149)	2.369 (2.102-2.662)	2.090 (2.036-2.145)	2.108 (2.059-2.157)
limb	7.74	6.51	4.103 (3.360-4.962)	4.638 (4.260-5.041)	4.781 (4.699-4.863)	3.691 (3.627-3.757)
including poly-dactyly	1.09	1.15	1.123 (0.752-1.612)	1.407 (1.202-1.636)	0.859 (0.825-0.894)	0.915 (0.883-0.948)
chromosomal abnormalities	1.05	0.97	1.123 (0.752-1.612)	1.591 (1.373-1.834)	1.767 (1.717-1.817)	1.689 (1.646-1.733)
teratogenic syndromes with malformations	0.50	0.85	0.426 (0.212-0.762)	0.553 (0.427-0.703)	0.105 (0.093-0.118)	0.109 (0.098-0.12)
Total	24.75	31.4	17.109 (15.55-18.27)	22.061 (21.227-22.920)	22.740 (22.562–22.920)	20.520 (20.368-20.674)

diovascular system of children born alive (from 5.86‰ in 2001-2005 to 10.48‰ in 2013-2017) among the populations of Kherson region. Moreover, these indicators are significantly higher than the prevalence of this nosological form in Ukraine (7.2 and 7.88 respectively) and in European countries (7.47 and 6.96‰ respectively). A similar trend is observed in the dynamics of the frequency of congenital malformations of the genital organs: an increase in the average annual indicator in Kherson region from 3.81 to 6.12‰ (in Ukraine – from 0.74 to 2.37‰), although in European countries this indicator has stabilized at the level of 2.09-2.11‰.

Discussion

Among all developmental anomalies the frequency of model forms and multiple defects is a sign that largely reflects the intensity of the mutation process in populations. Genetic factors and the mutational component (of at least 40%) largely contributes to the etiology of multiple congenital malformations [9].

The prevalence of malformations in the populations of Kherson region is inversely correlated with the prevalence of spontaneous abortions ($r = - 0.52 \pm 0.12$; $tr = 2.4 > t05 = 2.12$), which indicates the "sifting" effect of natural selection that eliminates nonviable genotypes in the embryonic period of ontogenesis [10].

The increase in the prevalence of developmental anomalies in the region is mainly due to the increase in the frequency of model malformations recorded by the EUROCAT ($r=0.69$, $p<0.05$). Fetal malformations that can affect the development of intrauterine and infant mortality and lead to human disability are called model congenital malformations or strict accounting malformations. They are highlighted by the EUROCAT for greater objectivity of research.

The prevalence of chromosomal abnormalities (1.0-1.17‰) is characterized by relative stability, which confirms the previously stated assumption about the constancy of the level of the mutation process in the region. Among the nosological forms of chromosomal defects the

largest specific weight is of Down syndrome (83.7%) and a frequency of 0.9-1.02‰.

The frequency of malformations of the musculoskeletal system in Kherson region is 6.51-7.74‰ (in Ukraine and Europe 4.1-4.64‰ and 4.1-3.37‰, respectively). In addition, an alarming trend was revealed of increased prevalence of multiple congenital malformations (from 0.5‰ to 0.85‰); their frequency significantly exceeds these indicators not only in Ukraine (0.43-0.55 ‰) but also in European countries (0.069-0.109 ‰).

The cluster of nosological forms of defects with a stable prevalence in Kherson region, which does not exceed the corresponding figure in Ukraine and European countries, should include malformations of the nervous system, congenital malformations of the face, neck, ears, cleft, lip and palate, other malformations of the digestive system, polydactyly (1.09‰ in 2001-2005 and 1.15‰ in 2013-2017; in Ukraine – 1.12 and 1.41‰; in European countries – 0.86 and 0.92‰, respectively), chromosomal abnormalities (in Kherson region – 1.05‰ and 0.97‰; in Ukraine – 1.12 and 1.59‰; in European countries – 1.77 and 1.69‰ respectively).

The prevalence of malformations of the respiratory system in Kherson region (0.38‰ in 2001-2005 and 0.35 ‰ in 2013-2017, respectively) significantly exceeds this level in Ukraine for the same period (0.04‰ and 0.12‰, respectively) and is at the level of prevalence in European countries (0.26‰ and 0.33‰, respectively).

Conclusion

The frequency of congenital malformations in Kherson region over the past 20 years is: for newborns – 31.57±1.25‰; for live births – 31.38±1.11‰; for stillbirths – 197.7±0.65 per 10.000. The increase in the prevalence of congenital malformations in the region is mainly due to an increase in the frequency of model malformations6 which are subject to mandatory registration by the EUROCAT system ($r=0.69$, $p<0.05$). In the structure of congenital malformations defects of the cardiovascular system (31.77%), of the musculoskeletal system (25.14%) and of genital organs (17.5%) are leading. The frequency of malformations of the cardiovascular system, genitals and musculoskeletal system, multiple congenital malformations in Kherson region significantly exceeds the prevalence of these nosological forms in Ukraine and European countries. Among hereditarily determined forms the frequency of chromosomal abnormalities (1.0-1.17‰) is characterized by relative stability, which confirms our hypothesis about the constancy of the level of mutation process in the region. Among the nosological forms of chromosomal defects the largest share is for Down syndrome (83.7%) with a frequency of 0.9-1.02. Reducing the burden of congenital anomalies in the population is possible through integration of research in epidemiology, genetic demography and epigenetics.

Conflict of Interests

Author declares no conflict of interest regarding this study.

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ДИНАМІКА ЧАСТОТИ ТА ОСОБЛИВОСТІ СТРУКТУРИ ВРОДЖЕНИХ ВАД РОЗВИТКУ НА ПІВДНІ УКРАЇНИ (МОНІТОРИНГОВЕ ДОСЛІДЖЕННЯ)

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ХЕРСОНСЬКИЙ ДЕРЖАВНИЙ УНІВЕРСИТЕТ. ХЕРСОН. УКРАЇНА

Вступ. *В Україні в умовах несприятливої демографічної ситуації актуальним є моніторинг народжуваності дітей з вродженими вадами розвитку для виявлення регіональних особливостей епідеміології та розробки методів пренатальної діагностики та прогнозування.*

Мета. *Мета дослідження – охарактеризувати динаміку частоти і виявити особливості структури вроджених вад розвитку новонароджених дітей у Херсонській області за 20-річний період (2000-2019 рр.), порівняти поширеність різних нозологічних форм вад розвитку в регіоні, в Україні і в країнах Європи.*

Методи. *Методи дослідження: епідеміологічний, медико-статистичний.*

Отримані результати*. У Херсонській області середня популяційна частота вроджених вад розвитку за останні 20 років становить: для новонароджених – 31,57±1,25‰; для народжених живими – 31,38±1,11‰; для мертвонароджених – 197,7±0,65 на 10 000. У структурі провідне місце займають вади розвитку серцево-судинної системи (31,77%). опорно-рухового апарату (25,14%). статевих органів (17,5%). Збільшення поширеності вроджених вад розвитку в регіоні в основному пов'язано зі збільшенням частоти модельних вад, що реєструються EUROCAT (r=0.69. p<0.05). Підвищення загальної частоти вроджених вад відбувається за рахунок збільшення кількості народжених дітей з вадами серцево-судинної системи (на 4,67‰), статевих органів (на 1,21‰), інших вад розвитку (на 1,55‰), множинних вад (на 0,37‰).*

Висновки. *Результати моніторингу свідчать про зростання поширеності вроджених вад розвитку в Херсонській області за 20-річний період на 7,94‰. Причиною такого явища може бути скорочення чисельності населення через негативний природний і механічний приріст. Поширеність спадково обумовлених вад залишається на постійному рівні. У регіоні частота деяких нозологічних форм вад значно перевищує цей показник в Україні і країнах Європи: вад серцево-судинної системи – в 1,5 рази, статевих органів – майже в 3 рази, опорно-рухового апарату – майже в 2 рази.*

КЛЮЧОВІ СЛОВА: вроджені вади розвитку; хромосомна патологія; популяція; новонароджені діти.

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VITAMIN D AND UROLITHIASIS IN CHILDREN

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Background. *Urolithiasis is currently one of the topical issues of contemporary urology and medicine in general. This is primarily due to the high prevalence of urolithiasis; according to several population studies it ranges from 3.5 to 9.6%. At the same time, there is a steady increase in its incidence. Therefore, the matter of urolithiasis is one of the most urgent in present-day medicine.*

Objectives. *The aim of the research was to study the content of a polymorphic genetic marker of the vitamin D receptor gene related to development and relapse of urolithiasis in children.*

Methods. *The content of a polymorphic genetic marker of the vitamin D receptor gene related to development and relapse of urolithiasis in 100 children was investigated.*

Results. *The results of the study prove that the vitamin D receptor gene assists in revealing disorders that promote urolithiasis development.*

Conclusion. *Comparative analysis of the frequency of distribution of Fok1 genotypes of the vitamin D receptor gene polymorphism showed that statistical significance of the association (p=0.02) of f allele according to the dominant inheritance model (total Ff+ff genotypes) was established in the group of patients with urolithiasis compare to the corresponding indicator of the control group (63%).*

KEYWORDS: vitamin D, urolithiasis, VDR gene, gene polymorphism, dysuria.

Introduction

It is established that urolithiasis is polyetiological. The final step of stone formation is salts crystallization in a urine supersaturated solution. However, a variety of mechanisms take part in pathogenesis before this, i.e. disorders of renal circulation, disturbance of urinary tract urodynamics, and inflammation in them [2, 3].

Urolithiasis is defined as a metabolic disease that is caused by several endogenous and/or exogenous factors and is characterized by occurrence of stones in the urinary system [3]. It is clear that stones in the urinary tract are a clinical manifestation of the disease; removal of the stone does not stop its progression, and it develops further if the causes are not eliminated [19].

Calcium stones are the most common, that is more than 80% of cases. Correspondingly, most of the calcium stones (about 85-90%) are calcium oxalates, 1-10% – calcium phosphates [7]. Recently, a tendency to decrease in the incidence of phosphate stones in the world structure was noticed. Probably, this is caused by a decrease in the occurrence of infected stones because of advances of minimally invasive surgery for urolithiasis and use of the latest antibacterial therapy [1,14].

Thus, metabolic disorders are significant in the pathogenesis of urolithiasis. Ever more, there are uric acid stones (up to 10% of all urinary stones); they include uric acid and its salts, along with mixed stones (up to 5% of all calcium stones) that contain calcium salts together with uric acid and/or its salts [18].

Violation of calcium metabolism is one of the risk factors for occurrence and relapse of urolithiasis; its regulation in the human body is a complex process. Three major hormones: parathyroid hormone (PTH), calcitonin, and metabolite of vitamin D, 1,25-dihydroxycholecalciferol-1,25 – the most important, are involved in calcium homeostasis maintenance [12].

Vitamin (hormone) D is a controlling anabolic hormone having antioxidant properties and an exclusive systemic metabolic effect [12]. Then again, seasonal fluctuations in vitamin D rate (high levels in summer and autumn and low – in winter and spring, similarly to annual testosterone cycles) take place [19]. On the other hand, regulation of the expression of

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hormone D metabolism genes variates according to the androgens level. Hence, androgen deficiency intensifies adverse health effects caused by vitamin D deficiency [10].

The receptor of vitamin D is encoded by the vitamin D receptor gene characterized by genetic polymorphism, i.e. different allelic variations of this gene in humans [4]. Bsm I, Fok I, Taq I are the most important polymorphisms of the vitamin D receptor gene involved in progress of diseases [18, 19]. Several studies associate VDR gene polymorphism with urolithiasis. Some publications prove the significance of occurrence of the ApalAA genotype that determines sensitivity to vitamin D in the formation of calcium stones in the urinary organs [7, 14]. It is also established that in patients with urolithiasis the HLA B13, B22 and B35 genes are more common than in healthy individuals [2].

The huge range of biological effects of vitamin D, its involvement in carbohydrate, fat, purine metabolism, as well as its anabolic, antiproliferative, immunomodulatory effects, the investigation of cases of its deficiency is essential.

Methods

To perform the tasks set, a clinical examination of 100 children with urolithiasis and 100 practically healthy children was conducted, as well as the collection and analysis of statistical data on urolithiasis in children of different age groups to identify the gender, age-related and family frequency of urolithiasis, nutrition and lifestyle features, as well as the seasonality of this pathological process. Ultrasound and X-ray examinations of the urinary organs of patients were performed.

The selection of patients was carried out on the basis of the diagnosis established in the clinic with the written consents of the probands. Blood samples were collected from the patients with urolithiasis (100 samples) and a control group of practically healthy children (100 samples). Venous blood (1 ml) was kept in 0.5 ml of sodium citrate solution at -20 °C.

The material for the research was provided by the Republican Specialized Scientific and Practical Medical Center of Pediatric Surgery of Samarkand.

Table 1 presents data on distribution of the patients according to age in the groups under consideration.

Table 1 shows that among the patients, school-age children prevailed – 69 (69%). The reason is the fact that it is the age when metabolic disorders are the most often manifested; it is associated with the transition of children to a general diet, violation of drinking regime, etc., and in the younger group – the diet is quite rational and metabolic changes are less pronounced.

The distribution of patients in both groups depending on gender is presented in Table 2.

The data presented in Table 2 shows that according to the gender distribution of patients, incidence of urolithiasis is higher among boys – 68 (68%) children than girls – 32 (32%).

The most common complaints of patients with urolithiasis were pain in the lumbar region, fever, hematuria, turbidity of urine, increased or decreased urination. Acute urinary retention and spontaneous discharge of concretions were noted in some cases. In dislocation of concretions in the ureter n/w the pain was present in the iliac region on the same side. The patients with renal insufficiency had charac-

Table 2. Division of patients depending on gender

Gender	Control group (n=100)		Main group (n=100)		Total (n=200)	
	n	%	n	%	n	%
Girls	35	35	32	32	35	17.5
Boys	65	65	68	68	165	82.5

Table 1. Age-related division of patients with urolithiasis and healthy children

Age of patients (years)	Control group (n=100)		Main group (n=100)		Total (n=200)	
	n	%	n	%	n	%
0-3	17	17	31	31	48	24
4-11	43	43	43	43	86	43
12-17	46	46	26	26	72	36

teristic symptoms, i.e. headache, drowsiness, poor appetite.

At admission pain syndrome was evidenced in 89 (89%) patients. Also, a severe pain syndrome of renal colic was present in 15 (15%) patients. The nature and localization of the pain syndrome depended on the child's age, presence of concomitant concretions in the urinary tract. In the patients of a younger age group, abdominal pain was typical. Older patients complained for lumbar pain more often, sometimes complained of irradiation of pain along the ureter.

In 3 (3%) children suffering from concretion in the lower third of the right ureter, pain syndrome led to unjustified appendectomy performed at the place of residence. In these patients ureterolithotomy was associated with some technical difficulties due to an adhesive process.

Dysuric manifestations were evidenced in 28 (28%) patients and mostly characterized by frequent and painful urination. Acute urinary retention was present in 5 (5%) patients. Dysuric manifestations were nearly 2 times more common in younger patients than other. Acute urinary retention in all patients was managed by inserting an Ad'mer catheter; in 3 (3%) patients, after the catheter was removed, spontaneous discharge of the concretion was evidenced.

According to the localization of the concretions, the following were identified: renal calculi in 43 patients (left-13, right – 21, on both sides – 9); ureter stones in 12 patients (c/3 ureter – 1, b/3 ureter – 1, n/3 ureter – 10); bladder stones in 9 patients (recurrent bladder stone in 1 of them); stones of the proximal urethra in 4 patients; multiple urolithiasis in 10 patients. Combined urolithiasis with urinary disorders were revealed in 22 children.

Genomic DNA was separated from the whole blood of patients diagnosed for urolithiasis and almost healthy individuals of the control group. It was performed by means of reagents kit Diatom DNA Prep 200 (IsoGen Laboratory LLC) according to a standard protocol. This kit comprised a lysing agent with guanidine thiocyanate, which was designed to destroy cells, solubilize cell debris and denature cell nucleases. With a lysing reagent the DNA was absorbed on the NucleoS™ sorbent, then washed from salts and proteins with an alcohol solution. The DNA, which was eluted from the Extra-Genome sorbent, was used for further analysis.

PCR was performed by means of special oligonucleotide primers and a reagents kit for PCR amplification of DNA GenePak™ PCR Core (IsoGen Laboratory LLC). We used Master Mix tubes ready for amplification, which comprised a lyophilized state Taq DNA polymerase, deoxynucleose triphosphates, and magnesium chloride with final concentrations of 1 u, 200 microns, and 2.5 mM, respectively, and an optimized buffer system intended for PCR amplification. 5 µl of a primers mixture, a final concentration of 0.5 µm, 10 µl of diluent PCR, and 5 µl of the test DNA were added to the Master Mix tubes. For PCR, the GeneAmp ® PCR system 9700 with a 96-cell block (Applied Biosystems) was used. The amplification program for the VDR gene involved 5 minutes of predenaturation at 95 °C, 34 cycles: 94 °C – 30 sec, 66 °C – 30 sec, 72 °C – 30 sec, and a final elongation for 7 minutes at 72 °C. The temperature-time mode of amplification for the Urokinase gene was: 5 minutes of preliminary denaturation at 95 °C, 40 cycles: 95 °C – 30 sec, 56 °C – 30 sec, 72 °C – 30 sec; as well as final elongation at 72 °C for 7 minutes.

Results

Bioinformatic search for the nucleotide sequences of these genes was carried out in the Ensemble Genome Browser genomic database. After PCR amplification of the VDR gene fragment, the PCR-derived products were subjected to PCR analysis using Fok I endonuclease (manufactured by NPO Sibenzyme) (Fig. 1, Table 3).

The genotypes of the VDR gene polymorphisms were interpreted on the basis of different band patterns on the electrophoregram (Fig. 2).

After photodocumentation, the subsequent restriction fragments were genotyped according to the presence of corresponding fragments.

Table 3. Chromosomal structure and localization of the studied gene

Gene, polymorphism	Primers	Nucleotide sequence	Restrictases
Vitamin D receptor Fok-I polymorphism	Pr_VDR_F	AGCTGGCCCTGGCACTGACTCTGCTCT	Fok-I
	Pr_VDR_R	ATGGAAACACCTTGCTTCTTCTCCCTC	

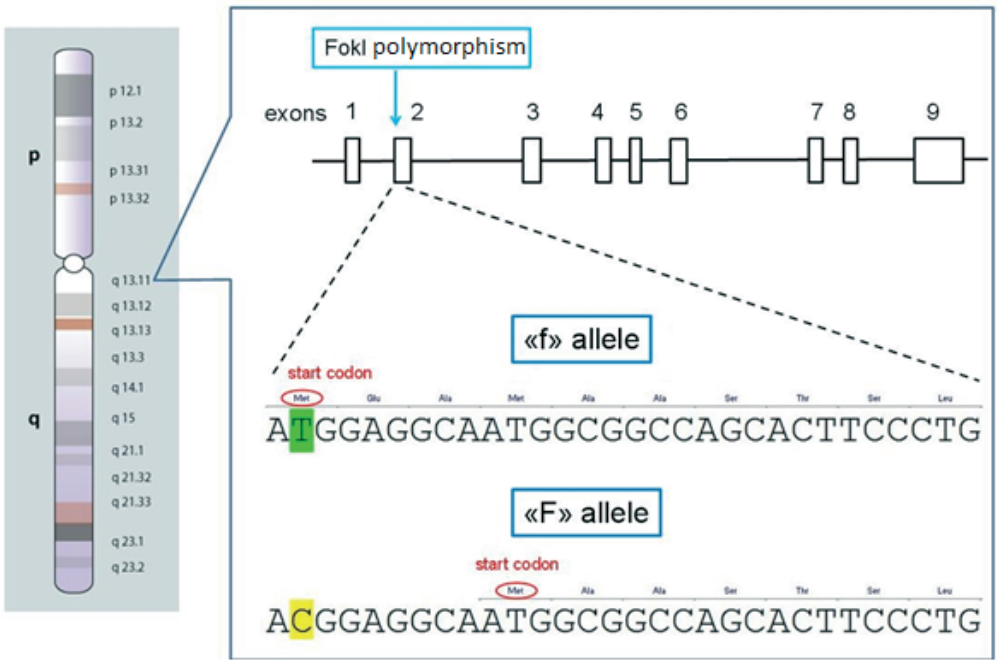

Fig. 1. *VDR gene Fok-I polymorphism.*

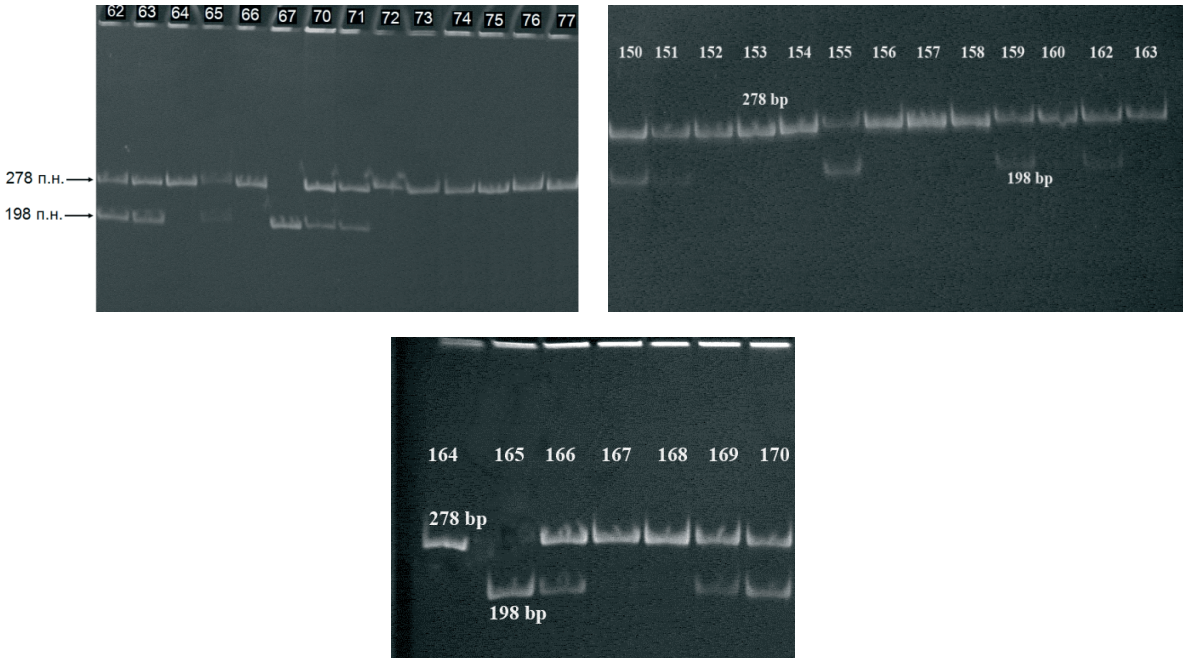

Fig. 2. *FDRF analysis of the Fok1 polymorphism of the VDR gene.*

Genotyping analysis of VDR gene PDRF products (restriction products) was performed.

One fragment weighing 278 bp indicated that this sample was a carrier of the homozygous genotype WT (Wild Type), and three fragments weighing 278 bp, 198 bp and 80 bp – a carrier of the heterozygous genotype WT/MUT, the presence of two fragments weighing 198 bp and 80 bp – a carrier of the homozygous genotype MUT (Mutant) (Fig.3).

The frequency distribution of the VDR gene polymorphism Fok1 genotypes in the control group corresponded to the Hardy-Weinberg distributions.

A comparative analysis of the frequency distribution of the VDR gene polymorphism

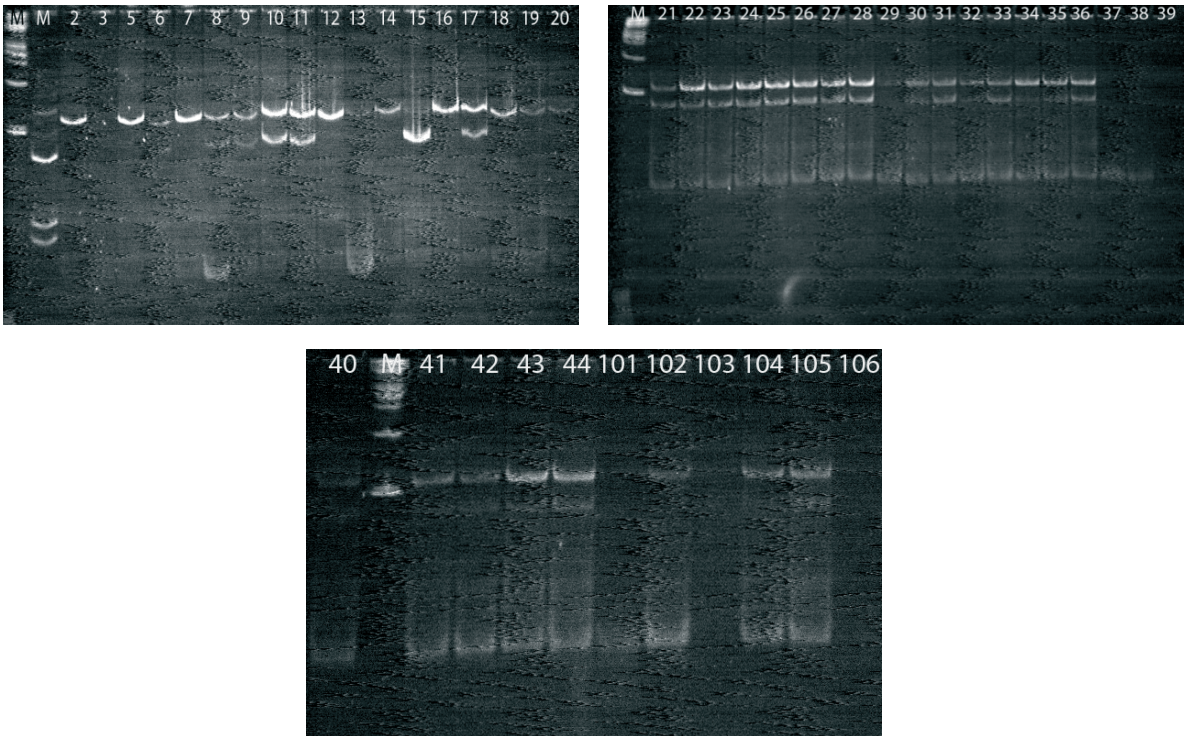

Fig. 3. *PDRF analysis of the Fok1 polymorphism of the VDR gene.*

Fok1 genotypes showed a statistically significant association (p=0.02) of the f allele regarding the dominant inheritance model (Ff +ff genotypes) in the group of urolithiasis patients compare to the corresponding indicators in the control group (Fig. 4).

As shown on the diagram, the genotype of heterozygotes F/F of the Fok1 polymorphism of the VDR gene was the most often recorded in the control group (53%), and in the group of urolithiasis children, a decrease tendency was established, no confidence between the indicators (47%) (p>0.05) was evidenced. The F/f+f/f polymorphism Fok1 of the VDR gene was the most informative genotype in urolithiasis, which occured almost 2 times more often compare to the control group (p<0.01).

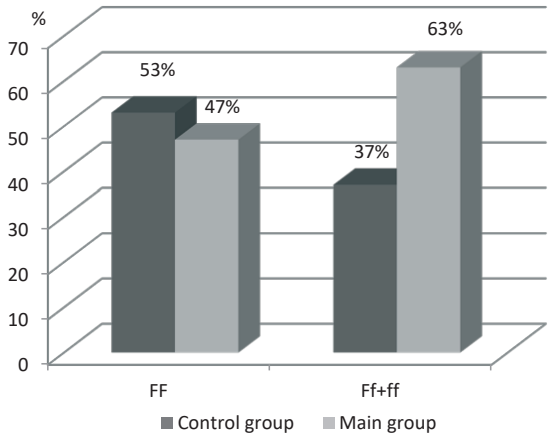

Fig. 4. *Frequency distribution of Fok1 polymorphism genotypes of the VDR gene in the control group and in patients with urolithiasis.*

The frequency distribution of the heterozygotes F / F genotype was 44.4%; in the control group – 15%, respectively (χ^2=0.47; p=0.24; OR=1.35; 95% CI 0.57-3.17; df=1). f allele detection increased the risk of urolithiasis in children in 2.4 times compare to the F allele (95% CI=0.68-2.93, df=1).

Allele determines the vitamin D receptor synthesis (427 amino acids); it is defined as *f, and the shorter form (424 amino acids) of the receptor is VDR*F. The study of the patient samples proved that the ff-normal genotype was revealed in 83 patients; a homozygous variant of the mutant Ff genotype – in one case, a heterozygous FF genotype – in 127 cases by the VDR gene polymorphic marker FokI (3663T>C).

The allelic form of VDR*F*f was associated with a frequency of 74% and urolithiasis manifestations in the studied group of patients; the *f allele occurrence was 93 compare to the *F allele – 89.

It is established that calcium urolithiasis is associated with the VDR*F*F genotype; in the individuals with this genotype the manifestations of urolithiasis at an early age are significantly more frequent; in the carriers of the VDR*f*f genotype the association with the development of urolithiasis is significantly lower; and in the individuals homozygous for the VDR*F*f genotype – intermediate.

The analysis of the VDR gene genotype frequencies proved that the analysed genotypes distribution in our population corresponded to the Hardy-Weinberg distribution (RHB) (χ^2=5.14; p=0.03) (Table 4).

Thus, the attained results prove that the VDR gene is significant in revealing the disorders that contribute to urolithiasis development.

Table 4. Statistical analysis of the genetic association of the VDR gene genotypes with urolithiasis according to the dominant inheritance model (test χ^2, df=2)

Genotypes	Cases	Control	χ^2	p	OR	
	n=100	n=94			Case	95% CI
Genotype F/F	0.370	0.532	5.14	0.02	0.52	0.29-0.92
Genotype F/f+f/f	0.630	0.468			1.93	1.09-3.43

Discussion

Many scientists have proved that a violation of the metabolism of vitamin D leads to an imbalance in the phosphorus-calcium metabolism, which is a direct risk factor for the development of urolithiasis. A decrease in the level of vitamin D leads to a deterioration of health due to a range of physiological processes. However, many guidelines on urolithiasis provide recommendations on the limited administration of vitamin D due to the fear of development of increased lithogenesis. Some researchers proved that 1.25 (OH)2D3 has a direct effect on calcium excretion [8]. Currently, the relationship between the level of vitamin D and nephrolithiasis is debatable; the data obtained are contradictory, especially in paediatrics.

Schlingmann and co-authors stated that in children with urolithiasis, significant nephrocalcinosis and hypercalcemia had conditions of suppressed serum PTH and a significant increase in the level of 1.25-dihydroxyvitamin D3 due to CYP24A1 mutations [13].

Consequently, VDRs are encoded by the VDR gene with genetic polymorphism, i.e. the presence of various allelic variants of this gene in the population [5]. Bsm I, Folc I, Taq I were the most significant polymorphisms of the VDR gene involved in disease development. The prevalence of VDR gene polymorphism has racial and ethnic differences. In one population, one genotype of a polymorphic marker prevails, in another population – another genotype of a polymorphic marker [11, 15, 17]. In this regard, it is relevant to study the distribution of genotypes of the polymorphic marker Fok I of the VDR gene in the Uzbek population in children with urolithiasis.

The Fok I locus is characterized by a T / C transition in the start codon (ATG) of the second exon of the VDR gene, in which the length of the protein molecule of the receptor decreases due to the displacement of the start codon by three triplets. In the case of thymine (T) or the "f" allele, the M1 form of a molecule of 427 amino acids is synthesized; in the case of cytosine (C) or the "F" allele, a shorter and, accordingly, more active M4 form of the receptor with a length of 424 amino acids is synthesized [9]. According to the US researchers, in children with the FF genotype, bone mineral density was on average by 8.2% higher than in those with the recessive homozygous genotype (ff) and by 4.8% higher than in children with the Ff genotype [16].

A group of scientists guided by S. Azab conducted a study of the association of BsmI polymorphism of the VDR gene with the risk of development of nephropathy in patients with systemic lupus erythematosus; it showed that the BB (AA) genotype is a risk factor for this complication. However, there was no significant association of VDR gene variants with other clinical manifestations, laboratory profiles of systemic lupus erythematosus, the disease activity index or the level of 25-hydroxyvitamin D in the blood serum of patients [6].

According to the results of out study, it was established that in the Uzbek population the genetic markers of predisposition to urolithiasis are: genotypes Ff+ff of the VDR gene. A comparative analysis of the frequency distribution of the VDR gene polymorphism Fok1 genotypes proved a statistically significant association (p=0.02) of the f allele regarding the dominant inheritance model (Ff+ff genotypes) in the

group of urolithiasis patients compare to the corresponding indicators in the control group. The genotype distribution frequencies of F/F heterozygotes were 44.4%, and 15% – in the control group, respectively (χ^2 =0.47; p=0.24; OR=1.35; 95% CI 0.57-3.17; df=1). Revealing of the f allele increased the urolithiasis risk in children in 2.4 times, compare to the F allele (95%) (CI = 0.68-2.93, df=1).

The heterozygosity of F/F mutations may predispose to formation of stones; therefore, screening should be carried out to determine the level of 25(OH)D in serum (which will be high) and levels of 24, 25(OH) vitamin D (which will be low), before prescribing vitamin D supplements to the patients with kidney stones to prevent exacerbation of calciuria. Those to be screened and genetically tested are the patients with high or above average serum calcium levels and reduced PTH levels.

The molecular genetic method of prediction of urolithiasis occurrence allows identifying a disease predisposition at any age: just about from the birth of a person, since the genotype of an individual does not change throughout life. Moreover, disease predisposition can be established by means of this method if no clinical or biochemical manifestations are present, specifically at the earliest preclinical stage of the pathology development. So, the earlier the genetic marker is detected, the more reliable and timely measures can be taken to prevent the disease.

Conclusions

A comparative analysis of the frequency distribution of VDR gene polymorphism Fok-1 genotypes showed a statistically significant association (p=0.02) of the f allele regarding the dominant inheritance model (Ff +ff genotypes in total) in the group of urolithiasis patients compare to this indicator in the control group that was 63%. In the Uzbek population, the genetic markers of predisposition to urolithiasis are: genotypes Ff+ff of the VDR gene. It is appropriate to test this genotype as a comprehensive program of urolithiasis prevention in Uzbekistan.

Conflict of Interests

Authors declare no conflict of interest.

Author's Contributions

Yusupov Shukhrat Abdurasulovich, Shamsiev Azamat Mukhitdinovich – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Shamsiev Jamshed Azamatovich*, *Pulotov Parviz Amridinovich* – data curation, writing – reviewing and editing, investigation, formal analysis.

ВІТАМІН Д І СЕЧОКАМ'ЯНА ХВОРОБА В ДИТЯЧОМУ ВІЦІ

Ш.А. Юсупов[1], А.М. Шамсиев[2], Ж.А. Шамсиев[3], П.А. Пулотов[4]

1, 2, 3, 4 – САМАРКАНДСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ ІНСТИТУТ, САМАРКАНД, РЕСПУБЛІКА УЗБЕКИСТАН

Вступ. *Сечокам'яна хвороба одна з найбільш актуальних проблем сучасної урології та медицини в цілому. Це пов'язано в першу чергу з високою поширеністю сечокам'яної хвороби, яка, за даними декількох популяційних досліджень, становить від 3,5 до 9,6%. При цьому відзначається неухильне зростання захворюваності.*

Мета. *Вивчити вміст поліморфного генетичного маркера гена рецептора вітаміну Д асоційованого з розвитком і рецидивуванням сечокам'яної хвороби у дітей.*

Методи. *Методом ПЛР проведено генетичне дослідження поліморфного генетичного маркера гена рецептора вітаміну Д асоційованого з розвитком і рецидивуванням сечокам'яної хвороби у 100 дітей.*

Результати. *результати, отримані в цьому дослідженні, вказують на те, що ген VDR сприяє визначенню порушень, що сприяють розвитку сечокам'яної хвороби*

Висновки. *В результаті проведеного порівняльного аналізу розподілу частот генотипів fok1 поліморфізму гена VDR встановлена статистично значуща асоціація (p=0.02) алеля f за домінантною моделлю спадкування (сумарно генотипи Ff+ff) в групі хворих з уролітіазом порівняно з відповідним показником в групі контролю, який склав 63%.*

КЛЮЧОВІ СЛОВА: вітамін Д, сечокам'яна хвороба, ген VDR, поліморфізм генів, дизурія

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CLINICAL OUTCOMES AND ADVERSE DRUG REACTIONS IN COVID-19 PATIENTS TREATED WITH HYDROXYCHLOROQUINE AND AZITHROMYCIN ALONE OR COMBINED

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Background. *Use of Hydroxychloroquine with or without Azithromycin is repurposed in SARS-CoV-2 in the absence of definitive treatment.*

Objective. *To evaluate the association between the use of Hydroxychloroquine and Azithromycin when given alone or in combination on clinical outcomes and adverse drug reactions among lab confirmed SARS CoV-2 positive patients admitted in a COVID tertiary care hospital of a University Medical college.*

Methods. *a retrospective observational comparative study was conducted. COVID-19 positive patients admitted in study hospital for management of COVID-19 were enrolled into the study. The patients were categorized into 4 treatment groups based on having received the following treatment during hospitalization: (A) Hydroxychloroquine with Azithromycin, (B) Hydroxychloroquine without Azithromycin (Hydroxychloroquine alone), (C) Azithromycin alone, and (D) Neither drug, defined as no receipt of either Hydroxychloroquine or Azithromycin in the record; other medications may have been dispensed.*

Results. *800 patients were enrolled. Mean±Standard deviation of duration of hospital stay (in days) for study Group A was 11.37±7.11, for Group B was 8.37±4.77, for Group C was 18.22 ± 5.69 and for Group D was 6.12±2.97. Mortality in Group A was 29.74%, Group B – 33.16%, Group C – 0% and in Group D – 1.32%.*

Conclusion. *Among hospitalized patients with COVID-19 treatment, Group C was associated with good clinical outcome. However, the interpretation of these findings may be limited by the observational design.*

KEYWORDS: COVID-19; hydroxychloroquine; azithromycin; SARS CoV-2

Introduction

In December 2019, several cases of pneumonia like disease were reported in the Wuhan city of China [1, 2]. The World Health Organisation (WHO) named this disease COVID-19. The causative agent for COVID-19 is the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV2).

SARS-CoV-2 is the newest of the family and is currently the cause of COVID-19 across the world [3]. Within months after its onset in China, this virus had spread involving most of the countries of the world. In March 2020, WHO declared it as a pandemic [4]. According to the recent available data by the end of December 2020, approximately 95 million populations worldwide and 10 million individuals in India have been diagnosed as COVID-19 positive. Until December 2020, the recovery rate in Indian population was 95.77% while the mortality rate was 1.45 %.

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The sources of infection of SARS-CoV-2 are reported to be infected animal hosts and infected humans. Bats [7] are considered to be initial hosts of this virus strain [5]. Main modes of transmission for interhuman spread of SARS CoV-2 are respiratory droplets and contact transmission. Patients of COVID-19 commonly present with symptoms like fatigue, cough, fever, myalgia, and diarrhoea.

Most of the people infected with the virus experience mild-to-moderate respiratory illness and recover without requiring any special treatment. However, elderly and those with underlying medical problems or diseases are more likely to develop serious illness. Research published till date has shown evidence that COVID-19 cause cytokine storm [6]. Some reports also revealed that patients of COVID-19 are associated with hyper inflammation and increased production of cytokines such as interleukin (IL)-1, 2, 6, 8, 10, and 17 [4, 7]. This may be the reason of tissue damage in the lungs of moderate to severely infected patients. Reports have also suggested that cytokine storm may

cause cellular demise and tissue injury in cardiac system which may lead to cardiovascular arrest [5]. Conditions like ARDS and cardiac arrest require an emergent medical attention in an intensive care unit.

Since no drug therapy has been specifically and conclusively established for the prevention, control, and cure at the time of its onset. So, several drugs have been repurposed to manage the rapidly deteriorating public health situation.

Many initial researches published during the early months of 2020 had suggested that Hydroxychloroquine is highly effective in both prophylaxis and treatment of COVID-19 positive patients. In vitro studies have demonstrated that Chloroquine and Hydroxychloroquine can inhibit viral replication at multiple points in the initial phase of viral infection [8]. It is postulated to exert a direct antiviral activity by increasing intracellular pH resulting in decreased phagolysosome fusion, impairing viral receptor glycosylation [9].

Various other studies showed the potential role of Azithromycin in treatment of COVID-19 patients. Azithromycin is a potent immunomodulator with significant antiviral properties. Azithromycin, a macrolide antibiotic, has in-vitro antiviral properties such as decreased viral replication, blocking entrance into host cells, and a potential immunomodulating effect [10].

According to other researches, combination of both the drugs may be more effective in curing the disease but some have also contradicted this line of treatment and several studies have even supported the statement that combination of both is harmful to the patient because the combination may add or increase the severity of their adverse effects.

All the above reasons led us to carry out this pilot study including drugs Hydroxychloroquine and Azithromycin. The aim of this study was to evaluate clinical outcome and adverse drug reactions among hospitalised laboratory confirmed COVID-19 positive patients treated with Hydroxychloroquine and Azithromycin either given alone or in combination.

Methods

This study received ethical approval from institutional ethics committee.

Study design: A retrospective, observational, comparative study.

Study population: All laboratory confirmed COVID-19 positive patients admitted in study hospital from March 2020 to July 2020.

Sample size: COVID-19 positive patients have been admitted in study hospital for management of COVID-19. Out of these, all those patients, who matched the inclusion and exclusion criteria, were enrolled in the study.

Inclusion and Exclusion criteria

Inclusion criteria:

1. All laboratory confirmed COVID-19 positive patients admitted in RUHS-HMS between March-July 2020.

2. Patients of both genders and above the age of 12 years old.

Exclusion criteria:

1. Patients whose hospital stay was less than 5 days (due to any reason).

2. Incomplete case files.

Study Groups:

Patients were categorized into 4 treatment Groups based on having received the following treatment during hospitalization:

(A) Hydroxychloroquine with Azithromycin,

(B) Hydroxychloroquine without Azithromycin (Hydroxychloroquine alone),

(C) Azithromycin alone, and

(D) Neither drug, defined as no receipt of either Hydroxychloroquine or Azithromycin in the record; other medications may have been dispensed.

Results

A total of 800 case records of lab confirmed COVID-19 positive patients admitted to RUHS Hospital of Medical Sciences from March to July were reviewed and enrolled in the study.

All the study Groups were compared via ANOVA test. This comparison included the parameters such as age, systolic blood pressure, diastolic blood pressure, SPO_2, and duration of hospital stay. Mean±standard deviation of each parameter of all groups were calculated. The obtained results of ANOVA test for all the study groups has p value less than 0.05, which represents a higher significance for the study. Observations and results of this test is shown in Table 1.

1. Gender distribution of patients as per groups

Results and observations obtained are presented in Fig. 1.

2. SPO_2 distribution as per group

Mean±Standard deviation of SPO_2 for study Group A was 89.6±7.1, for Group B was 90.24±7.44, for Group C was 91.63±5.34 and for Group D was 96.12±3.62. Results and observations obtained are presented in Fig. 2.

Table 1. ANOVA of the groups (N=800)

Parameters	ANOVA	Significance
Age	12.46	0.000001
Systolic BP	3.07	0.0271
Diastolic BP	3.01	0.02971
SPO_2 (in %)	19.01	0.000001
Hospital Stay	103.03	0.000001

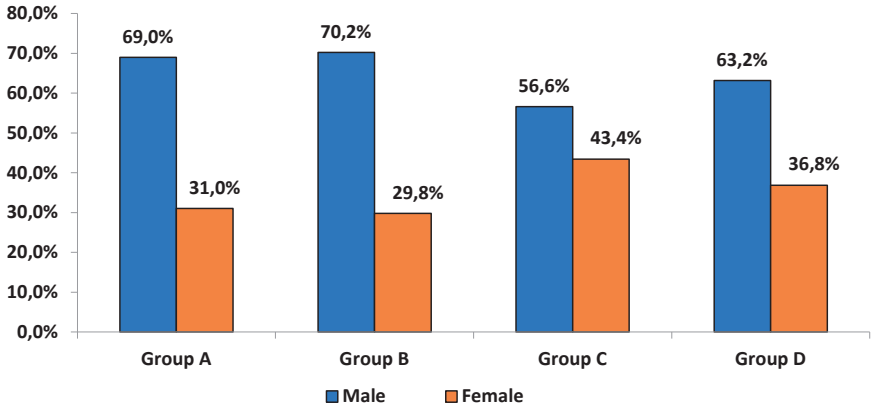

Fig. 1. *Gender distribution of patients as per groups (N=800).*

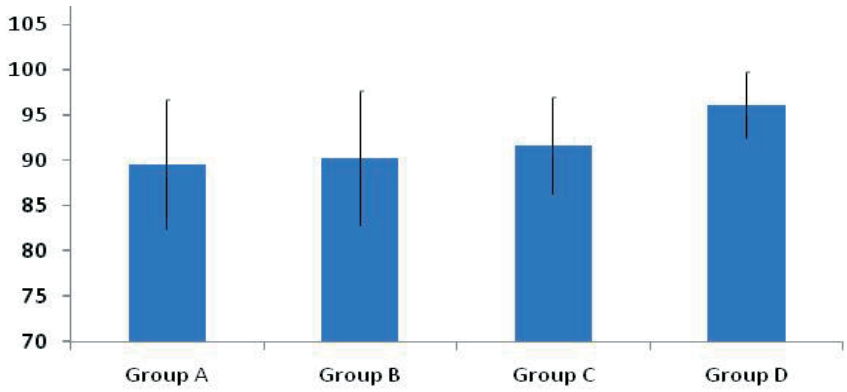

Fig. 2. *Mean and SD of SPO_2 (in %).*

3. Duration of Hospital stay distribution as per group

Mean±Standard deviation of duration of hospital stay (in days) for the study Group A was 11.37±7.11, for Group B – 8.37±4.77, for Group C – 18.22±5.69 and for Group D – 6.12±2.97. Results and observations obtained are presented in Fig. 3.

4. Severity of illness in study population

Severity of illness in study population in the study is classified as:

Asymptomatic – flu-like symptoms, patients are not hospitalized, and recover at home.

Mild symptoms – runny nose, sore throat, congestion, and dry cough.

Moderate symptoms – high fever, tiredness and fatigue, and chest pain.

Patients with severe symptoms – respiratory distress syndrome (shortness of breath, increased blood pressure, and decreased oxygen saturation).

Patients in the critical stage – Severe Acute Respiratory Syndrome (SARS) (high fever, chest pain, and breathlessness).

Patients with mild-moderate symptoms were categorized in one group and patients with severe symptoms and patients with critical stage were categorized in another group.

Chi-square test was applied for evaluation which was 72.73, and p value for this was

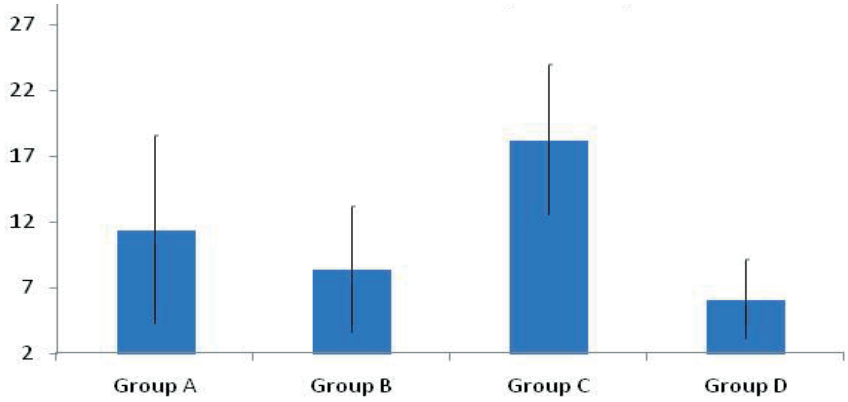

Fig. 3. *Mean and SD of hospital stay (in days).*

0.0000001, which was highly significant for our study. Severity of illness was highly significant with p-value 0.000001 (chi-square=72.73). Results and observations obtained are presented in Fig. 4.

5. Comparison of the clinical outcome of all the groups on the basis of illness severity and presence or absences of co-morbidity (Table 2).

6. Adverse events observed during study

Total numbers of adverse events observed in this study were 3. Two patients experienced itching over the body and they were associated to Group A and 1 patient of Group B experienced diarrhoea. Observations and results are shown in Table 3.

Discussion

For the evaluation of the treatment efficacy in our study two variables were examined. The first was the duration of hospital stay and the second was outcome (discharged or death). In the present study, duration of hospital stay for Group A was 8 days (IQR: 5-16), Group B – 7 days (IQR: 5-9), Group C – 18 days (IQR: 15-20), and Group D – 5 days (IQR: 5-6). The Mean±SD for Group A, B, C, D was 11.37±7.11, 8.37±4.77, 18.22±5.69, and 6.12±2.97 respectively. p value was 0.000001 and was highly significant. Group D had the shortest duration of hospital stay. Possible reasons for this could be that in Group D, 98.68% were mild-moderately ill. Also, SPO_2 which was an important clinical feature in COVID-19 was maximum for Group D (median 97.5, IQR: 95-99, and mean±SD 96.12±3.62) with a p value of 0.000001. Therefore, we can infer that milder disease severity and least deranged clinical features in Group D may have resulted in faster recovery and a shorter duration of hospital stay. Another reason can be that in Group D only 14.47% patients were having underlying comorbidities. This could also be important factor since presence of co-morbidities is now known to adversely affect the course of illness.

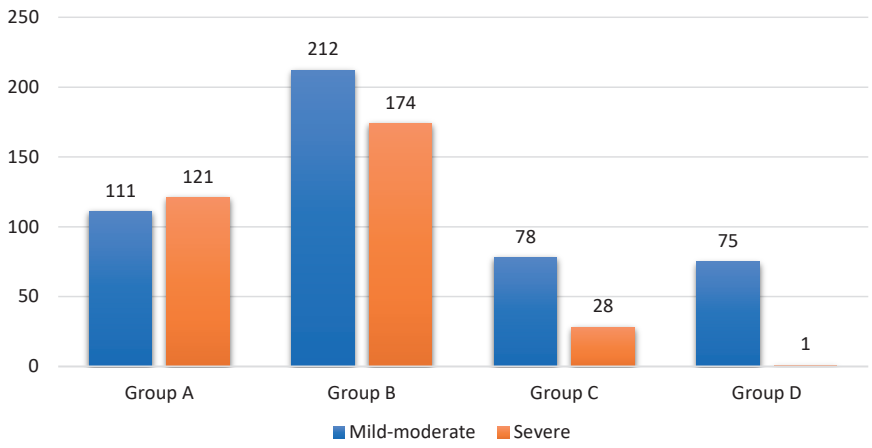

Fig. 4. *Distribution of illness severity (N=800).*

Table 2. Comparison of clinical outcome among all groups

	A			B			C			D		
	Hospital stay	Recovery	Death	Hospital stay	Recovery	Death	Hospital stay	Recovery	Death	Hospital stay	Recovery	Death
Mild-moderate comorbid patients	16.1	27.03% (N=30)	0% (N=0)	8.3	13.67% (N=29)	0% (N=0)	18.94	88.46% (N=69)	0% (N=0)	6.9	13.33% (N=10)	0% (N=0)
Mild-moderate patients without comorbidity	10.6	72.97% (N= 81)	0% (N=0)	8.4	86.32% (N=183)	0% (N=0)	8.25	11.53% (N=9)	0% (N=0)	6.3	86.66% (N=65)	0% (N=0)
Severe comorbid patients	11.97	33.88% (N=41)	42.14% (N=51)	7.7	16.66% (N=29)	56.32% (N=98)	21.04	100% (N=28)	0% (N=0)	14	0% (N=0)	100% (N=1)
Severe patients without comorbidity	7.9	9.09% (N=11)	14.8% (N=18)	8.2	9.77% (N=17)	17.24% (N=80)	0	0% (N=0)	0% (N=0)	0	0% (N=0)	0% (N=0)

Table 3. Adverse events observed during study

ADR	Group A	Group B	Group C	Group D
Diarrhoea	0	1	0	0
Itching	2	0	0	0
NIL	230	385	106	76
Total	232	386	106	76

The longest duration of hospital stay was in Group C which was having Median 18, IQR: 15-20 and mean±SD for 18.22±5.69. Despite of having borderline SPO_2 (Median 92, IQR:88-96 and mean±SD 91.63 ±5.34) with 73.58% patients of this Group being mild-moderately ill, still this Group had the longest duration of hospital stay. Possible reasons could be that 92.45% patients of this group were having underlying comorbidities.

Second variable which was observed in our study was clinical outcome. Two possible clinical outcomes were considered; recovered and discharged – meaning a good clinical outcome, and death -denoting a poor clinical outcome.

Samia Arshad et. al [9] found in a retrospective observational study that overall mortality was 18.1%, 20.1% by treatment with the combination of Hydroxychloroquine with Azithromycin, 13.5% with Hydroxychloroquine alone, 22.4% with Azithromycin alone and 26.4% with neither drug. According to their results Hydroxychloroquine provided 66% hazard ratio reduction and Hydroxychloroquine with Azithromycin 71% compared to neither treatment ($p<0.001$).

In the present study good clinical outcome was observed in Group C. In Group C 100% of the patients were discharged whether in Group A only 66.37% were discharged. Various factors could cause this. Chi square test was applied to measure the significance of illness severity in the population. Results of this test showed p=0.000001, it means severity of illness was highly significant. In Group C 98.68% patients were mild moderately ill and only 1.34% were severely ill. Another reason could be that SPO_2 as SPO_2 was 91.63±5.34 (p value 0.000001), which showed high significance. It may have led to decreased mortality in this Group.

To validate this overall result sub-Group analysis was performed. Thus it was established that in the patients, who were mild to moderately ill with comorbidities, maximum recovery or good clinical outcome were observed in Group C (88.46%). Similarly, when severely ill comorbid patients were evaluated again maximum recovery or good clinical outcome were observed in Group C (100%). It was also observed that in both groups of comorbidities (mild-moderate and severe) the longest duration of hospital stay was in Group C. In Group C the mean of

hospital stay for mild-moderate comorbid patients was 18.24 and for severely ill co-morbid patients it was 21.04. It proved that in our study effective treatment in the co-morbid patients was observed in Group C among all patients but it took more time to recover comparatively to other groups.

However, in patients without co-morbidities maximum recovery was evidenced in Group B. For Group B patients, who were mild-moderately ill with no co-morbidities, recovery was 86.32% and for severely ill without co-morbidity, recovery was 9.77%. In Group D recovery in mild-moderate non-co-morbid patients was 86.6%, which was slightly higher than in Group B, but for severely ill non-comorbid patients, recovery rate was higher in Group B. So, the overall recovery for non-comorbid patients was higher in Group B. The duration of hospital stay for mild-moderately ill Group B patients with comorbidities was 8.3 and for patients without comorbidities it was 8.4. For severely ill Group B patients with comorbidities it was 7.7 and for patients without comorbidities – 8.2. This data also revealed that patients of Group B without comorbidities had longer duration of hospital stay than the non-co-morbid patients.

Poor clinical outcome was measured by number of deaths. The highest mortality was observed in Group B with 33.16% death. Factors which influenced this result could be that 70.2% of patients of this Group were males. Males were more prone to lung infection due to their habits like smoking. Smoking habit is associated with males in Indians comparative to females. Therefore, this could be a reason for a greater number of deaths. In this Group 45.07% patient were severely ill. So, risk was higher for them compare to mild-to-moderate ill patients. 40.9% patients with underlying comorbidities were present in this Group which can have led to poor clinical outcome.

Sub-Group analysis revealed that there were no deaths in mild-moderately ill patients in both co-morbid and non-co-morbid groups. All the deaths were associated with severely ill patients. The highest mortality among all severely co-morbid patients was associated with Group D, which was 100%. There were no patients in Group D with non-co-morbidity. After that, the maximum deaths of non-co-comorbid patients were seen in Group B (17.24%).

Results of our study revealed that Group C treatment in comorbid patients were more effective and similar treatment in Group D was not safe. Recovery ratein mild-moderately ill comorbid patients was higher (27.03%) in the Group A compare to Group B (13.67%). However, in mild-moderately ill non-co-morbid patients, higher recovery was observed in Group B (86.32%) compared to Group A (72.97%). In severely ill co-morbid patients decreased mortality (42.14%) and increased recovery (33.88%) was seen in Group A compare to Group B, where mortality was 56.32% and recovery was 16.66%. In non-co-morbid severely ill patients, recovery in both groups (A – 9.09% and B – 9.77%) was almost the same but mortality was lesser in Group A (14.80%) compare to Group B (17.24%). These findings revealed that on the whole Group C treatment was the best among all and among groups A and B, Group A was better than B.

Similar results have been shown in the study by Matthieu Million et. al [11]. They also conducted a retrospective analysis of early treatment of COVID-19 patients with Hydroxychloroquine and Azithromycin. A poor clinical outcome was observed in 4.3%. They concluded that this combination was safe and associated with low fatality rate in patients.

Adverse events distribution in the study

In the present study safety of the treatment was also observed by adverse events. Very few adverse events were reported and they were mild which were associated with Group A and B. Reason for this is because of retrospective study, we are unable to analyse all those events which were not mentioned in case record files.

Conclusion

The present study concluded that no deaths were observed in mild-moderately ill patients with or without comorbidity. Among four groups, treatment in Group C (Azithromycin-500 mg OD for 5 days) had better results. Between groups A (Hydroxychloroquine with Azithromycin) and B (alone Hydroxychloroquine 400 mg BD on day 1 followed by 200 mg BD on day 2 to 5), treatment in Group A had better outcome. The duration of hospital stay was longer for comorbid patients compare to patients with no comorbidity.

Limitations

Though, we did our best to make this study without any blemish but several limitations make the scope for future study. It was a retrospective observational study. Therefore, regarding the data we had to rely on the case records and the mentioned records. There might be a few findings which were not

mentioned in the records but may have been present in the patients. We could not note them. Time constraint was the major limitation. So, we were unable to analyse the data on the basis of each sub-group. The data were incomplete for some patients because services were overwhelmed. CT scans, ECG and potential cofounders such as inflammatory markers associated with severity of the disease were not frequently measured/recorded. Mortality was limited to in-hospital death and patients, who were discharged or referred, were assumed to still be alive during study period. Because of retrospective analysis, we were not able to record all adverse events. Therefore, the evaluation of the safety was not adequate. For this, prospective randomized controlled trials may have been conducted at that time.

Conflict of interest

Authors declare no conflict of interest.

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Author's contribution

Heena Rathi, Priyanka Rathi – conceptualization, methodology, formal analysis, writing – original draft; *Heena Rathi, Mohit Biyani* – data curation, investigation; *Heena Rathi, Priyanka Rathi, Mohit Biyani* – writing, reviewing and editing.

НАСЛІДКИ ТА ПОБІЧНІ РЕАКЦІЇ ПРИ ЛІКУВАННІ ПАЦІЄНТІВ З COVID-19 ГІДРОКСИХЛОРОХІНОМ ТА АЗИТРОМІЦИНОМ ОКРЕМО ТА В КОМБІНАЦІЇ

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Вступ. *Застосування гідроксихлорохіну самостійно або у комбінації з азитроміцином – одна з опцій терапії SARS-CoV-2 за відсутності чітко визначеного лікування.*

Мета. *Оцінити вплив застосування гідроксихлорохіну та азитроміцину у якості монотерапії чи у комбінації на клінічні результати та частоту розвитку побічних реакцій серед пацієнтів з лабораторно підтвердженої інфекцією SARS-CoV-2, які були госпіталізовані до спеціалізованої COVID-лікарні при медичному коледжі університету.*

Методи. *Було проведене ретроспективне спостережне порівняльне дослідження. У дослідженні брали участь госпіталізовані пацієнти з лабораторно підтвердженим діагнозом COVID-19. Пацієнти були розділені на 4 групи, базуючись на лікуванні яке вони отримували під час госпіталізації: (A) гідроксихлорохін з азитроміцином, (B) гідроксихлорохін без азитроміцину (лише гідроксихлорохін), (C) лише азитроміцин та (D) жоден з препаратів не призначався; могли застосовуватися інші ліки.*

Результати. *Було залучено 800 пацієнтів. Середнє значення±стандартне відхилення тривалості перебування в лікарні (у днях) для досліджуваної групи A становило 11,37±7,11, для групи B – 8,37±4,77, для групи C – 18,22±5,69 та для групи D – 6,12±2,97. Смертність у групі A становила 29,74%, групі B – 33,16%, групі C – 0%, а групі D – 1,32%.*

Висновок. *Серед госпіталізованих пацієнтів з COVID-19 лікування у групі C (лише азитроміцин) було пов'язане з позитивними клінічними результатами. Однак інтерпретувати висновки остаточно неможливо через обмеження і рамки проведеного дослідження.*

КЛЮЧОВІ СЛОВА: COVID-19; гідроксихлорохін; азитроміцин; SARS-CoV-2.

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RADIATION EXPOSURE IN ACCESSORY PATHWAY ABLATION PROCEDURES IN CARDIAC ELECTROPHYSIOLOGY: A RETROSPECTIVE ANALYSIS

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Background. *Radiofrequency catheter ablation (CA) has been the treatment of choice in patients with accessory pathway (AP)-mediated tachycardias. Most of these procedures are done under fluoroscopic guidance, leading to significant radiation exposure to the patient and the laboratory personnel. In this analysis, we have looked at the amount of radiation exposure in AP CA procedures performed without the support of a three-dimensional electroanatomic mapping system. We have analyzed changes in exposure indices over the study period and the impact of change in fluoroscopy frame rate (FFR).*

Objectives. *The objectives of this study are to quantify radiation exposure in accessory pathway ablation procedures; to analyze the radiation exposure trend over time; and to evaluate the effect of fluoroscopy frame rate reduction on the radiation exposure indices in these procedures.*

Methods. *All the AP ablation procedures performed at our institute from January 2016 to December 2019 were retrospectively analyzed. The collected data were age, sex, location of APs based on successful site of ablation on fluoroscopy, procedure time, fluoroscopy time, and dose-area product (DAP). Effective dose (ED) was estimated from DAP. The data of procedures performed before January 2018 ("pre" group) were compared with those of the procedures performed after that date ("post" group). Pre-group procedures were performed at an FFR of 7.5 frames per second (fps), and post-group procedures – at an FFR of 3.75 fps.*

Results. *The total number of procedures included in the analysis was 635. The mean age of the patients was 39±14 years, and 401 of them (63%) were males. The most common location of the APs was left lateral (38%). Procedure time and radiation indices showed a significant decrease over the study period ($p < 0.001$). Post group procedures had significantly shorter procedure time and lower radiation exposure than pre group procedures.*

Conclusions. *A decrease in the FFR was associated with a significant reduction in radiation exposure in AP ablation procedures*

KEYWORDS: accessory pathway; catheter ablation; dose-area product; fluoroscopy time; radiation exposure.

Introduction

The ablation of accessory pathways (AP) using radiofrequency energy was first introduced by Borggrefe and colleagues in 1987 when they ablated a right-sided accessory pathway for the first time in humans using radiofrequency energy [1]. Since then, radiofrequency ablation has been the treatment of choice in patients with accessory pathway-mediated tachycardias [2,3]. Although radiofrequency ablation for AP-mediated tachycardias is a highly efficacious and safe treatment option, some of the adverse effects of this procedure are not because of the procedure itself but because of the ionizing radiation used to visualize the catheters during the procedure. Ionizing radiation exposure affects the patient and the laboratory personnel because of the fluoroscopic imaging used during cardiac electrophysiology (EP) procedures.

The ionizing radiation exposure can cause long-term hazardous effects, including malignancy, cataract formation, thyroid dysfunction, dermatitis, germline mutations, etc. This is due to double-strand breaks induced in the DNA backbone brought about by the free radicals

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generated due to the exposure of ionizing radiations [4].

The hazard of radiation exposure increases with the higher radiation dose, which in turn increases with the longer duration of EP procedure for the patient and increase in the number of EP procedures for the laboratory staff. Thus, it is essential to evaluate the benefit of the intended EP procedure over the risk of expected exposure to ionizing radiation and minimize the radiation exposure levels to minimum achievable levels for both the patients and the laboratory staff to have an overall beneficial outcome of the EP procedure [4].

One way to decrease radiation exposure in cardiac electrophysiology procedures is to use a lower fluoroscopy frame rate. We have previously reported of the impact of fluoroscopy frame rate reduction in complex catheter ablation procedures performed under a three-dimensional electroanatomic mapping system [5]. In this study, we analyzed radiation exposure indices in our laboratory's AP ablation procedures. We have also analyzed the impact of fluoroscopy frame rate reduction on radiation exposure indices during these procedures.

The objectives of this study are: to quantify radiation exposure in accessory pathway ablation procedures; to analyze the radiation exposure trend over time; and to evaluate the effect of fluoroscopy frame rate reduction on the radiation exposure indices in these procedures.

Methods

A retrospective analysis of accessory pathway ablation procedures carried out in a tertiary care referral institute in Southern India was performed. The data were collected from January 2016 to December 2019. The data available were age, sex, location of successful ablation, fluoroscopy time, and dose area product (DAP).

Procedures without such data were exclu_ded. Patients with multiple accessory pathways were also excluded from the analysis. The procedure time for catheter ablation procedures was defined as the time from the administration of a local anesthetic agent to removing catheters from the patient's body. Our institute has an ongoing cardiac electrophysiology fellowship program, and the fellows assist in all the catheter ablation procedures performed in our laboratory. Four physicians performed the catheter ablation procedures with an experience of 21, 11, 6, and 4 years in interventional electrophysiology. All the catheter ablation procedures were performed without three-dimensional electroanatomic mapping. The institute has an active EP fellowship program running since 2009, and the fellows assist in all the procedures performed in the laboratory. The laboratory is equipped with the Philips Allura Xper FD 10 system (Philips Healthcare, the Netherlands), and all the procedures were performed using the same system.

The fluoroscopy frame rate used in the laboratory was 7.5 fps before February 2018. From the beginning of February 2018, fluoroscopy at 3.75 fps have been used. The location of the accessory pathways was determined by the location of successful ablation in the left anterior oblique (LAO) projection, as shown in Figure 1.

The Effective Dose (ED, mSv) was estimated from DAP provided by the X-ray system by multiplying DAP with the following conversion factors depending on the age of the patient: 5-10 years of age: 1.0; 10-15 years of age: 0.6; 15-20 years of age: 0.4; adult females: 0.28 and adult males: 0.2. Lifetime attributable risk (LAR) of cancer incidence and mortality was derived from the ED by multiplying it with 0.0001/mSv (the standardized BEIR VII conversion factor) [5].

The data were summarized using standard descriptive statistics and presented as the arithmetic means with standard deviation (SD) or median with interquartile range, as appropriate. Nonparametric statistical tests like the Kruskal-Wallis test and the Mann-Whitney U test were used to analyze the procedure time and radiation exposure parameters over the study period and the effect of fluoroscopy fra-

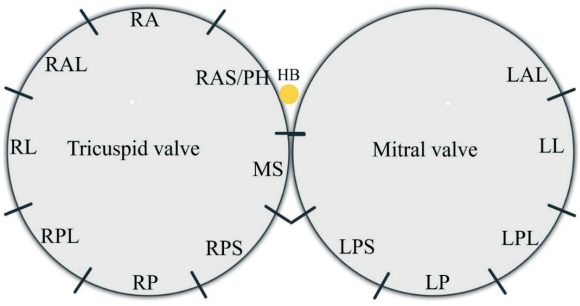

Fig. 1. *Location of accessory pathways as seen on a left anterior oblique (LAO) projection.*
HB: His Bundle; LAL: left anterolateral; LL: left lateral; LPL: left posterolateral; LP: left posterior; LPS: left posteroseptal; RPS: right posteroseptal; RP: right posterior; RPL: right posterolateral; RL: right lateral; RAL: right anterolateral; RA: right anterior; RAS/PH: right anteroseptal/para-Hisian; MS: midseptal.

me rate over various parameters, respectively. The level of significance was fixed at p<0.05.

Institutional ethical committee approval was not applied for this retrospective analysis. Informed consent was obtained from all patients before the procedures, which included the clause that their data could be used for scientific purposes in the future.

Statistical Package for the Social Sciences (SPSS), version 20, was used to perform statistical analysis.

Results

Six hundred and thirty-five pathway ablation procedures were included in the analysis. The mean age of the patients was 39±14 years. The mean age of male patients (n=401; 63% of the total) was 39±13 years, and female patients (n=234; 37% of the total) were 40±14 years.

The location of the accessory pathways and the associated procedure time and radiation indices are presented in Table 1. The number of the pathways ablated from the left side was 372 (58.6%), and that from the right were 263 (41.4%). The most common location of the ablated pathways was left lateral, which was 38.4 % of all the pathways, and the second most common location was right posteroseptal, which was 27.2% of all the pathways. The least common location of accessory pathways was mid septal (0.3%) followed by right anterolateral (0.5%) and right anterior (0.5%) locations.

Trends in various indices over the study period are shown in Table 2. 15% (96) of all the procedures were performed in 2016. In the years 2017, 2018, and 2019 the proportion of the procedures performed was 23% (149), 31% (196), and 31% (194), respectively.

Trends in the indices before (pre group) and after (post group) the change in fluoroscopy frame rate are shown in Table 3. 41% of the procedures (n=260) were performed under fluoroscopy frame rate of 7.5 fps, and 59% of the procedures (n=375) were performed under fluoroscopy frame rate of 3.75 fps.

Discussion

Table 1. Procedure time and radiation indices based on the successful location of ablation on fluoroscopy in left anterior oblique projection

Pathways	No. (%)	Procedure Time (min)	Fluoroscopy Time (min)	DAP (cGy/cm^2)	ED (mSv)	LAR, %
LAL	18 (2.8)	55 (45-98)	10 (8-14)	538 (313-706)	1.1 (0.8-1.5)	0.01 (0.008-0.02)
LL	244 (38.4)	55 (45-75)	11 (8-17)	796 (480-1343)	1.8 (1.1-3.1)	0.02 (0.01-0.03)
LPL	34 (5.4)	55 (44-81)	12 (7-17)	787 (483-1884)	1.9 (1.0-4.1)	0.02 (0.01-0.04)
LP	22 (3.5)	58 (48-66)	13 (7-19)	1014 (456-1570)	2.3 (1.1-3.5)	0.02 (0.01-0.03)
LPS	54 (8.5)	80 (55-106)	20 (10-29)	1637 (795-2384)	3.6 (1.8-5.3)	0.04 (0.02-0.05)
RPS	173 (27.2)	60 (43-90)	13 (8-19)	988 (465-1609)	2.1 (1.1-3.6)	0.02 (0.01-0.04)
RP	12 (1.9)	60 (36-94)	15 (7-26)	1372 (411-3420)	2.7 (0.8-8.2)	0.03 (0.01-0.08)
RPL	24 (3.8)	69 (55-100)	19 (11-26)	1220 (738-2448)	3.5 (1.8-5.5)	0.04 (0.02-0.06)
RL	27 (4.3)	90 (70-120)	27 (15-43)	1534 (848-2529)	3.6 (1.7-7.5)	0.04 (0.02-0.08)
RAL	3 (0.5)	105 (45-105)	17 (11-17)	701 (288-701)	2.8 (0.6-2.8)	0.03 (0.005-0.3)
RA	3 (0.5)	65 (60-65)	21 (18-21)	1596 (723-1596)	4.4 (1.5-4.4)	0.04 (0.01-0.4)
AS/PH	19 (3.0)	65 (46-80)	15 (7-18)	555 (441-862)	1.2 (0.9-2.3)	0.01 (0.01-0.02)
MS	2 (0.3)	100 (45-100)	21 (7-21)	1549 (377-1549)	3.1 (0.8-3.1)	0.01 (0.007-0.01
Total	635 (100.0)	60 (45-90)	13 (8-19)	889 (488-1660)	2.1 (1.1-3.7)	0.02 (0.01-0.04)

Notes: LAL: left anterolateral; LL: left lateral; LPL: left posterolateral; LP: left posterior; LPS: left posteroseptal; RPS: right posteroseptal; RP: right posterior; RPL: right posterolateral; RL: right lateral; RAL: right anterolateral; RA: right anterior; RAS/PH: right anteroseptal/para-Hisian; HB: His bundle; MS: midseptal.

Table 2. Change in procedure time and radiation indices over the study period

Year	Number	Procedure time (mins)	Fluoroscopy time (mins)	DAP (cGy/cm^2)	ED (mSv)	LAR, %
2016	96	75 (51-94)	19 (11-25)	1254 (751-2527)	3.1 (1.6-5.8)	0.03 (0.02-0.06)
2017	149	75 (60-110)	17 (12-30)	1604 (789-2713)	3.5 (1.8-5.7)	0.04 (0.02-0.06)
2018	196	60 (45-80)	12 (9-17)	798 (445-1284)	1.8 (1.0-3.1)	0.02 (0.01-0.03)
2019	194	50 (40-65)	9 (6-12)	596 (335-1079)	1.4 (0.8-2.4)	0.01 (0.008-0.02)
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3. Impact of fluoroscopy frame rate change on procedure time and radiation indices. Pre-group: procedures performed at 7.5 fps; post-group: procedures performed at 3.75 fps

	Number	Procedure time (mins)	Fluoroscopy time (mins)	DAP (cGy/cm^2)	ED (mSv)	LAR, %
Pre	260	75 (55-104)	18 (12-27)	1432 (780-2564)	3.2 (1.8-5.7)	0.03 (0.02-0.06)
Post	375	55 (40-75)	10 (7-15)	698 (392-1176)	1.6 (0.9-2.7)	0.02 (0.01-0.03)
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001

The main findings of our study are:

a. There was a significant increase in the number of catheter ablation procedures performed in our laboratory over the study period.

b. The procedure time and the radiation indices (fluoroscopy time, DAP, ED, and LAR) significantly decreased over the study period ($p<0.001$ for all the parameters).

c. The trend of a significant decrease in procedure time and radiation exposure continued when the data were divided into two groups based on the fluoroscopy frame rate ($p<0.001$ for all the parameters).

The radiation indices reported in different studies of catheter ablation procedures are presented in Table 4. The radiation indices reported are comparable to that of the published data.

Injury caused by exposure to ionizing radiation can be classified into two groups: a) the deterministic effects and b) the stochastic effects. Deterministic effects are dose-dependent (e.g., cataracts and skin injuries). A threshold radiation dose is a radiation dose below which the deterministic effects are not produced.

On the other hand, even a tiny radiation dose involves an increased risk of stochastic effects (e.g., cancer), and the chances of suffering that effect are directly proportional to the radiation dose.

As no radiation dose is safe, the policy of maintaining radiation exposure level is dependent on the "as low as reasonably achievable" (ALARA) principle. [4]

Some of the factors that influence the extent of ionizing radiation exposure in a cardiac EP laboratory are:

1) Operator dependent: a) experience of the operators, b) training of operators with simulators, c) radiation awareness of the staff, d) C-arm projection used during the procedure, e) fluoroscopy frame rate, f) cine duration, g) cine substitution by stored fluoroscopy, h) fluoroscopy use during catheter removal from the body, i) collimation of the X-ray system, j) pelvic radiation, k) written report of the patient's radiation exposure during the procedure.

Table 4. Reported radiation exposure in catheter ablation of supraventricular tachycardias (SVT). Adapted from [9]

Study	Type of study	Number of patients	Fluoroscopy time (mins)	DAP (cGy/cm^2)	Effective Dose (mSv)
Smith IR et al. [10]	Retrospective	AVNRT 270 AVRT 135	2.1 (1.3-4.5) 23.8 (13.4-45.3)	260 (170-610) 2690 (1600-5410)	–
Rogers DP et al. [7]	Observational	Pre DRM 147 (AVNRT/AVRT) Post DRM 257 (AVNRT/AVRT)	–	2040±2690 800±1030	3.3 1.24
Heidbuchel H et al. [4]	EHRA practical guide	–	–	–	4.4 (1.6-25)
Casella M et al. [6]	Multicentre randomized	Pre DRM 128 Post DRM 134	14.32 (9.08-22.43) 0 (0-0.2)	2036 (54–5297) 278 (80–791)	8.87 (3.67–22.01) 0 (0–0.08)
See J et al. [11]	Observational	Pre DRM AVRT 55 Post DRM AVRT 44	49.0 ± 36.3 14.1 ± 13.4	3292 ± 3282.7 654.4 ± 645.5	–
Casella M et al. [9]	Retrospective	979 (SVT)	13(6-21)	1721(727-3884)	4.1(1.8-9.1)
Our data	Retrospective	Pre 260 Post 375	18 (12-27) 10 (7-15)	1432 (780-2564) 698 (392-1176)	3.2 (1.8-5.7) 1.6 (0.9-2.7)

2) Patient dependent: a) body habitus of the patient, b) arrhythmic lesion to be ablated.

3) Technology dependent: a) X-ray system, b) combination with computed tomography (CT), c) three-dimensional electroanatomic mapping systems, d) shielding of the laboratory personnel. [4]

The factors that lead to decreased radiation exposure in the cardiac EP laboratory are:

1) Operator dependent: a) expert operators, b) operators who have been trained with simulators, c) radiation aware staff, d) predominant usage of right anterior oblique C-arm projection than an anteroposterior of left anterior oblique projection, e) low frame rate of fluoroscopy (<6 fps), f) short cine duration, g) frequent cine substitution by stored fluoroscopy, h) catheter withdrawal from the body without using fluoroscopy, i) optimized and adapted collimation, j) avoidance of pelvic radiation, k) a written report of the patient's radiation exposure that includes air kerma or dose area product received during the procedure.

2) Patient dependent: lean body habitus of the patients, supraventricular tachycardia ablation than atrial fibrillation ablation or ventricular tachycardia ablation,

3) Technology dependent: cardiac EP tuned X-ray system which has been adequately maintained and inspected for quality control, no preprocedural or rotational computed tomography, the predominant use of three-dimensional electroanatomic mapping systems, proper shielding which includes above and below the table or cabin shielding [4].

One of the most efficient ways to decrease radiation exposure during the ablation of accessory pathways is to perform these procedures with the help of three-dimensional electroanatomic mapping, which has been shown to decrease radiation exposure in such procedures significantly.

No-Party trial was the first multicentre, prospective, randomized trial that compared conventional fluoroscopy-guided catheter ablation procedures with procedures performed using the three-dimensional electroanatomic mapping system in the patients undergoing EP study for supraventricular tachycardias, 36% of whom were AP ablation procedures [6]. In this trial, three-dimensional electroanatomic mapping significantly reduced fluoroscopy time and ED (0 seconds and 0 mSv) when compared to the procedures performed under fluoroscopic guidance (859 seconds and 8.87 mSv) ($p<0.00001$).

But the use of three-dimensional electroanatomic mapping systems is restricted due to various logistic reasons (availability, lack of expertise); we need to find alternative ways to decrease radiation exposure during catheter ablation procedures [4].

Rogers AJ et al. conducted a study to evaluate the effect of radiation dose-reduction maneuver on the radiation exposure in EP laboratory. Atrioventricular nodal reentrant and AP-mediated tachycardia was 64% of catheter ablation procedures. They showed that with the simple maneuver of removing a secondary radiation grid to improve image quality and reducing the fluoroscopy pulse rate from 12.5 to 6.25 pulses/second, a radiation dose reduced from 20.4 to 8.0 $Gycm^2$. Additionally, the risk of radiation-related fatal malignancy was reduced by 63 due to the implementation of the maneuver. This study showed the importance of selecting electrophysiology laboratory parameters in reducing radiation exposure during catheter ablation procedures [7].

Voskoboinik A et al. conducted a prospective study to analyze the trends in radiation exposure during AF ablation at a single center over 12 years. A significant and progressive decrease in the fluoroscopy time and ED was observed over time. The significant decrease in the patient and operator radiation exposure was attributed to increased operator experience, higher annual case volume, technology evolution over time, and recent use of contact force-sensing catheters. [8]

More recently, Casella M et al. have reported fluoroscopy data from their retrospective analysis of various EP and device implantation procedures at a large volume laboratory over 7 years. Fluoroscopy time, DAP, and ED showed a statistically significant reduction trend for all EP procedures. Based on the obtained results, it was proposed that a combination of operator awareness about the fluoroscopy-associated risk and technological advancement can be used for optimizing the use of fluoroscopy in EP procedures [9].

One of the significant limitations of this study, other than being a retrospective analysis, is that one can argue that a significant decrease in the procedure duration can solely explain the changes in the radiation exposure in the procedure time, which is also reflected in the decrease in the fluoroscopy time. If there is a significant decrease in the procedure time, the radiation exposure will automatically decrease.

Some of the other limitations of this study are that data regarding some of the other factors that influence radiation exposure during procedures (e.g., body mass index of the patients, cine exposure duration during the procedures) performed under fluoroscopy was not available. This study is based on retrospective data collected from a single center, and the results cannot be generalized to other centers. As already alluded to, radiation exposure also depends on the individual expertise of operators, but data regarding the radiation exposure of individual operators was not available.

We propose that in our study, the decrease in the radiation indices is not solely because of the reduction in fluoroscopy frame rate but because of other factors, too, one being a more than 200% increase in the yearly number of ablation procedures performed over the study period. Any modern electrophysiology laboratory will face the same scenario.

Conclusions

A reduction in the fluoroscopy frame rate and improvement in the electrophysiology laboratory workflow leads to a significant decrease in the radiation exposure during catheter ablation procedures of accessory pathway-mediated tachycardias.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Muzaffar Ali: formal analysis, writing – original draft, investigation, formal analysis. *Bharatraj Banavalikar:* writing – reviewing and editing, investigation, formal analysis. *Milan Kumar Ghadei:* formal analysis, writing – original draft, investigation, formal analysis. *Anju Kottayan:* formal analysis, writing – original draft, investigation, formal analysis. *Deepak Padmanabhan:* writing – reviewing and editing, investigation, formal analysis. *Jayaprakash Shenthar*: conceptualization, methodology, writing – reviewing and editing

ІОНІЗУЮЧЕ ОПРОМІНЕННЯ ПРИ РАДІОЧАСТОТНІЙ АБЛЯЦІЇ ДОДАТКОВИХ ШЛЯХІВ ПРОВЕДЕННЯ У ЕЛЕКТРОФІЗІОЛОГІЇ СЕРЦЯ: РЕТРОСПЕКТИВНИЙ АНАЛІЗ

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Вступ. *Радіочастотна катетерна абляція (КА) метод вибору лікування у пацієнтів з тахікардією, додатковими шляхами (ДШ). Більшість таких процедур проводиться під флюороскопічним наглядом, що призводить до значного опромінення пацієнта та персоналу лабораторії. У цьому аналізі ми розглянули радіаційне опромінення в процедурах КАДШ, виконаних без підтримки тривимірної системи електроанатомічного відображення. Ми проаналізували зміни показників експозиції за період дослідження та вплив зміни частоти кадрів флюороскопії (FFR).*

Мета. *Метою цього дослідження є (1) кількісна оцінка радіаційного опромінення в процедурах абляції додаткових шляхів, (2) аналіз тенденції радіаційного опромінення з часом та (3) оцінка впливу зменшення частоти кадрів флюороскопії на показники радіаційного опромінення під час цієї процедури.*

Методи. *Ми ретроспективно проаналізували всі процедури абляції ДШ, проведені в нашому інституті з січня 2016 року по грудень 2019 року. Зібрані дані: вік, стать, місце розташування ДШ на основі успішного місця абляції на флюороскопії, час процедури, час флюороскопії та індекс доза опромінення відносно площі (DAP). Ефективну дозу (ЕД) оцінювали за DAP. Дані процедур, проведених до січня 2018 р. (група "До"), порівнювались із даними процедур, проведених після цієї дати (група "Після"). Процедури групи "До" виконувались із частотою FFR 7,5 кадрів в секунду (fps), а процедури групи "Після" – з частотою FFR 3,75 fps.*

Результати. *Загальна кількість процедур, включених до аналізу, становила 635. Середній вік пацієнтів становив 39±14 років, і 401 з них (63%) були чоловіками. Найчастіше зустрічалися лівобічні ДШ (38%). Показники тривалості процедури та опромінення зменшилися протягом періоду дослідження (p<0,001). Процедури групи "Після" мали значно коротший час проведення КА та менший радіаційний вплив, ніж процедури групи "До".*

Висновки. *Зменшення FFR було пов'язане зі значним зменшенням радіаційного опромінення в процедурах абляції ДШ.*

КЛЮЧОВІ СЛОВА: додаткові шляхи проведення; катетерна абляція; дозування опромінення; час флюороскопії; радіаційне опромінення.

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MAIN INDICATORS OF THE OXIDANT-ANTIOXIDANT SYSTEM AND THEIR RELATIONSHIP WITH THE FORCE OF THE RESPIRATORY MUSCLES IN ADULT PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA

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Background. *Currently, pneumonia is the 4th-5th in the world in the structure of death causes after cardiovascular and cancer diseases, cerebrovascular pathology, injuries and poisonings.*

Objective. *The aim of the study was to evaluate the indicators of the oxidative-antioxidant system and their relationship with the strength of the respiratory muscles in adult patients with community-acquired pneumonia.*

Methods. *The study was carried out in the period 2017-2020 at the therapeutic department of the Municipal Non-Profit Enterprise "City Clinical Multidisciplinary Hospital No. 25" of Kharkiv City Council. The study involved 52 adult patients with community-acquired pneumonia (CAP) aged 18 to 80 years old. The control group consisted of 20 apparently healthy humans. The activity of malondialdehyde, catalase and superoxide dismutase, level of reduced glutathione, glutathione reductase and glutathione peroxidase were determined. The assessment of the RM strength was investigated by recording the maximum static pressures at the level of the mouth and nose with "closed" airways using a MicroRPM apparatus on the 1st and 10th days of illness.*

Results. *Dysfunction of expiratory respiratory muscles prevailed in patients with non-severe CAP, and inspiratory respiratory muscles – in the patients with severe CAP. Significant negative correlations of malondialdehyde with indicators of respiratory muscles strength and positive correlations with glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase were established.*

Conclusions. *The relations between prooxidant and antioxidant indicators and respiratory muscles strength complements the concept of the body systemic response on pulmonary inflammation – one of the markers of respiratory muscles dysfunction.*

KEYWORDS: community-acquired pneumonia; respiratory muscles; oxidative-antioxidant system.

Introduction

Community-acquired pneumonia (CAP) is still one of the most urgent issues of contemporary medicine. CAP leads to a steadily high and growing morbidity. According to the WHO data in recent years, lower respiratory tract infections have become the third leading cause of death in the world, and over the past 30 years, the incidence rate has risen [1]. The mortality rate in hospitalized patients with a severe form of the disease varies from 14 to 40 % and significantly increases among patients over 60 years old [2]. According to the literature, the number of disease forms with severe or protracted course is increasing with frequent complications of pneumonia such as abscess formation, acute respiratory failure, infectious toxic shock, etc. [3, 4, 5]. In each 3-4 patients with CAP, the disease has a protracted course [1, 6, 7].

That is why study continues for clarifying individual relations in the pathogenesis of CAP, improving programs for its diagnosis and therapy. One of the leading factors in the pathogenesis of CAP is the excessive production of reactive oxygen intermediates (ROI), which is associated with bacterial or viral-bacterial aggression and insufficiency of the compensatory potential of antioxidant defence (AOD). An imbalance in the oxidative-antioxidant system is one of the key factors in the development of oxidative stress (OS), which plays an important role in the implementation of the molecular-cellular mechanisms of the pathogenesis of respiratory diseases [8, 9]. Free radical processes are typical general biological protective reactions of the body, normally providing energy metabolism, proliferation, differentiation of cells, gene expression, immune and adaptive responses, etc. [10]. At the

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same time, excessive ROI initiates lipid peroxidation (LPO) with subsequent damage of cellular membranes, uncoupling oxidative phosphorylation, formation of energy insufficiency, disturbance of enzymatic activity of the detoxification systems [11, 12, 13]. Some studies have established that OS is the most important component of endogenous intoxication – an obligate manifestation of CAP, which has a significant effect on its clinical course. In the pathogenesis of CAP the glutathione redox system (enzymes form thioredoxin and glutaredoxin-dependent complexes) play a special role that maintain intracellular homeostasis, which resists the destructive effects of oxidative stress factors [12]. It has been proven that low glutathione intracellular concentration contributes to imbalance between prooxidants and antioxidants in the lungs, aggravation of inflammatory reactions and complications development.

Also, one of the most important issues in the pathogenesis of CAP, is dysfunction of the respiratory muscles (RM). Formation of RM dysfunction at CAP is characterized by the effect of local (alveolar inflammation) and systemic (endogenous intoxication) factors on muscle contraction [2, 12]. It is assumed that these changes are caused by the destabilization of cell membranes, decrease in the rate of myopotential conduction and violation of the potassium and calcium intracellular transport, which provide muscle contraction [11, 13]. At the same time, there is no data in the scientific literature about influence of free radical processes on the functional status of RM in patients with CAP.

The aim of the study was to evaluate the indicators of the oxidative-antioxidant system and their relationship with the strength of the respiratory muscles in adult patients with community-acquired pneumonia.

Methods

The study involved 52 patients with CAP aged 20-60 years (mean age 36.5±10.3), who were treated in the therapeutic department of the Municipal non-profit enterprise "City Clinical Multidisciplinary Hospital No. 25" of Kharkiv City Council in 2017-2020. Diagnosis of CAP was based on the results of clinical, radiological, microbiological and laboratory studies. Non-severe CAP (NCAP) was diagnosed in 56 (72%) patients, severe CAP (SCAP) – in 22 (28%). Unilateral subsegmental inflammatory infiltration of the lung tissue was recorded in patients with NCAP, and SCAP was characterized by the presence in one or both lungs of multisegmental, lobar, or bilobar infiltrates. The etiological structure of CAP is mainly represented by *Streptococcus pneumonia* – 57.4%, *Haemophilus influenza* – 23.4%, *Mycoplasma pneumonia* – 13.4%, *Chlamydophila pneumonia* – 5.8%. The patients were treated according to the Recommendations of the International Society of Pulmonologists and the F.H. Yanovskyi National Institute of Phthisiology and Pulmonology (Kyiv, 2019). The average hospitalization period was 10.80±0.67 days. All the patients signed informed consent.

The control group involved 45 apparently healthy humans (AHH) of the same age (mean age 39.5±12.5).

The intensification of LPO processes was determined by the level of the final product – malondialdehyde (MDA) in erythrocytes [14]. The total antioxidant activity (AOA) was assessed by the integral index in blood plasma. The intensity of the first line of AOD was investigated by the activity of the enzymes catalase and superoxide dismutase (SOD). Catalase activity was determined by the rate of utilization of hydrogen peroxide in the reaction mixture [15], and SOD in erythrocytes – by the ability to suppress the reduction of nitro blue tetrazolium. The state of the redox system was studied by the level of reduced glutathione (RG), the activity of glutathione reductase (GR), and glutathione peroxidase (GPO) in whole blood [16].

The assessment of the RM strength was carried out by recording the maximum static pressures at the level of the mouth and nose with "closed" airways using a MicroRPM apparatus (CareFusion, Great Britain) on the 1st and 10th days of illness. The maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and sniff nasal inspiratory pressure (SNIP) were evaluated. MIP and SNIP characterized the strength of the inspiratory muscles, and MEP – the expiratory muscles. The correlation of SNIP with the level of transhiatal pressure can refer it to the indicators of the functional activity of the diaphragm. The maximum rate of expiratory and inspiratory pressures rises in the oral cavity (maximal rate of pressure development – $MRPD_{exp}$ and $MRPD_{insp}$) was assessed using the additional software PUMA (Micro Medical, UK). The RM strength was assessed in the sitting position after 3-fold execution of respiratory movements with the fixation of maximum result. The

required values (RV) for MEP, MIP, SNIP were calculated using a previously developed model [17].

To analyse the statistical significance of differences between the groups, statistical processing of the research results was carried out depending on the distribution origin of the data as follows: if the distribution was close to normal, the analysis was performed using the methods of variation statistics, the Statistica 8.0 software package – the statistical two-way ANOVA method (Fisher LCD post-hoc test). If it was significantly different from normal, the differences between the groups were determined using the Kruskal-Wallis ANOVA and median test method. Correlation analysis was carried out in the same Statistica 8.0 package by means of parametric and nonparametric methods depending on the type of distribution. The statistical significance of the differences between the indicators of the control and experimental groups was determined by the Student's and Kruskal-Wallis's criteria using the Excel program. The level of $p<0.05$ was statistically significant [18].

Results

Group 1 involved 36 (69.2%) patients with NCAP. Their metabolic profile was characterized by minimal violations of the oxidative-antioxidant balance. The patients of this group showed slight decrease of RG and moderate increase of redox system RG-GPO and GR enzymes at normal values of SOD and catalase (Table 1).

The analysed indicators showed adequate response to antioxidant protection, which ensured the neutralization of ROI and LPO products, which was confirmed by normal MDA values in erythrocytes.

Among these patients, the absolute values of the strength indices of expiratory RM were significantly lower in the group of AHH (MEP – on 54.6%), and the limitation of their inspiratory function was less evident (MIP – on 17%, SNIP – on 7%) (Fig. 1, 2). In this case, the ratio of measured and RM was 78% for MEP and 88% for MIP and SNIP, which indicated a predominantly expiratory variant of RM dysfunction.

Group 2 involved 16 (30.8%) patients with SCAP, whose indicators were characterized by the most significant imbalance, as evidenced by significant increase of MDA level (by 29%) against the background of decrease in AOA (by 18%) and catalase (by 59%).

The inhibition of the RG redox system was expressed by decrease in the concentration of RG in the blood (by 48%) and decrease in GPO (in 1.7 times compare to the AHH). At the same time, compensatory increase in the content of GR (by 11%) and SOD (by 45%) did not ensure the restoration of oxidative-antioxidant homeostasis.

The study of the RM functional state in the patients of group 2 showed that with increase of the CAP severity and imbalance in the oxidative-antioxidant system, the degree of their strength signs deviation from the level of AHH significantly increased and reached the maximum level. In this case, the indicators of MEP and $MRPD_{exp}$ in relation to the AHH group decreased in 2.0 times, SNIP – in 1.5, and MIP – in 1.75 times, which indicated about development of pronounced expiratory-inspiratory type RM dysfunction. The obtained results confirmed that the power of inspiratory RM was more evidence limited in SCAP with a significant weakening of functional activity of the diaphragm.

The analysis of paired correlations showed that at the height of disease, there were multidirectional relations of varying intensity between the indicators of RM strength and

Table 1. Oxidative-antioxidant indicators of patients with CAP in various groups, ME (95% CI)

Indicators	Apparently healthy humans (n=45)	Patients with CAP (n=52)	
		Group 1	Group 2
MDA, μmol/1gHb	7 [6,9; 71,0]	7,3 [7,1; 7,6]	9 [8,9; 9,2]*
AOA, %	70,3 [68; 72,0]	68,6 [66,2; 70,1]	59,4 [57,5; 61,8]*
MDA/AOA, c.u.	0,14 [0,13; 0,16]	0,13 [0,11; 0,15]	0,16 [0,15; 0,17]*
RG, mol/1gHb	6,7 [6,4; 7,1]	5,8 [5,6; 6,0]	4,5 [4,2; 4,8]*
GPO, μmol RG/1gHb/hour	128 [127; 133]	149 [142; 155]*	74 [71,5; 76]*
GR, μmol NADPH/1gHb/hour	154,3 [153,5; 156]	164 [158; 169]*	171,3 [160; 181]*
Catalase, %	80 [75; 84]	77 [73; 81]	51,2 [48,6; 54]*
SOD, %	54 [51; 58]	57,3 [56; 59]	78,3 [77; 80]*

*Notes: * – p<0,05 statistically significant difference compare to the apparently healthy humans.*

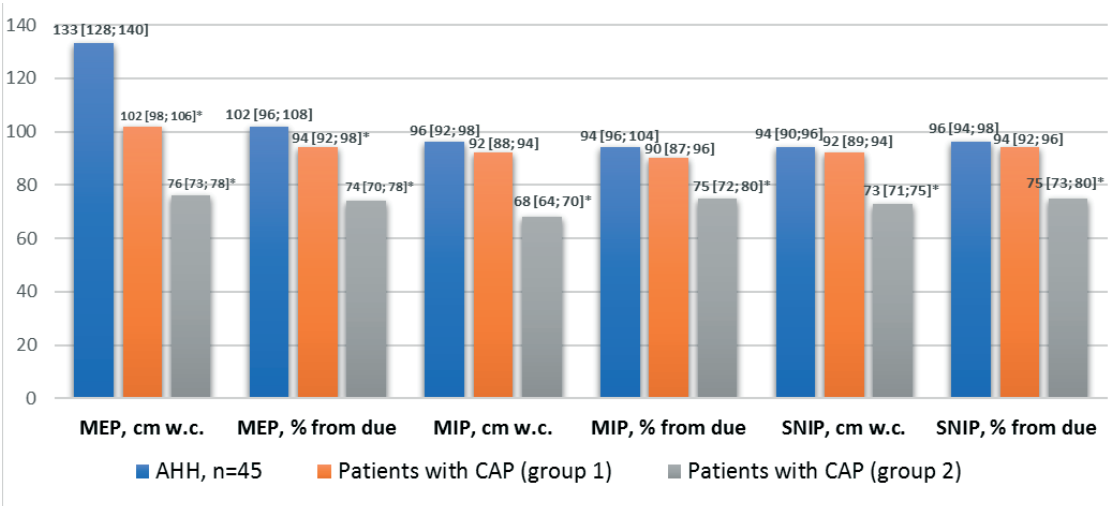

Fig. 1. *Indicators of RM strength in patients with community-acquired pneumonia in various groups, ME 95% CI)*
*Notes: * – p<0.05 statistically significant difference compare to the apparently healthy humans.*

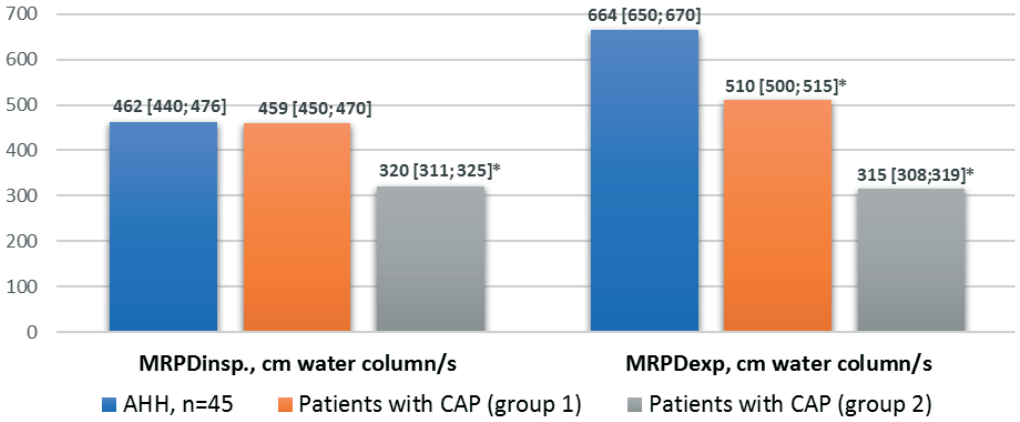

Fig. 2. *Indicators of RM strength in patients with community-acquired pneumonia in various groups, ME (95% CI)*
*Notes: * – p<0.05 statistically significant difference compare to the apparently healthy humans.*

some indicators of the oxidative-antioxidant status. Thus, negative correlations of the average strength of MDA with MEP (r=-0.56), MDA with $MRPD_{exp}$ (r=-0.53), MDA with SNIP (r=-0.61) and MDA with MIP (r=-0.58) were evidenced. Direct correlations were found between the indicators MIP with GPO (r=0.76), SNIP with catalase (r=0.65), SOD with SNIP (r=0.51), GR with MEP (r=0.48). The obtained result indicates about the heterogeneity of the effect of prooxidant and antioxidant factors on the functional status of RM and confirm their role in the development of DM dysfunction.

On the 10th day, the patients of the group 1 showed tendency to restoration of the strength indicators to the level of the AHH. At the same time, only MIP, SNIP, and $MRPD_{insp}$ indicators reached the values of AHH group, while MEP and $MRPD_{exp}$ significantly differed, which indicated persisting signs of isolated expiratory RM dysfunction. In the patients of the group 2 on the 10th day of studying, the medians of all indicators of the RM strength were minimal compare to other groups.

Discussion

Analysis of the scientific literature showed that the role of DM in the pathogenesis of CAP is not fully understood. It was noted, that decrease in the peak expiratory flow rate at the height of CAP is associated not with increase of bronchial resistance to expiratory air flow, but with DM dysfunction.

In the works of Geltser B.I. et al. (2019) the pathophysiological significance of endogenous intoxication in the development of DM dysfunction in alveolar inflammation has been proven [12]. Its development in this case is associated with the effect of systemic factors of acute inflammation on DM, including pro-

ducts of oxidative stress, excessive proteolysis, bacterial toxins, pro-inflammatory cytokines and other inflammatory mediators that impair the efficiency of respiratory myofibrils. In previous study of Segizbaeva M. O, Aleksandrova N. P. (2014) it was found that in CAP, even endogenous intoxication could cause limitation of the contractile function of DM [13].

In resistive respiration, intensely contracting myocytes are able to transform into metabolic bridgehead producing spectrum of proinflammatory cytokines. At the same time, breathing resistance is considered as an "immune challenge" to the body with excessive synthesis of inflammatory mediators. It has been established that DM fatigue can also develop in healthy individuals with intense respiratory muscle stress [12, 13].

It is established, that the deficiency of glutathione enzymes contributes to damage of cell membranes and macromolecules – proteins, lipids, DNA and can determine the development of extrapulmonary manifestations of alveolar inflammation, including through damage to the myofibrils of skeletal muscles [7].

The obtained results can identify pathophysiological determinants that combine changes in the oxidative-antioxidant system and the RM functional activity in CAP. This is an imbalance in the LPO-AOD system, characterized by excess lipoperoxides and hypofunction of the first and second lines of AOD. Deficiency of the key intracellular antioxidant – glutathione, contributes to increase in OS, which becomes as key factor in the initiation of the molecular-cellular mechanisms of alveolar inflammation and RM dysfunction. Criteria for the severity of CAP in most cases corresponded to the severity of changes in the prooxidant and antioxidant system. At the same time, the heterogeneity of prooxidant processes among patients with NCAP, who were included in the group 1, may be due to individual typological characteristics of the organism, including genetic [19]. The important role of individual components of LPO-AOD (MDA, GPO, GR, catalase, SOD) in the development of expiratory and inspiratory RM dysfunction was confirmed by the results of correlation analysis. The presence of interrelations between prooxidants and antioxidants and indicators of RM strength, complements the concept of the body systemic response on pulmonary inflammation – one of the markers of RM dysfunction.

Conclusions

An imbalance in patients with community-acquired pneumonia in the system of lipid peroxidation and antioxidant defence, which is characterized by overproduction of lipoperoxidases and hypofunction of antioxidant defence has been established. Increased oxidative stress in patients with community-acquired pneumonia causes deficiency of the key intracellular antioxidant – glutathione. Glutathione is key factor in the initiation of the molecular and cellular mechanisms of alveolar inflammation and respiratory muscles dysfunction in patients with community-acquired pneumonia. The relations between prooxidant and antioxidant indicators and the respiratory muscles strength complements the concept of the body systemic response on pulmonary inflammation – one of the markers of respiratory muscles dysfunction. The obtained results will optimize the diagnosis of community-acquired pneumonia and its severity.

Conflicts of Interest

Authors declare no conflict of interest.

Authors' Contributions

Vladyslav Bereznyakov – investigation, conceptualization, formal analysis, writing – original draft.

ОСНОВНІ ПОКАЗНИКИ ОКСИДАНТНО-АНТИОКСИДАНТНОЇ СИСТЕМИ І ЇХ ВЗАЄМОЗВ'ЯЗОК З СИЛОЮ ДИХАЛЬНИХ М'ЯЗІВ У ДОРОСЛИХ ХВОРИХ, ЩО СТРАЖДАЮТЬ НА ПОЗАЛІКАРНЯНУ ПНЕВМОНІЮ

В.І. Березняков

ХАРКІВСЬКА МЕДИЧНА АКАДЕМІЯ ПІСЛЯДИПЛОМНОЇ ОСВІТИ, ХАРКІВ, УКРАЇНА

Вступ. *В даний час пневмонії займають 4–5 місце в світі в структурі причин смерті після серцево-судинних і онкологічних захворювань, цереброваскулярної патології, травм і отруєнь.*

Мета дослідження – *оцінити показники оксидантно-антиоксидантної системи та їх взаємозв'язок з силою дихальних м'язів у дорослих хворих, що страждають на позалікарняну пневмонію.*

Методи дослідження. *Дослідження проведено у період 2017-2020 рр. на базі терапевтичного відділення Комунального некомерційного підприємства "Міська клінічна багатопрофільна лікарня № 25" Харківської міської ради. У дослідженні брали участь 52 дорослих пацієнтів, що страждали на ПП, віком 18 до 80 років. Контрольну групу складали 20 практично здорових людей. Визначено активність малонового диальдегіду, каталази та супероксиддисмутази, рівень відновленого глутатіону, глутатіонредуктази та глутатіонпероксидази. Оцінку сили дихальних м'язів досліджували шляхом реєстрації максимальних статичних тисків на рівні порожнини рота та носа при "закритих" дихальних шляхах за допомогою апарату MicroRPM на 1-у та 10-у добу захворювання.*

Результати. *Дисфункція експіраторних дихальних м'язів переважала у пацієнтів з нетяжкою формою ПП, а інспіраторних дихальних м'язів – у пацієнтів з тяжкою формою ПП. Були встановлені негативні кореляції між рівнем малонового диальдегіду та показниками сили дихальної мускулатури, а також позитивні кореляції – з глутатіонредуктазою, глутатіонпероксидазою, каталазою та супероксиддисмутазою.*

Висновки. *Виявлені взаємозв'язки між про- і антиоксидантними показниками і силою дихальних м'язів доповнюють уявлення про системну реакції організму на легеневе запалення, одним з маркером якої є дисфункція дихальних м'язів.*

КЛЮЧОВІ СЛОВА: позалікарняна пневмонія; дихальні м'язи; оксидантно-антиоксидантна система.

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SIGNIFICANCE OF DETECTION OF FREE/TOTAL PSA RATIO AND OTHER BIOCHEMICAL PARAMETERS IN PATIENTS WITH BPH, CARCINOMA PROSTATE AND ITS CLINICOPATHOLOGIC CORRELATION

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Background. *Benign prostatic hyperplasia (BPH) can raise prostate-specific antigen (PSA) levels two to three times higher than the normal level. An increased PSA level does not indicate Prostate Cancer (PCa), but the higher the PSA level, the higher the chance of having PCa. Detection and treatment have been profoundly affected by the advent of Free/Total PSA ratio testing.*

Objectives. *The aim of the study was to estimate free, total PSA levels and its ratio for serum levels of calcium, acid phosphatase and alkaline phosphatase in patients with BPH and PCa; to correlate clinical, biochemical and histopathological findings in the above patients.*

Methods. *PSA levels were detected by Chemiluminescent assay; serum calcium – by Modified Arsenazo method; serum acid phosphatase – by Doumas et al method; and Alkaline phosphatase – by Lowry et al method.*

Results. *Present study found high levels of total PSA in BPH and PCa. Levels of free PSA were high in BPH as compared to PCa rate. Free/Total PSA ratio is reduced considerably in PCa as compared to BPH. Serum acid phosphatase and alkaline phosphatase were considerably higher in PCa as compared to BPH. Serum calcium levels did not show significant difference in control and study groups.*

Conclusions. *It was established that patients with PCa have a greater fraction of bound PSA and a lower percentage of free PSA than in those without PCa. Therefore, in clinical practice Free/Total PSA ratio helps clinicians to decide if a biopsy is necessary.*

KEYWORDS: benign prostatic hyperplasia (BPH); prostate cancer (PCa); prostate-specific antigen (PSA); free/total PSA.

Introduction

Benign prostatic hyperplasia (BPH), also called prostate enlargement, is a noncancerous increase in size of the prostate gland. Symptoms may include frequent urination, trouble starting to urinate, weak stream, inability to urinate, or loss of bladder control. Complications can include urinary tract infections, bladder stones, and chronic kidney problems [1, 2]. The clinical diagnosis of BPH typically depends on a history of LUTS (lower urinary tract symptoms), digital rectal examination. The degree of LUTS does not necessarily correspond to the size of the prostate. On rectal examination, significant finding of symmetric and smooth enlarged prostate gland is suggestive diagnosis of BPH. However, if the prostate gland feels asymmetrical, firm, or nodular, this raises concern for prostate cancer (PCa) [2, 3]. Urinalysis is typically performed when LUTS are present and BPH is suspected to evaluate for signs of a urinary tract infection, glucose in the urine (suggestive of diabetes), or protein in the urine (suggestive of kidney disease). Blood tests including kidney function tests and prostate specific antigen (PSA) are often ordered to evaluate for kidney damage and PCa, respectively. A total PSA test measures all the PSA, including both the bound and the free-floating antigens. A free PSA test, on the other hand, only measures PSA that is floating freely in the bloodstream and not bound to a different protein. Comparing the two results can help them understand the risk of PCa being present [1, 3]. BPH and PCa are both capable of increasing blood PSA levels and PSA elevation cannot differentiate these two conditions well. If PSA levels are tested and are high, then further investigation is warranted. Measures including PSA density, free PSA, rectal examination, and transrectal ultrasonography may be helpful in determining whether a PSA increase is due to BPH or PCa [2, 4, 5]. Ultrasound examination of the testes, prostate, and kidneys is often performed, again to rule out cancer and hydronephrosis [2-4]. US Food and Drug Association

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(FDA) approved PSA as an auxiliary diagnostic test in the management of the patients diagnosed with PCa. Since its clinical introduction to the present time, evaluation of serum PSA level is one of the most widely used tests in urology practice [5, 6, 7].

In conclusion Serum PSA level is very important for urologists. Introduction of PSA test into clinical practice has enabled early diagnosis of PCa, and provided important information about its staging, and postoperative follow-up period.

The present study was performed with the aim to study free and total PSA levels in patients with BPH and PCa. To aid in the early detection and diagnosis of PCa.

The study was carried out with the following objectives: to estimate free, total PSA levels and calculate Free/Total PSA ratio in patients with BPH and PCa, to study the serum levels of calcium, acid phosphates and alkaline phosphatase in patients with BPH and PCa and to correlate clinical, biochemical and histopathological findings in these patients.

Methods

The present prospective study was conducted in the Department of Biochemistry at Dr D. Y. Patil Medical College Hospital and Research Centre, Pune-18.

Study period: July 2012–September2014.

Study design: The study was designed as a Prospective case – control study.

Ethics statement

Written informed consents were obtained from all patients and healthy controls. Study protocols were approved by the institutional ethics committee of Dr. D. Y. Patil Vidyapeeth Pune. An informed consent was also obtained from the study population which consisted of two groups aged over 50 years old.

Study group and control

1. Control group comprises 30 healthy adults above the age of 50 years.

2. Study group comprises 35 cases of benign prostatic hyperplasia and 35 cases of Carcinoma Prostate.

Inclusion criteria: Male patients above the age of 50 years who presented with urinary complaints and showed prostatic enlargement on USG.

Exclusion criteria: Patients, who were diabetic, hypertensive and had a history of any major surgery, were excluded.

Collection of blood samples: Under all aseptic precautions about 5 ml of venous blood was collected in a plain bulb and allowed to clot for one hour at room temperature, centrifuged at 2000 rpm for 10 min. Serum was separated and analysed immediately for Free PSA, Total PSA, serum calcium, serum acid posphatase and alkaline phosphatase

Methods utilized:

Free and total PSA levels: Chemiluminescent assay.

Serum Calcium: Modified Arsenazo method.

Serumacid phosphatase: Doumas et al method.

Alkaline phosphatase: Lowry et al method.

Measurement of Total PSA (Chemiluminescence immunoassay method).

Electro-chemiluminescence immunoassay (ECLIA) for the quantitative determination of Total PSA in human serum and plasma [6,7,8,9,10,11].

Principle: A chemical moiety is disclosed which comprises a chemical, biochemical, or biological substance attached to one or more electrochemiluminescent organometallic compounds. Substances of interest are attached to one or more ruthenium containing or osmium containing labels or other electrochemiluminescent labels. Methods for detecting small amounts of chemical moiety using chemiluminescent, electrochemiluminescent and photoluminescent means. The chemiluminescent reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. Electrochemiluminescent technology can accommodate many immunoassay principles while providing superior performance [6,7].

Measurement of Free PSA (Chemiluminescence method [8,9].

Electro-chemiluminescence immunoassay (ECLIA) for the quantitative determination of free PSA in human serum and plasma. Measurement of free PSA is used to determine the free/total PSA ratio (% free PSA) which can be clinically useful in evaluating the need for prostate biopsy in two ways:

A) Individual risk assessment: The % free PSA is significantly lower in patients having prostate cancer than those with benign diseases or normal controls. The probability of finding prostate cancer with total PSA and with decreasing % free PSA. Therefore % free PSA improves the sensitivity and specificity in patients with total PSA values in this "gray zone".

B) Alternatively a single cut-off may be used for men in all age groups. It was found that the

proportion of unnecessary biopsies can be reduced by 20 % when using a threshold for % free PSA of 25 %.

Serum Calcium: Modified Arsenazo method (end point method) [10,11].

System reagent for the quantitative determination of calcium concentrations in human serum, plasma and urine on Beckman Coulter AU analysers.

Principle: Calcium reacts with a dye arsenazo at specific pH to form bluish purple coloured complex. The intensity of colour formed is directly proportional to the amount of calcium present in the sample.

This Calcium procedure is based on calcium ions (Ca^{2+}) reacting with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]- bisbenzenear-sonic acid) to form an intense purple coloured complex. Magnesium does not significantly interfere in calcium determination using Arsenazo III. In this method the absorbance of the Ca-Arsenazo III complex is measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

Quality control: During operation of the Beckman Coulter AU analyser at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Appropriate qualified urine controls should be established and utilized during urine analysis.

Results are automatically printed out for each sample in mg/dL at 37°C. For SI units (mmol/L) the results must be multiplied by 0.25.

Serum Acid Phosphatase: Doumas et al method [12-15].

Methodology: Kinetic method.

Acid Phosphatase α-Naphtyl Phosphate. Kinetic Quantitative determination of Acid Phosphatase.

Principle: Hillmann method – acid Phosphatase activity present in the sample is determined according to the modified method of Hillmann.

α-naphtyl-phosphate+H_2O → ACP α-naphtol +Phosphate

α-naphtol + Fast Red TR → Azo Dye

α-naphtol reacts with a diazoted compound forming a colour with a maximum of absorbance at 405 nm. Tartrate is used as specific of the prostatic fraction.

Quality Control: Sera control are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and technique for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Alkaline Phosphatase Assay [16-18].

Alkaline Phosphatase assay on the ARCHITECT c Systems™ and the AEROSET System.

The Alkaline Phosphatase assay is used for the quantitation of alkaline phosphatase in human serum or plasma.

Principle of procedure: Several substrates have been used to measure alkaline phosphatase activity such as glycerophosphate, phenyl phosphate and p-nitrophenyl phosphate. Bowers and McComb improved the method of Bessey *et al.* to include a kinetic measurement. Tietz et al. optimized this method to include a chelated metal-ion buffer of zinc, magnesium, and HEDTA. This Alkaline Phosphatase procedure is a modification of this method. Alkaline phosphatase in the sample catalyses the hydrolysis of colourless p-nitrophenyl phosphate (p-NPP) to give p-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the p-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Statistical analysis: At the end of study all data was compiled and analysed statistically using Annova method, Tukey's test.

Results

Serum Free PSA, Total PSA, Free/Total PSA ratio along with serum calcium, acid phosphatase, alkaline phosphatase were estimated in 30 controls (Group I) and 35 cases each of benign prostatic hyperplasia (Group II) and carcinoma Prostate (Group III). The results showed that Total PSA levels, calcium, acid phosphatase, alkaline phosphatase were higher in Group III as compared to Group I and II. Free PSA levels and Free/Total PSA ratio was decreased in Group III as compared to Group II. (Table 1).

The chart depicts the Mean±SD distribution into controls (Group I), cases (Group II) and Group (III).

Number of controls were 30 and cases were 35 each in Group II and Group III. Age of patients was considerably higher in Group III as compared to Group II (Table 2).

Table 3 shows comparison of Mean±SD values of serum Free PSA in Group I, II and III. Levels of Mean±SD values of Free PSA in Group III were significantly lower as compared to Group II.

Table 4 shows distribution of Mean±SD values of Total PSA in Group I, II and III.

Mean±SD values of total PSA were found to be increased in Group III as compared to Group I and II.

Table 5 is depicting Free/Total PSA ratio in terms of Mean±SD in Groups I, II and III.

Mean values of serum calcium in patients with carcinoma was considerably higher as compared to benign prostatic hyperplasia and control group. Test results obtained were significant (p<0.05) (Table 6).

Chart depicts intergroup comparison of serum alkaline phosphatase between cases and controls. Mean±SD values of alkaline phosphatase were higher in Group III as compared to Groups I and II.

Table 1. Comparison of age in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	68.07	7.95	5.04	<0.01
Group II (Control)	35	64	4.92		
Group III (Control)	35	68.26	5.82		

Note: Gr. I vs Gr. II: p<0.05 & Gr. I vs Gr.III: p<0.05
p<0.05: Significant
p<0.01: Highly significant
p>0.05 and p>0.01 Not Significant

Table 2. Comparison of free PSA in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	0.68	0.56	39.56	<0.0001
Group II (Control)	35	2.43	1.10		
Group III (Control)	35	1.59	0.55		

Gr. I vs Gr. II: p<0.0001andGr. I vs Gr.III: p<0.0001 and Gr.II vs Gr.III: p<0.0001

Table 3. Comparison of total PSA in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	1.95	1.24	35.93	<0.0001
Group II (Control)	35	8.51	5.20		
Group III (Control)	35	66.83	58.29		

Gr. I vs Gr. II: p<0.0001 & Gr. I vs Gr.III: p<0.0001

Table 4. Comparison of free/total PSA ratio in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	0.34	0.15	95.29	<0.0001
Group II (Control)	35	0.32	0.09		
Group III (Control)	35	0.04	0.03		

Gr. I vs Gr. II: p<0.0001 & Gr. I vs Gr.III: p<0.0001

Table 5. Comparison of serum calcium in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	9.19	0.41	4.20	<0.05
Group II (Control)	35	9.18	0.48		
Group III (Control)	35	9.52	0.71		

Gr. I vs Gr. II: p<0.05 & Gr. I vs Gr.III: p<0.05
p<0.05: Significant
p<0.01: Highly significant
p>0.05 and p>0.01 Not Significant

Table 6. Comparison of serum acid phosphatase in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	4.65	0.93	36.31	<0.0001
Group II (Control)	35	5.30	1.51		
Group III (Control)	35	18.52	12.67		

Gr. I vs Gr. II: p<0.001andGr. I vs Gr.III: p<0.0001

Table 7. Comparison of serum alkaline phosphatase in study groups

Group	Number	Age (Yrs.)		F Value	P Value
		Mean	SD		
Group I (Control)	30	51.90	13.09	42.20	<0.0001
Group II (Control)	35	71.20	29.58		
Group III (Control)	35	112.49	33.6		

Gr. I vs Gr. II: p<0.0001andGr. I vs Gr.III: p<0.05 and Gr.II vs Gr.III: p<0.0001

Discussion

PCa is a major cancer and leading cause of death in men. As with any cancer, early detection followed by treatment increases the disease-free survival rate significantly. Early diagnosis is very important in management of any type of cancer and infections. After introduction of serum PSA measurements into clinical use, the incidence rate of early diagnosis has increased, and a shift between stages was achieved. Nowadays, 70-80% of the diagnosed cancers are organ-confined [18,19].

Serum PSA level was also helpful in the staging of PCa. serum PSA values between 0-4 ng/mL can detect 80% of the organ-confined disease, while PSA values between 4-10 ng/mL, and above 10 ng/mL were found in 70% and 50% of the cases with organ-confined disease. Serum PSA levels also provide us helpful information about lymph node involvement. Lymph node involvement has been also reported in men with serum PSA levels of <10 ng/mL (5%), 10-20 ng/mL (18%), and >20 ng/mL (20%) in respective percentages. Therefore, if serum PSA level is below 25 ng/mL, there is no need to perform CT or MRI, and bone scanning is not required for men with serum PSA levels below 20 ng/mL. Besides, it has been demonstrated that in men with Gleason score ≤6, clinical stage T1/T2, and serum PSA levels below 10 ng/mL, lymph node dissection is not required [1,3,4, 12,18,19].

The current prospective study was conducted with the aim to estimate Free PSA, Total PSA, Free/Total PSA ratio in patients with benign BPH and PCa and correlate with the histopathology report. In the present study age wise distribution in control group is 68.07±7.95, while in BPH it was 64±4.92 and in PCa 68.26±5.82. There was no significant difference in the age wise distribution among all the three groups. Mean values of Free PSA levels in benign prostatic hyperplasia were 2.43±1.10 ng/ml, in carcinoma prostate – 1.59±0.55 ng/ml as compared to 0.68±0.56 ng/ml in the control group. The result was statistically highly significant (p<0.0001). In the present study, free/total PSA ratio is reduced in carcinoma prostate as compared to benign prostatic hyperplasia. Histological grades of prostate

biopsy showed a negative correlation with free/total PSA ratio in our study.

Lakhey M et al (2010) reported serum free PSA is elevated marginally in patients with BPH without inflammation, determined the relationship between free PSA and histological findings in biopsy specimens of patients with prostate disease. Active inflammation and high-grade lesions are associated with free PSA level more than 5 ng/ml [18].

Ari Adamy et al (2011) reported that with prostate specific antigen included in progression criteria prostate specific antigen at confirmatory biopsy (HR 1.29, 95% CI 1.14-1.46, p<0.0005) and positive confirmatory biopsy (HR 1.75, 95% CI 1.01-3.04, p=0.047) were independent predictors of progression [19].

Measurement of serum PSA levels is very important during post-treatment monitorization of PCa. Besides based on preoperative serum PSA levels, a biochemical recurrence can be seen within 10 years after surgery. Accordingly, recurrence rates of 10, 20, and 50% are anticipated in men with preoperative serum PSA levels of <2.6 ng/mL, 2.6-10 ng/mL, and above 10 ng/mL, respectively. Serum PSA levels are important in patients who had undergone androgen suppression therapy because of metastatic disease. If serum PSA level does not drop below 4 ng/mL 7 months after androgen suppression therapy, median life-expectancy of these patients is only one year. If serum PSA levels of these patients drop below 0.2 ng/mL, then median life-expectancy longer than 6 years can be predicted. If after radical prostatectomy or radiotherapy, serum PSA levels of the patients without radiological metastases rise above 0.2 ng/mL within the first 8 months of androgen suppression, prostate cancer mortality increases 20-fold. Still PSA doubling time shorter than 3 months is a very bad prognostic finding. Increase in serum PSA levels after prostatectomy aids in differentiation between local and systemic recurrences. If PSA doubling time increases after the first 2 postoperative years, and PSA doubling time is longer than 11 months, then local recurrence should be conceived with an 80% probability. However, if PSA levels rise within the first postoperative year, and PSA doubling time is 4-6 months, the systemic disease should be thought of with a 80% probability.

Similar results were obtained in a prospective study by Muhittin Serdar et al (2002) reported patients with serum total PSA between 4.1-9.9 ng/ml, if none of the PSA-based parameters is positive, biopsy can be postponed and the patients should be followed-up; on the other hand, patients with three positive parameters should be biopsied. If only one or two of the parameters are positive, the patient's age, race, and clinical findings should be considered in decision-making. Hence, the combined use of all markers can increase sensitivity and specificity [20].

Cut off value of free/total PSA ratio is 0.14 (14%) in the present study which has sensitivity of 94.3% and specificity of 99%. Patients with less than 0.14 ratio had Carcinoma prostate as compared to those above with cut off which showed benign prostatic hyperplasia. Most PSA in the blood is bound to other proteins. Normally more than 23% of serum PSA is in its free form (free PSA). Benign prostatic tumours may be associated with elevated PSA, especially free PSA. With prostate carcinoma, however, the proportion of free PSA decreases. One theory hypothesizes that prostate carcinoma produces not only PSA but also proteins to which the PSA is bound. As a result, portion of free PSA decreases. Histological grades showed a negative correlation between free/total PSA ratio and histological grade of carcinoma.

A lower ratio suggests prostate carcinoma while higher ratio suggests BPH by Borros Arneth [21]. In our study, total PSA and free/total PSA ratio were in accordance with study by D Weckermann et al in which the patients with prostate cancer showed high total PSA and reduced free/total PSA ratio as compared to BPH patients. Mione R et al found that percent free PSA was more effective than total PSA in the differential diagnosis between carcinoma prostate and BPH. Percent free PSA is superior to total PSA in distinguishing between primary Carcinoma prostate from BPH, in patients with total PSA between 2-30 ng/ml [22].

According to Manabu Kuriyama et al [23] the diagnostic significance of free/total PSA has been reported to improve diagnostic accuracy of prostate cancer in the group with considerably elevated serum PSA values. As serum PSA values increased, f/t was lower in the carcinoma prostate 56 group than in non-prostate carcinoma group. However, f/t values were lower in prostate carcinoma group than in non-prostate carcinoma group. The positive predictive value for prostate cancer increased to 54% in total PSA alone. Cut off value of free/total was 0.155 for obtaining relatively high specificity. In the present study, sensitivity was 85% and specificity was 56.5%.

Levels of calcium in serum were significantly correlated with serum levels of free PSA. It is unlikely that serum PSA acts to increase serum calcium, whose serum levels are under strict homeostatic control. Specifically, if serum levels of PSA increased serum levels of calcium, the men with advanced prostate cancer would develop hypercalcemia.

In this study probability estimates for prostate cancer in the percent free PSA ranges of ≤10 ng/ml is 58.3%. Although diagnostic improvements have been made through the use of PSA quotient, the diagnosis of prostate carcinoma can be made only after tumour biopsy and histopathology examination. Since the introduction of prostate-specific antigen testing over two decades ago, there has been a steep increase in the prostate cancer incidence, especially the incidence of localized low risk disease. Use of the percentage free PSA can enhance the specificity of PSA testing and decrease the number of unnecessary biopsies. However, the cut off free/total PSA in our case was 18.0%. Use of percent free PSA has been shown to improve specificity in the detection of prostate (4.1-10 ng/ml). In our study 17 (22%) patients had total PSA levels between 4-10 ng/ml. Of these one of the patients had PSA level 6.6 ng/ml, which on biopsy was reported as carcinoma prostate. Remaining 16 patients were reported as BPH on biopsy. Other parameters like serum calcium showed mean values of 9.91±0.41mg/dl in the control group. In BPH and PCa mean values of serum calcium were 9.18±0.48 mg/dl and 9.52±0.71 mg/dl, respectively. In the present study acid phosphatase values were higher in Group III (Carcinoma) as compared to group I and II. However, with advent of Prostate Specific Antigen (PSA) measurement of acid phosphatase in the diagnosis, staging and monitoring of prostate cancer has taken a back stage. Human prostatic acid phosphatase is a prostate epithelium specific differentiation antigen found in large amounts initially in seminal fluid. In our study 5 (14.28%) out of 35 patients were found to have slightly elevated acid phosphatase levels. Only one patient showed bone metastases. Similar study by Gutman and his colleagues made the critical observation that serum prostate acid phosphatase activity was significantly higher in prostate cancer patients, particularly those with bone metastases.

Limitation of the study is that in the present study per rectal examination was not done, as it was not advised by clinicians.

Conclusions

Present study found high levels of Total PSA in BPH and PCa. The levels of free PSA were high in BPH as compared to PCa rate. Free/Total PSA ratio was reduced considerably in PCa as compared to BPH. Serum acid phosphatase and alkaline phosphatase were considerably higher in PCa as compared to BPH. Serum calcium levels did not show significant difference in the control and study groups. We concluded that the patients with PCa have a greater fraction of bound PSA and a lower percentage of free PSA than in those without PCa. There was a negative correlation found between free/total PSA ratio and the histopathologic findings. The lower is the ratio, the higher is the grade of malignancy. Therefore, in clinical practice Free/Total PSA ratio helps clinicians to decide if a biopsy is necessary.

Conflict of Interests

The authors declare no conflict of interest.

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Author's Contributions

Dr. Shilpa Joshi – conceptualization, methodology, practical work, formal analysis; *Dr. Mona Tilak – g*uidance in research work, investigation and analysis; *Dr. Savita Jadhav – w*riting original draft, reviewing and editing draft, finalization of draft.

КЛІНІКО-ПАТОЛОГІЧНА КОРЕЛЯЦІЯ ТА РОЛЬ СПІВВІДНОШЕННЯ ВІЛЬНОГО/ЗАГАЛЬНОГО ПРОСТАТОСПЕЦИФІЧНОГО АНТИГЕНУ ТА ІНШИХ БІОХІМІЧНИХ ПОКАЗНИКІВ У ПАЦІЄНТІВ З ДОБРОЯКІСНОЮ ГІПЕРПЛАЗІЄЮ ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ ТА КАРЦИНОМОЮ ПРОСТАТИ

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Вступ. *Доброякісна гіперплазія передміхурової залози (ДГПЗ) може супроводжуватися відвічі-втричі підвищеним рівнем (ПСА). Підвищений рівень ПСА не вказує на рак передміхурової залози (РПЗ), чим вищий рівень ПСА, тим більша ймовірність виникнення РПЗ. Поява тесту на співвідношення вільного/загального ПСА мала значний вплив на частоту виявлення та вибір тактики лікування РПЗ.*

Мета. *Оцінити співвідношення вільного/загального ПСА, рівень сироваткового кальцію, кислої та лужної фосфатаз у пацієнтів з ДГПЗ та РПЗ. Провести кореляцію клінічних, біохімічних та гістопатологічних даних у вищевказаних пацієнтів.*

Методи. *Визначення ПСА проводилося за допомогою хемілюмінесцентного аналізу; сироватковий кальцій визначався модифікованим методом арсеназо. Кисла фосфатаза сироватки визначалася методом за Doumas et al., лужна фосфатаза – за Lowry et al.*

Результати. *Дослідження виявило високі рівні загального ПСА при ДГПЗ та РПЗ. Рівні вільного ПСА були вищими при ДГПЗ порівняно з РПЗ. Співвідношення вільного/загального ПСА значно знижується при РПЗ порівняно з ДГПЗ. Рівні сироваткової кислої та лужної фосфатаз значно підвищуються при РПЗ порівняно з ДГПЗ. Рівень кальцію в сироватці крові не відрізнявся в контрольній та досліджуваній групах.*

Висновки. *Пацієнти з РПЗ мають більшу частку зв'язаного ПСА та менший відсоток вільного ПСА, порівняно з пацієнтами без РПЗ. Тому в клінічній практиці співвідношення вільного/загального ПСА може допомогти клініцистам вирішити чи потрібна біопсія.*

КЛЮЧОВІ СЛОВА: доброякісна гіперплазія передміхурової залози (ДГПЗ); рак передміхурової залози (РПЗ); простатоспецифічний антиген (ПСА); вільний/загальний ПСА.

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SYSTEMATIC REVIEW ON THE PSYCHOMETRIC, RELIABILITY AND VALIDITY PROPERTIES OF TRANSLATED NEUROPATHIC PAIN SCREENING TOOLS (DN4, LANSS AND PDQ) 1 JANUARY 2005 – 19 JULY 2019

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Background. *Different neuropathic pain screening tools (DN4, LANSS and PDQ) have been developed, translated into several local languages, and validated. To determine the reliability of these tools and their ability to differentiate between diagnosing neuropathic pain quality from nociceptive pain, a systematic review was conducted to synchronize properties and suggest the reliability of the translated version of these neuropathic pain-screening tools.*

Objective. *To conduct an evidence-based systematic review to assess the psychometric, reliability and validity of the translated version of DN4, LANSS and PDQ between January 2005 and 2019.*

Methods. *Two independent reviewers adopted the use of online (Internet) search machine (Pubmed, Scopus and Web of Science) to search for the relevant articles based on JBI (Joanna Briggs Institute) inclusion criteria. Data extracted from the articles were synthesis in tabular form.*

Results. *Twenty-six articles were included from DN4 (n=11), LANSS (n=8) and PDQ (n=4) translated from English language to eight local languages. The sensitivity and specificity of the DN4 studies ranged from 75% to 98% and 37.3% to 96%, respectively. The internal reliability (α) of the translated version of the DN4 ranged from 0.55-0.862. The sensitivity and specificity of the LANSS studies ranged from 75% to 98% and 37.3% to 96%, respectively. The internal reliability (α) of the translated version of the LANSS ranged 0.67-0.96. The sensitivity and specificity of the PDQ studies ranged from 75% to 98% and 37.3% to 96%, respectively. The internal reliability (α) of the translated version of the PDQ ranged 0.81-0.86.*

Conclusions. *All the translated instruments reviewed showed good internal consistency of the items, high sensitivity and Positive predictive value (PPV) but not to a suitable level compared with the original version. Therefore, these screening tools are suggested to be used in conjunction with the clinical testing for appropriate diagnosis of patients with neuropathic pain quality.*

KEYWORDS: neuropathic pain; positive likelihood; negative likelihood; positive predictive value; negative predictive value.

Introduction

Neuropathic pain (Np) is classified as one of the worse pains reported by chronic pain patients [1]. An estimated 1 out of 10 chronic pain patients develop neuropathic pain, depending on the population study [2]. The prevalence may be as high as 51.9 % in the patients being managed for chronic pain clinic [3]. Evidence indicates that neuropathic pain affects both physical and emotional state of the patients [4], thereby. This type of pain decreases the quality of life of patients [4, 5] and results in a negative interaction with society in general [6]. Neuropathic pain is associated with lesion or disease of the somatosensory pathway that leads to abnormality observed at the peripheral and central region of the system function (hyperalgesia or allodynia) [7]. The common symptoms associated with neuropathic pain are: sharp, burning, pins and needles, tingling, painful cold, numb and shooting [2].

Diagnosing standards among pain physicians and researchers of neuropathic pain in chronic pain patients have been a challenge [8]. The five Np screening tools are LANSS [9], Neuropathic Pain Questionnaire [10], Douleur Neuropathique 4 'DN4' [11], ID pain [12] and PainDETECT [13]. These instruments have been validated and adapted in different languages from different countries.

Among these instruments, DN4, LANSS and PainDETECT are the most commonly used tools in the assessment of the quality of neuropathic

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pain in chronic pain patients due to their high sensitivity and specificity, short duration of the assessment, easy understanding of the terms and application by the pain experts [14, 15]. Translation of these tools from the original language to local languages is essential for good communication and effective assessment of pain quality between the researcher or pain expert and the patients.

Critically appraising the data measurement of these instruments may be valuable for the clinician and researchers in decision making based on evidence from peer-reviewed articles that adopted these instruments in their studies. Therefore, the aim of this study was to conduct a systematic review on the translated version of the Douleur Neuropathique en 4 Questionnaire (DN4), Leeds Assessments of Neuropathic Symptoms and Signs (LANSS) and the PainDETECT Questionnaire (PD-Q) tools with the objective to evaluate their psychometric, reliability and validity properties.

Methods

Study design: systematic review of studies was conducted according to PRISMA guidelines [16]. The systematic review was conducted using a developed protocol registered on PROSPERO (CRD42015016752) by the authors. PICO method was adopted to define our study question:

P (Patient or population): Patients with chronic pain

I (Intervention): Diagnostic screening tool

C (Comparator): None

O (Outcome): Psychometric and diagnostic properties of neuropathic pain screening tools: DN4*, LANSS**, and PD-Q (* Includes the DN4-interview, ** Includes the self-complete (S)-LANSS)

Study Inclusion Criteria

The following article selection criteria were used:

- **Language of publication:** No restrictions;
- **Geographic location:** No restrictions;
- **Publication date:** 1 January 2005 to 31 July 2019;
- **Publication type**: Original articles and abstracts;

Search strategy

The search strategy was as follows:

Databases: PubMed, Scopus, Web of Science.

Secondary search: Reference lists of selected publications were checked.

Search terms: ("Douleur Neuropathique" OR DN4 OR DN-4 OR "Leeds Assessment of Neuropathic Signs and Symptoms" OR LANSS OR PainDetect OR "Pain Detect" OR PDQ or PD-Q) AND pain AND (neuropathy OR neuropathic OR neuralgia OR neuritis OR central OR stroke OR spinal) AND (translation OR adaptation OR validation OR reliability OR validity).

Data management

Search results were transferred to Mendeley Desktop Reference Manager (Elsevier), where all references retrieved were combined, and duplicates were removed.

Screening

Initial screening of the articles included was done by title and abstract and was performed by TF (Temitope Fagbohun) and checked by PK (Peter Kamerman). The excluded articles were removed, and the reason for their exclusion was recorded. The full text of all retained studies was then screened by TF and PK and a consensus list of studies was generated to include into the review.

Data extraction

The following data were extracted:

1. Bibliographic information;
2. Study characteristics:

a. Name of the translated questionnaire;

b. Language of translation;

c. Setting (study population;)

d. Study methods;

e. Measures of reliability (Reliability of the screening tools was determined by the following measures: Test-retest reliability (intraclass correlation coefficient, Pearson's or Spearman's correlation coefficient), inter-rater reliability (Cohen's Kappa lowest and highest score), and internal consistency (Cronbach's alpha));

f. Diagnostic properties (Measures of diagnostic performance: Diagnostic performance was assessed by measures of sensitivity, specificity, positive likelihood, negative likelihood, positive predictive value, and negative predictive value.

Results

A total of 1,493 articles were obtained from the initial electronic databases search and 27 articles were finally included in the final review. The details of the study identification and selection process in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement [16] are described in Fig. 1. One hundred and twenty-two articles were excluded due to duplications. The abstract and the title of 1,371 articles were screened, 1,337 articles were excluded due to not meeting the inclusion

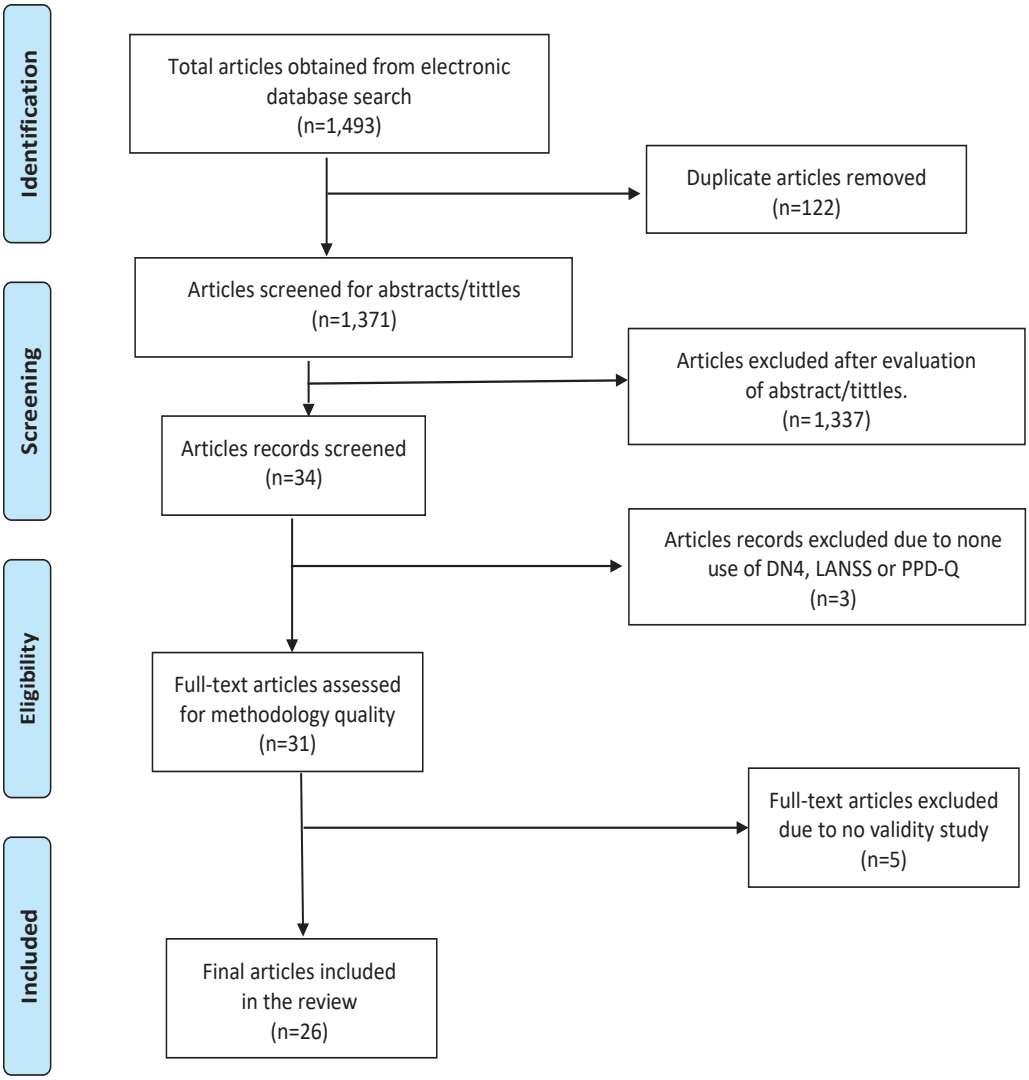

Fig. 1. *Flow chart of the final selected articles.*

criteria of this study. Thirty-four (34) articles were further screened for full-text inclusion, and three (3) articles were excluded for not applying DN4, LANSS or PainDETECT instrument neuropathic pain screening tools. Thirty (30) articles were further screened for check of validity test; five (5) articles were further excluded due to no validity test. Twenty-six (26) articles were included in this review for extraction.

Summary of the articles included

Twenty-six articles where included in this review – 11 DN4 articles [17-27], 8 LANSS articles [20, 24, 28-33], 4 PD-Q articles [15, 34-36] and 3 S-LANSS [28, 37, 38]. The total sample size reported was 2,075. Out of this, 1,056 were diagnosed with neuropathic pain, 874 – nociceptive pain and 55 were patients with mixed pain. Eighty-two (82) participants had mixed pain included in the neuropathic pain participants [24] (Table 1).

DN4

Description of the DN4 articles

Eleven (11) studies were included in the DN4 screening tool in this review (Table 2). Two (2) studies [17, 21] further evaluated the reliability and validity properties of the tools at different cut off. The DN4 was translated to eight different languages which includes: the Arabic language (n=2) [17, 21], Brazilian Portuguese (n=1) [26], Korean (n=2) [19, 20], Spanish (n=2) [24, 25], Farsi (n=1) [22], Greek (n=1) [23], Italian (n=1) [27] and Japanese (n=1) [18]. The total sample size reported n=1,756. Out of this, n=880 was diagnosed with neuropathic pain, n=731 – nociceptive pain and n=55 were patients with mixed pain. Eighty-two (82) participants had mixed pain included in the neuropathic pain

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Table 1. Description of the included studies

Author	Np Tool	Language	SAMPLE SIZE				Forward translation	No of forward translations	Backward translation	No of backward translations	Expert assessment	Pilot test
			Total	Neuropathic	Nociceptive	Mixed sample						
Matsuki et al. [18]	DN4	Japanese	187	100	87	0	Yes	1	Yes	1	Yes	No
Chatila et al. [17]	DN4	Arabic	195	99	96	0	Yes	3	Yes	NS	Yes	Yes
Terkawi et al. [21]	DN4	Arabic	124	77	47	0	Yes	5	Yes	2	Yes	Yes
Kim et al. [19]	DN4	Korean	83	43	40	0	Yes	-	Yes	-	-	-
Park et al. [20]	DN4	Korean	83	40	43	0	-	-	-	-	-	-
Sykioti et al. [23]	DN4	Greek	237	123	59	55	Yes	NS	Yes	NS	Yes	-
Madani et al. [22]	DN4	Farsi	175	86	89	0	Yes	5	Yes	3	Yes	Yes
Hamdan et al. [24]	DN4	Spanish	192	121	91	*See footnote	-	-	-	-	-	-
Spallone et al. [27]	DN4	Italian	221	50	61	0	NS	NS	NS	NS	NS	NS
Santos et al. [26]	DN4	Brazilian Portuguese	101	42	59	0	Yes	2	Yes	2	Yes	Yes
Perez et al. [25]	DN4	Spanish	158	99	59	0	Yes	2	Yes	2	Yes	NS
Batistaki et al. [28]	LANSS	Greek	100	58	42	0	Yes	2	Yes	2	Yes	Yes
Park et al. [20]	LANSS	Korean	213	113	100	0	Yes	2	Yes	2	Yes	Yes
Spanos et al. [30]	LANSS	Greek	70	35	35	0	NS	NS	NS	NS	NS	NS
Barbosa et al. [29]	LANSS	Portuguese	167	103	64	0	Yes	2	Yes	1	Yes	Yes
Hamdan et al. [24]	LANSS	Spanish	192	121	91	*See footnote	-	-	-	-	-	-
Türkel et al. [32]	LANSS	Turkish	148	99	49	0	Yes	NS	Yes	1	Yes	Yes
Schestatsky et al. [31]	LANSS	Brazilian Portuguese	90	34	44	12	Yes	2	Yes	1	Yes	NS
Yucel et al. [33]	LANSS	Turkish	101	49	52	0	Yes	1	Yes	1	Yes	No
Gudala et al. [15]	PainDETECT	Hindi	160	80	80	0	Yes	2	Yes	2	Yes	Yes
Matsubayashi et al. [36]	PainDETECT	Japanese	113	60	53	0	Yes	2	Yes	2	Yes	No
Alkan et al. [34]	PainDETECT	Turkish	240	80	80	80	Yes	2	Yes	2	Yes	No
De Andrés et al. [35]	PainDETECT	Spanish	221	71	71	79	Yes	2	Yes	2	Yes	NS
López-de-Uralde-Villanueva et al. [38]	S-LANSS	Spanish	182	71	111	0	Yes	2	Yes	2	Yes	Yes
Batistaki et al. [28]	S-LANSS	Greek	100	54	46	0	Yes	2	Yes	2	Yes	Yes
Koc and Erdemoglu [37]	S-LANSS	Turkish	154	137	107	0	Yes	2	Yes	NS	Yes	Yes

*Note. *82 (42%) had mixed pain, but were included in the neuropathic pain group*
NS – not specified

Table 2. Measures of validity for translated versions of the DN4

Author	Assessed Internal validity	Cronbach alpha	Assessed Test-retest reliability	ICC*	Correlation coefficient	Assessed Inter-rater reliability	Cohen's Kappa (lowest score)	Cohen's Kappa (Highest score)	Correlation Coefficient	ICC
Chatila et al. [17]	Yes	0.55 to 93	No	-	-	Yes	0.92 (brushing)	1.0 (hypoesthesia to brushing, hypo-esthesia to pinprick)	-	0.99
Chatila et al. [17]	Yes	0.86**	No	-	-	Yes	0.92 (brushing)	1.0 (hypoaesthesia for touch, hypoaesthesia for pinprick)	-	0.99
Terkawi et al. [21]	Yes	0.7	Yes	-	-	-	-	-	-	-
Terkawi et al. [21]	Yes	0.67	Yes	0.81	0.81 (Spearman)	No		-	-	-
Santos et al. [26]	Yes	0.76	No	-	-	Yes	0.92 (total score)	0.92 (total score)	-	-
Madani et al. [22]	Yes	0.862	Yes	0.957	-	Yes	0.416 (electric shocks)	0.826 (numbness)	-	0.957
Sykioti et al. [23]	Yes	0.65	Yes	0.956	-	Yes	0.818 (total score)	0.818 (total score)	-	-
Spallone et al. [27]	No	-	No	-	-	No	-	-	-	-
Matsuki et al. [18]	Yes	-	Yes	0.827	-	-	-	-	-	-
Park et al. [20]	Yes	0.819	Yes	0.813	-	Yes	0.823 (itching)	0.946 (numbness)	-	-
Kim et al. [19]	Yes	0.819	Yes	0.813	-	Yes	0.823 (itching)	0.946 (numbness)	-	-
Perez et al. [25]	Yes	0.7	Yes	0.949	-	Yes	0.68 (Not specified)	0.79 (Not specified)	-	0.926
Hamdan et al. [24]	No	-	-	-	-	No	-	-	-	-

***** *Intra-class correlation coefficient;**Only provided internal consistency for the entire questionnaire (by Kuder-Richdson formula).*

participants [24]. All the included articles that used DN4 instruments were published between 2007 and 2018 (Table 2).

Forward translation was reported in eight studies [17-19, 21-27] with the translation conducted in two studies in 5-times [21, 22], one study in 3-times [22], two studies in 2-times [25, 26], one study in 1-time [18]. Similarly, backward translation was reported in eight studies [17-19, 21-23, 25, 26]. One study conducted in 3-times [22], three studies conducted in 2-times [21, 25, 26], three studies were not specific on the number of times backward translation was done, and three studies did not report on the backward translation (Table 2). Expert assessment was involved in seven (7) studies [17, 18, 21-23, 25, 26] and four (4) studies conducted a pilot test [17, 21, 22, 26] (Table 2).

Validity and reliability of the DN4 instrument

Internal validity was reported in nine (9) studies [17-23, 25, 26] of the included eleven (11) studies (Table 2). Cronbach was reported in eight studies [17, 19-23, 25, 26]. Findings on test-retest validity were reported in seven (7) studies [18-23, 25] with ICC values between 0.81 and 0.96. One study reported the coefficients of correlation spearman value 0.81 [21]. The inter-rated reliability was conducted in seven (7) studies [17, 19, 20, 22, 23, 25, 26] in translated DN4, Cohen's kappa lowest scored values of 0.92 were reported for brushing in one (1) study [17], while total score values of 0.818 and 0.92 were reported in two (2) studies [23, 26], scored values of 0.823 was reported for itching in two studies [19, 20] (Table 2).

Cohens kappa high values of 1.0, 0.826-0.946 and 0.9 were reported for hypoesthesia to brushing and hypoesthesia to pinprick in two studies [17, 21] and numbness [19, 20, 22]. Also, total Cohens kappa highest score values of 0.818 and 0.92 (total score) were reported in two (2) studies [23, 26].

Sensitivity, specificity, negative and positive likelihood

Different cut offs were adopted to differentiate the neuropathic pain from nociceptive pain in this instrument (Table 3). Studies included at cut off of 3 showed sensitivity between 93.3-100%, specificity between 3-100%, Positive Likelihood between 5.2-5.5, Negative Likelihood between 0-3, PPV ranges between 84.3-85.6% and NPV of 72.1% – 97.5% in three studies.

At cut off 4, sensitivity reported ranges between 80-96%, while the specificity was between 6.8-95%, Positive likelihood 8.4-20.2 reported in three (3) studies, Negative Likelihood range between 0.1-0.2; PPV was between 63.9-95% and the NPV was between 69-95.5% reported in eight (8) studies. At cut off of 5, sensitivity reported ranges between 75-91% in four (4) studies, specificity was between 51-99%, Positive likelihood ranged between 5-150, Negative Likelihood was between 0.1-0.2, the PPV was between 84.3-93.7% and NPV was between 53.2-92.9%. Youden index values with cut off of three ranges between 0.46-0.92, cut off 4, was between 0.6-0.932 and cut off of 5 ranges between 0.6-0.89 (Table 2).

DN4-interview

Two studies were included in this review instrument [17, 27] conducted in the Arabic and Italian languages respectively (Table 1) and reported between 2012 and 2017 were participants in Arabic, and Italian population with a total sample size of 611. Patients with neuropathic pain (NP) were 248. The number of nociceptive pain patients' range was 253 patients, and none had mixed pain (MP). Forward translation was conducted in two studies thrice (3-times), and out of the three (3) studies that adopted this DN4-interview, one (1) study was not specific on the conduct and the number of times it was conducted. Similarly, backward translation was conducted in two (2) studies out of the three (3) studies adopted in DN4-interview, but the number of times conducted was not specific. Expert assessment involved, and a pilot study was conducted in two studies included.

Measurement of the validity of DN4-interview instrument

Internal validity was assessed in two studies with Cronbach alpha value between 0.55-93 and 0.86 (using Kuder-Richardson formula to assess the internal consistency of the whole questionnaire). In two studies Cohens Kappa lowest score value of 0.92 (brushing) and Cohen's Kappa Highest score values of 0.9 (electric shocks) and 1.0 (Hypoesthesia to brushing, hypoesthesia to pinprick).

Measurement of reliability of DN4-Interview

ROC was conducted in two studies included using this instrument (Table 6). At cut off of 2, the sensitivity was 99%, Specificity value of 58.3%, Positive Likelihood value 2.4, PPV of 71%, NPV of 98.2%. Two studies employ cut off 3 with sensitivity of 97%, Specificity value of between 82-82.3, Positive Likelihood value of 5.5., Negative Likelihood of 0, PPV of 85% and NPV of 96.3%. One study reported cut off value of 4 with sensitivity value of 84%, Specificity value

of 90%. Positive Likelihood value of 8.1, and Negative Likelihood of 0.2, PPV 89.2% and NPV of 84.3%. Youden index value at cut off of 2 was 0.56, cut off 3 was 0.79 and cut off 4 was 0.37.

LANSS

Description of the LANSS articles

Demographic characteristic of eight studies included using LANSS instrument in assessment of neuropathic pain (Table 1). Eight (8) studies [20, 24, 28-33] were included in this review. Two studies each were reported in the Greek [28, 30] and Turkish languages [32, 33]. Each of the following languages reported one study – Brazilian Portuguese [31], Korean [20], Spanish [24] and Portuguese [29]. Total sample size reported was 1,081. Out of this, 612 were diagnosed with neuropathic pain, while 477 were classified to have nociceptive pain. One study reported 42% of the neuropathic pain participants also had mixed pain [24]. Forward translation was conducted in four studies twice and one study once. Backward translation was conducted twice in two studies. Four studies conducted backward translation once. Six studies involved expert assessment while four studies conducted pilot studies.

LANSS measurement of validity

Internal validity assessment was conducted in six studies, with the Cronbach alpha ranging between 0.65-0.96 and the Test-retest reliability conducted in four studies (Table 4). One study reported the intra class coefficient value of 0.77 [28] with Pearson correlation coefficient reported in two studies (0.912-0.990 and 0.940). the inter-rated reliability was reported in two studies [28, 31] with Pearson value of 0.87 in one study [28].

Measure of reliability of LANSS instrument

Five studies conducted ROC at cut off of 12. Six (6) cut off values were reported to different neuropathic pain from nociceptive pain. Cut off 2, two (2) studies reported 80.2 and 89.8%, respectively. Specificity was 100% and 94.2%. One (1) study reported PPV of 93.6% and NPV of 90.74. While one (1) study reported Youden index value of 0.8. Two (2) studies reported cut off 2, sensitivity was 80.2 and 89.9, specificity was 94.2 and 100, NPV was 93.6. PPV was 90.74 with Youden index value 0.8. One (1) study reported cut off 7 with sensitivity of 91.2%, Specificity value of 83%, Positive Likelihood of 5.4, Negative Likelihood value of 0.1, PPV of 86% and NPV 89% with Youden index value of 0.74.

Cut off of 10.5 was reported in one (1) study with sensitivity of 88%, Specificity of 95%. One (1) study reported the use of Cut off 11 with sensitivity of 100%, specificity of 95.9%, PPV of 93.6 and NPV of 100. Four studies used cut off 12 with sensitivity ranging 72.6-98%, specificity range between 74-98%. Negative likelihood was reported in two (2) studies, NPV, at cut of 7 with value 5.4 and cut off 12 with value 36.3, Negative likelihood value 0.1 at cut off 7 and 0.3 at cut off 12, the sensitivity range between 72.6-98, specificity range between 74 98%. Positive likelihood was reported in a study with value of 36.3 and Negative Likelihood 0.3. Positive Predictive Value range between 85-99%, Negative Predictive Value of 76-96%. One study applied cut off 13, with sensitivity of 95%, specificity of 98%, PPV of 99 and Negative Predictive v=Value of 90.57. In addition, one (1) study reported the use of cut off 14 with Sensitivity 84, Specificity 82.8, PPV 88.7 and NPV 76.8.

Self-LANSS

Description

Three (3) included studies adopted LANSS-self between 2010-2016, one (1) study in the Greek language [28], one study in the Spanish language [38] and one study in the Turkish language [37] (Table 1). Internal validity was reported in three (3) studies with Cronbach alpha between 0.67-0.74. Test-retest validity was reported in two (2) studies with r-coefficient 964-Spearman, 0.97-Pearson, respectively. One (1) study reported inter-rater reliability and second r-coefficient. ROC was conducted in three (3) studies [28, 37, 38]. Three (3) different cut offs were used in this study labelled cut off 1, cut off 2, cut off 3, to distinguish neuropathic pain from nociceptive pain.

Reliability

ROC was conducted in three (3) studies (Table 7.3) with three different cuts off. One (1) study reported cut off 10 with sensitivity 78.8%, Specificity 76.6%, PPV 81.2%, NPV 73.9%. One (1) study adopted Cut off 10.5, Sensitivity 87%, Specificity 88%, Cut off 11, Sensitivity 90.1%, Specificity 72.1 %, Positive Likelihood value of 3.23, Negative Likelihood value of 0.2, PPV 67.4 and NPV 91.0% with Youden index value of 0.62. Three (3) studies adopted cut off 12 with sensitivity ranging between 72.3-88.7%, specificity ranging between 78.8-95.2%, Positive likelihood value of 3.8 and Positive likelihood value 0.2 was recorded in one (1) study (López-de-Uralde-Villanueva et al., 2018); PPV ranging between 70.8-96.2%, NPV between 69.4-91.4% with Youden index value 0.61 reported in one study. Cut off of 13 showed sensitivity of 81.7%, Specificity 79.3%, Positive likelihood value of 4,

Table 3. Measure of reliability for the DN4 instrument translated

Author	ROC*	Cut off used	Sensitivity (%)	Specificity (%)	Positive Likelihood	Negative Likelihood	PPV** (%)	NPV*** (%)	Youden Index
Chatila et al. [17]	Yes	3	98	81.3	5.2	0	84.3	97.5	0.79
		4	96	89	8.4	0.1	89.6	95.5	0.85
		5	93	96	22	0.1	85.8	92.8	0.89
Chatila et al. [17]	Yes	5	93	95.8	22	0.1	95.8	92.9	0.89
		4	96	89	8.4	0.1	98.5	95.5	0.85
Terkawi et al. [21]		-	-	-	-	-	-	-	-
Terkawi et al. [21]	Yes	4	88.3	75.4	-	-	-	-	-
Santos et al. [26]	Yes	3	1	37.3	-	-	85	80	0.627
		4	1	6.8	-	-	-	-	0.932
		5	91	5.1	-	-	-	-	0.854
Madani et al. [22]	Yes	3	95	88	8.5	0	89	95	83
		4	90	95	20.2	0.1	95	91	85
		5	83	99	150	0.2	99	86	82
Sykioti et al. [23]	Yes	3	93.3	52.5	-	-	85.6	72.1	0.46
		4	89	78	-	-	92.4	69.7	0.67
		5	75	85	-	-	93.7	53.2	0.6
Spallone et al. [27]	Yes	4	80	91.7	9.6	0.2	81.6	90.8	-
Matsuki et al. [18]		-	-	-	-	-	-	-	-
Park et al. [20]	Yes	3	100	88.2	-	-	-	-	0.882
		4	87	94	-	-	-	-	-
Kim et al. [19]	Yes	3	100	88.2	-	-	-	-	0.882
		4	87	94	-	-	-	-	0.812
Perez et al. [25]	Yes	4	79.8	78	-	-	85.9	69	0.58
		4	82	78	-	-	79	80.7	0.6
		4	82	78	-	-	63.9	90.2	0.6
Hamdan et al. [24]	Yes	4	95	97.2	-	-	-	-	0.92
	Yes	3	97	82.3	5.5	0	85	96.3	0.79

Table 4. Measures of validity for translated versions of the LANSS

Author	Assessed Internal validity	Cronbach alpha	Assessed Test-retest reliability	ICC	Correlation coefficient	Assessed Inter-rater reliability	Correlation coefficient	ICC
Schestatsky et al. [31]	Yes	0.67	No	-	-	Yes	-	0.97
Spanos et al. [30]	Yes	0.895	Yes	-	0.91 to 0.99* (Pearson)	No	-	-
Batistaki et al. [28]	Yes	0.65	Yes	-	0.940 (Pearson)	Yes	0.87 (Pearson)	-
Park et al. [20]	Yes	0.82	No	-	-	No	-	-
Barbosa et al. [29]	Yes	0.77	Yes	0.77	-	No	-	-
Hamdan et al. [24]	No	-	-	-	-	No	-	-
Yucel et al. [33]	No	-	No	-	-	No	-	-
Türkel et al. [32]	Yes	0.96	Yes	-	0.95 (Spearman)	#see footnotes	-	-

**range of r coefficients for each item: #Difficult to interpret test-retest, and inter-rater reliability because of the manner of reporting*

Table 5. Measures of reliability for translated versions of the LANSS

Author	*ROC*	Cut off used	Sensitivity (%)	Specificity (%)	Positive Likelihood	Negative Likelihood	PPV** (%)	NPV** (%)	Youden index
Schestatsky et al. [31]	No	-	-	-	-	-	-	-	-
Spanos et al. [30]	No	-	-	-	-	-	-	-	-
Batistaki et al. [28]	Yes	12	82.8	95.2	-	-	96	80	-
		10.5	88	95	-	-	-	-	-
Park et al. [20]	Yes	12	72.6	98	36.3	0.3	98	76	Not specified
		7	91.2	83	5.4	0.1	86	89	0.74
Barbosa et al. [29]		12	89	74	-	-	85	81	-
		14	84	82.8	-	-	88.7	76.8	-
Hamdan et al. [24]	Yes	2	80.2	100	-	-	-	-	0.8
Yucel et al. [33]	Yes	2	89.9	94.2	-	-	93.6	90.74	-
Türkel et al. [32]	Yes	11	100	95.9	-	-	98	100	-
		12	98	98	-	-	99	96	-
		13	95	98	-	-	99	90.57	-

Negative likelihood value of 0.2, PPV of 71.6%, Negative Predictive value 87.1 with Youden index value of 0.61.

PainDETECT

Description

Four (4) studies were included in the translated original using this instrument between 2012 to 2017 in population with local language Hindi [15], Japanese [36], Spanish [35] and Turkish [34]. Total sample size reported was 974, out of this, 371 participants had neuropathy, 364 and 239 participants were diagnosed with nociceptive and mixed pain respectively. Five (5) studies included reported forward translation, the translation was reported twice in five (5) studies. Backward translation was reported in five (5) studies conducted twice in five studies. Expert assessment was conducted in five studies. Pilot study was conducted in one (1) study [15].

PainDETECT validity characteristic

Internal validity of the terms was reported in the five (5) studies by Cronbach alpha reported ranges from 0.78-0.86. Test-retest validity was reported in five (5) studies and ICC reported ranges between 0.934-0.98 in five (5) studies included. Four (4) studies reported ROC [15, 34-36].

PainDETECT Reliability characteristic

Table 8.3: Four cut offs were reported in the studies included using PainDETECT instrument. Cut off 12, the sensitivity was between 84-93%, Specificity 66-68%, two studies reported PL 2.7 and 2.9; NL 0.1 and 0.2; PPV was reported by four (4) studies ranging between 73-87%, NPV 65-88% and Youden index value of 0.575 and 0.519 reported in two studies (Table 3).

Two (2) studies made use of cut off 17, Sensitivity 81%, Specificity 80 and 81%; Positive Likelihood 4.1 and 4.3; Negative predictive value 65 and 81 with Youden index values of 0.613 and 0.624. One (1) study reported cut off of 18, Sensitivity 83%, Specificity 91%, PPV 90% and NPV 84%. Three (3) studies adopted cut off of 19, specificity between 71-79%, Specificity 83-93 %, Positive likelihood reported in two (2) studies with values of 4 and 4.4, Negative likelihood 0.3 and 0.4, PPV reported in three (3) studies ranging between 82-90%, NPV between 55-79% with Youden index reported in two (2) studies of 0.531 and 0.613 (Table 3).

Discussion

The aim of the study was to conduct a qualitative systematic review to determine the psychometric property of translated, validation and reliability of neuropathic pain screening tools (LANSS, DN4 and PD-Q).

DN4 instrument

The participants' average sample size was above 30 in all the included studies, and this indicated that all the included studies had the sample size sufficient to represent the population and achieve the aim of the study, and the sample mean on normal distribution. Forward and backward translation were conducted in 90% of the included studies from local languages (Arabic, Brazillian-Portuguies, Farsi, Greek, Italian, Japanese, Korean and Spanish) into the English language and from English into local languages in the included studies. Tsang et al. [39] reported that this process is an important step in translation, the more times the translation the better chances of avoiding the error of bias. The reported Cronbach alpha value was higher than 0.6 that indicated an acceptable internal consistency among the items and accurate translation with exception of one study [17]. The involvement of expert assessment in over 80% of the included studies was in agreement with the set-out guidelines for the process of accurate translation [39]. Furthermore, a pilot study was conducted in most of the studies included which is an essential step in determination of the reliability of the items involved in the questionnaire and the validity of the test instrument. The value of Cohen's Kappa was low and high score reported by the pain expert was higher than normal values indicating a profound agreement among the pain experts. The average high sensitivity values and specificity values reported in the instrument pointed to a good validity of this instrument in differentiating neuropathic pain cohort from non-neuropathic pain groups.

The high average value of positive likelihood decreased as the cut off increases. The same pattern of decrease was observed in positive likelihood, however no obvious change in the negative likelihood was evidenced. Positive likelihood and negative likelihood were considered important factors in the measurement of sensitivity and specificity in test population. Considering the reports from the included studies, an optimum value sensitivity, specificity, positive predictive value was reported at cut off 4 at average value, which could make it a better cut off in agreement with Bouhassira et al. [11] in the original development of the DN4 instrument.

DN4-interview

The participants sample size in this review (n = 416) was greater than 30 in all the included

studies [17, 27] using DN4-interview instrument; this indicated that all the studies were statistically adequate, and the sample mean was on normal distribution. Forward and backward translation were conducted in 67% from local languages (Arabic and Italian) to the English language and from English to local languages at least (three times) which were reported as important steps in translation process, as mentioned previously, the more times the translation – the better chances of avoiding the error of bias. Tsang et al. [39] recommended a minimum of twice forward and backward translation for a good translation procedure. There was no specification on the number of times for backward translation that could lead to a possible limitation when using this instrument. The re_viewed Cronbach alpha value using this instrument (0.55-0.862) was averagely higher than 0.6 (the minimum Cronbach alpha value for a good internal consistency) that indicated an acceptable internal consistence among the items, a general internal consistency measured (dichotomized measurement of reliability) by the Kuder-Richardson formula (0.86) which is close to 1 as recommended for a good reliability [40] with exception of one study [17].

Moreover, expert assessment in over 80% of the included studies was also corroborating with the set-out guidelines for the process of adequate translation [39]. Inter-rated reliability review showed a close point (0.9) to 1 in bru_shing at low Cohen Kappa and 1 in hypoesthesia to brushing and pinprick. This indicates a high reliability in these two signs of measuring neuropathic pain and shows that this instrument is a good instrument and is consistent among the pain-expert. Therefore, this instrument could be used to distinguish neuropathic pain from non-neuropathic pain.

Pilot study was conducted in most of the studies (67%) included studies, this is an essential step in determination of the reliability of the items involved in the questionnaire and hence the validity of the test instrument. The optimum sensitivity (84-99%) and specificity (58-90%) reported in the instrument pointed to the fact that this instrument (DN4-interview) was a highly sensitive and valid in distinguishing neuropathic pain quality from non-neuropathic pain. Comparing the optimal test scores value of the translated DN4-interview instrument in the included studies with the original DN4-interview test score values, the performance of the translated was not as good as the original version.

LANSS

Our review on the psychometric translation properties using LANSS showed the sample size (n=90-213) indicating a good statistical sample. Forward and backward translation were conducted (80%) for the review [20, 28, 29, 31, 33] of the reviewed studies as compared with the original version of LANSS. Test-retest reliability was conducted in 55% of the studies within the pain experts. Only one study evaluated the intra-class correlation coefficient (0.77) [29]. This is contrary to the expectation from the original version, which showed that the included studies reported Cronbach alpha (0.67-0.9612) higher than 0.6, indicating a good internal consistency among the items. Forward and backward translation were conducted with the instrument good translation procedures in 75% of the included studies. Fifty percent (50%) complied with minimum of twice forward translation while twenty percent (20%) complied with minimum of twice backward translation. This shows that there were gaps in translation procedures in 70% of the included studies [24, 29, 31-33]. Inter-rated reliability was conducted in 20% of the included studies, which were considered as an important step in measurement of reliability in instrument testing and the validity of the instrument as compared with original LANSS translation procedure. This is in contrary to the setout procedure for a good neuropathic pain instrument.

The average sensitivity value (85%) and specificity values (92%) observed using this instrument indicated that LANSS was a very sensitive instrument and specific to measure the neuropathic components of a pain patient across all languages. Average Positive Predictive value (93.7%) showed that the instrument was an effective instrument in the determination of components of LANSS questionnaire that marked out neuropathic pain components. This agrees with the Bennett study on the development of LANSS neuropathic pain screening tool [9].

S-LANSS

The is a modification of the original LANSS instrument without the clinical examinations that was developed by Bennett et al. [41]. Our review indicated that the internal consistence measured by Cronbach alpha (0.67-0.74) showed high internal consistency among the items included in the instrument in a population sample size of average (n=145), which was statistically dependable sample size. Forward and backward translations were conducted in

Table 6: Measures of reliability for translated versions of the S-LANSS

Author	Assessed Internal validity	Cronbach alpha	Assessed Test-retest reliability	ICC	Correlation coefficient	Assessed Inter rater reliability	Cohens Kappa (lowest score)	Cohens Kappa (highest score)	Correlation coefficient	ICC
Batistaki et al. [28]	Yes	0.67	Yes	-	0.964 (Spearman)	Yes	-	-	0.54 (Pearson)	-
López-de-Uralde-Villanueva et al. [38]	Yes	0.71	No	-	-	No	-	--	-	-
Koc and Erdemoglu [37]	Yes	0.74	Yes	-	0.97 (Pearson)	No	-	-	-	-

Table 7. Measures of reliability for translated versions of the S-LANSS

Author	ROC*	Cut off used	Sensitivity (%)	Specificity (%)	Positive Likelihood	Negative Likelihood	PPV**(%)	NPV***(%)	Youden
Batistaki et al. [28]	Yes	12	86.2	95.2	-	-	96.2	83.33	-
		10.5	87	88		-	-	-	-
López-de-Uralde-Villanueva et al. [38]	Yes	11	90.1	72.1	3.23	0.1	67.4	91.9	0.62
		12	88.7	76.6	3.8	0.2	70.8	91.4	0.65
		13	81.7	79.3	4	0.2	71.6	87.1	0.61
Koc and Erdemoglu [37]	Yes	12	72.3	80.4	-	-	82.5	69.4	--
		10	78.8	76.6	-	-	81.2	73.9	-

*Receiver operating characteristic, ** – Positive predictive value, *** – Negative predictive value.*

Table 8. Measures of validity for translated versions of the PainDETECT

Author	Assessed Internal validity	Cronbach alpha	Assessed Test-retest reliability	ICC	Correlation coefficient	Assessed Inter-rater reliability	Cohens Kappa (Lowest score)	Cohen's Kappa (Highest score)	Correlation coefficient
Gudala et al. [15]	Yes	0.83	Yes	0.94	-	No	-	-	-
Matsubayashi et al. [36]	Yes	0.78	Yes	0.94	-	No	-	-	-
De Andrés et al. [35]	Yes	0.86	Yes	0.934	-	No	-	-	-
Alkan et al. [34]	Yes	0.81	Yes	0.98	-	-	-	-	-
Alkan et al. [34]	Yes	0.81	Yes	0.98	-	-	-	-	-

Table 9. Measures of reliability for translated versions of the PainDETECT

Author	ROC*	Cut off used	Sensitivity (%)	Specificity (%)	Positive Likelihood	Negative Likelihood	PPV**(%)	NPV***(%)	Youden
Gudala et al. [15]	Yes	19	79	93	-	-	91	81	-
		12	90	66	-	-	73	87	-
		18	83	91	-	-	90	84	-
Matsubayashi et al. [36]	-	-	-	-	-	-	-	-	-
De Andrés et al. [35]	Yes	12	93	68	2.9	0.1	87	80	0.609
		19	75	84	4.7	0.3	92	60	0.595
		17	81	81	4.3	0.2	91	65	0.624
Alkan et al. [34]	Yes	12	90	68	2.8	0.2	73	87	0.575
		19	78	83	4.4	0.3	82	79	0.6
		17	81	80	4.1	0.2	80	81	0.613
Alkan et al. [34]	Yes	12	84	68	2.7	0.2	86	65	0.519
		19	71	83	4	0.4	90	55	0.531

all the studies (two times) that was the minimum number of times it should be carried out; pilot study was conducted with involvement of expert assessment in all the included studies. This review proved Cronbach alpha (0.67-0.74 range) of above 0.6 recommended for a good internal consistency in translation research [42]. However, the Test – retest reliability was conducted (90%) of the included article, which is an important procedure to measure good translation procedure. Our review on this instrument showed an average sensitivity value (83.02%) and specificity (80.3%); these values indicated that the instrument was sensitive in determination of the neuropathic component of pain patients. The value of the sensitivity deceased as the cut off value increases from 12 to 13 in a population sample size of 154 [38] in association with decrease in the value of NPV as the cut off value deceased. This review showed an optimum value of sensitivity, specificity, and the positive predictive value at cut off 12, indicating a high performance of the instrument. This cut off 12 was also reported by the three studies, which could make it an acceptable cut off to distinguish neuropathic pain patients from non-neuropathic pain patients.

PD-Q

All the included studies conducted forward and backward translation twice (in agreement with the recommended standard). Most of the studies did not conduct any pilot study. This is contrary to the guideline for translation procedures, which is an important primary step. The internal consistency of the items measured by evaluating the Cronbach alpha values (0.78-0.86) showed a high internal consistency among the items listed on the instrument. Our review showed that Test-retest reliability was conducted in all the included studies with the average value of interclass correlation coefficient (0.95), suggesting a high validity of the instrument in all the translated versions of PD-Q reviewed in the diagnosis of neuropathic pain from non-neuropathic pain. The studies showed an average sensitivity value (82.3%) and specificity (80.5%) that indicated that PDQ was a sensitive instrument in the determination of neuropathic pain component, and the items were very specific in the determination of the symptoms of neuropathic pain.

Conclusions

The original DN4 and LANSS had the most evidence for their psychometric, reliability and validity properties in peer-reviewed articles. These tools were designed to assess the neuropathic pain quality in a test population through differentiating signs and symptoms between neuropathic and non-neuropathic pain patients. Furthermore, these screening questionnaires may provide an indication of the presence of neuropathic pain quality; however, they cannot replace a clinical assessment. It is clear from the studies that most of the instruments do not assess psychometric, reliability and validity properties effectively. For those that were assessed, not all of them were satisfactory, and most of the findings were supported by low or very low level of evidence. In conclusion, we recommend that both the clinical assessment and neuropathic pain-screening tool are pivotal in the diagnosis of neuropathic pain component in pain patients in clinical settings.

Recommendations

These three neuropathic pain screening tools (DN4, LANSS and PDQ) translated version as performed ultimately well in other local languages at their test population but none of these has been developed in the African Language, it will be a valuable interest also to evaluate the performance of this tool by determining the reliability and validity properties.

To increase the sensitivity, reliability and validity of these screening tools, efforts should be taken to carry out forward and backward translation more than twice from original version of the tools. Additionally, the use of language translation experts in both the original version and the translated local version of the original screening tool should be used for transcription and translation process. Moreover, translation into as many local languages as possible should be made to ensure consistency of the methodology and the properties should be measured by the tools.

This would be especially valuable in the sub-Saharan African region where most of the population might not be proficient in the lingua franca. We have just completed the translation of the DN4 screening tool into IsiZulu (a commonly spoken Nguni African language in South Africa) in our research group. In future studies, we will assess the sensitivity, reliability, and validity of this translated version and evaluate its feasibility in line with other previously translated versions.

Conflict of Interests

There are no conflicts of interest between the author and other researchers that contributed to data extraction.

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Autho's Contributions

Temitope Richard Fagbohun – Conceptualization, methodology, formal data collection and analysis, writing – original draft, writing – reviewing and editing.

СИСТЕМАТИЧНИЙ ОГЛЯД МЕТОДІВ ПСИХОМЕТРІЇ, НАДІЙНОСТІ ТА ВАЛІДНОСТІ ПЕРЕКЛАДЕНИХ ОПИТУВАЛЬНИКІВ ДЛЯ СПОСОБІВ СКРИНІНГУ НЕЙРОПАТИЧНОГО БОЛЮ (DN4, LANSS І PDQ) 1 СІЧНЯ 2005 – 19 ЛИПНЯ 2019

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Вступ. *Для скринінгу нейропатичного болю використовують різні опитувальники (DN4, LANSS та PDQ), які були перекладені на декілька локальних мов та валідовані. Щоб визначити надійність цих засобів та їх здатність відрізняти нейропатичний біль від ноцицептивного при діагностиці, було проведено систематичний огляд для синхронізації властивостей та припущення про надійність перекладеної версії цих засобів скринінгу нейропатичного болю.*

Мета. *Провести обґрунтований систематичний огляд для оцінки психометрії, надійності та валідності DN4, LANSS та PDQ у період з січня 2005 по 2019 рік.*

Методи. *Двоє незалежних рецензентів провели пошук відповідних статей у Pubmed, Scopus та Web of Science на основі критеріїв включення JBI (Інститут Джоани Бріггс). Дані, отримані зі статей, були синтезовані у вигляді таблиці.*

Результати. *В огляд були включені двадцять шість статей з DN4 (n=11), LANSS (n=8) та PDQ (n=4), перекладених з англійської мови на вісім місцевих мов. Чутливість та специфічність шкали DN4 коливалися від 75% до 98% та 37,3% до 96% відповідно. Внутрішня надійність (α) перекладеної версії DN4 коливалася в межах 0,55-0,862*

Чутливість та специфічність шкали LANSS коливалися від 75% до 98% та 37,3% до 96% відповідно. Внутрішня надійність (α) перекладеної версії LANSS перебувала в межах 0,67-0,96

Чутливість та специфічність шкали PDQ коливалися від 75% до 98% та 37,3% до 96% відповідно. Внутрішня надійність (α) перекладеної версії PDQ знаходилася в межах 0,81-0,86.

Висновки. *Усі перекладені інструменти продемонстрували хорошу внутрішню узгодженість елементів, високу чутливість та позитивне прогностичне значення, однак не досягали рівня оригіналів. Тому для належної діагностики пацієнтів з нейропатичним болем ці скринінгові інструменти пропонується використовувати разом з клінічним обстеженням.*

КЛЮЧОВІ СЛОВА: нейропатичний біль; позитивна ймовірність; негативна ймовірність; позитивне прогностичне значення; негативне прогностичне значення.

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A CROSS SECTIONAL STUDY OF LIPID PROFILE IN NON-DIABETICS WITH STROKE IN URBAN CHITRADURGA

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Background. *The amount of evidence regarding the relation between serum lipids, lipoproteins and cerebrovascular accident is not adequate. The atherogenecity of diabetics and non-diabetics are different. Therefore, non-diabetic patients were included in the study.*

Objective. *To study lipid abnormalities in non-diabetic stroke patients in our setup.*

Methods. *The study was carried out at the Department of General Medicine, BMCH, Chitradurga, during the period from June 2020 to December 2020. The lipid profile and the fasting blood sugar rates of 50 stroke patients without diabetes were studied. Their serum samples were assessed for fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) by using standard biochemical methods.*

Results. *The age distribution of the subjects was from 19 to 72 years with a mean age of patients 54.8±15.75 years. Among patients 31 (62%) were males and 19 (38%) were females. Among the study subjects 58% were hypertensive, 76% were smokers, 32% were alcoholics and 34% had family history of cerebrovascular accident. Among ischemic stroke group, the most common deranged value in the ischemic group was decreased HDL deranged in 54.1% of patients; the second most common deranged value – increased VLDL deranged in 40.5%. Among the hemorrhagic group the most common deranged value was also decreased HDL, which was deranged in 46.1% of patients and the second most common deranged value – increased total cholesterol, which was deranged in 53.8% patients.*

Conclusion. *Lipid profile should be considered while predicting the risk of stroke.*

KEYWORDS: lipid profile; dyslipidemia; non-diabetic stroke; serum cholesterol.

Introduction

As per World Health Organization, stroke is defined as a clinical syndrome consisting of rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, with duration lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin [1].

Stroke is the second leading cause of death worldwide causing 6.2 million deaths in 2011 [2]. Stroke is a medical emergency which is an acute neurological injury that occurs due to vascular pathology and presents as a brain infarction or haemorrhage. The modification of risk factors in stroke has brought down both mortality and morbidity of stroke remarkably in the last 30 years. Dyslipidemia is a major risk factor for stroke. It has been established that reduction of total cholesterol, LDL cholesterol, trigycerides, VLDL cholesterol and increasing HDL cholesterol by drugs decreases the incidence of stroke.

In our study, lipid profile was studied in non-diabetic patients with stroke. Diabetes itself is associated with hyperlipidemia and increased atherosclerosis which makes it an undisputed risk factor for stroke. The atherogenecity of diabetics and non-diabetics are different. With this background, we conducted the study of lipid profile in the non-diabetics with stroke in our setup.

Methods

Patients with stroke and non-diabetic attending OPD and IPD of the Department of General Medicine of Basaveshwara Medical College and Hospital, Chitradurga, were enrolled in the study. Patients with haemorrhagic strokes, embolic strokes, past/present H/O diabetes mellitus or history of head injury or usage of anti-coagulant drugs were excluded from the study.

The study took place over the period of 6 months from June 2020 to December 2020.

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Sample size estimation was performed using open epi software version 2.3.1 at 95% confidence level, 80% power of the study, proportion of cases with stroke with raised lipid parameters as 70% [3]. Sample size estimated was 37 inflated to 50.

A brief personal history and medical history, including brief histories about co-existing disease states, family history of diabetes mellitus, and history of hypertension, past and present illnesses, dietary pattern, addiction and medication were recorded in self prepared questionnaire. Detailed general physical examination, systemic examination and neurological examination were performed to all the patients and were recorded.

The investigations done for the patients at presentation included fasting lipid profile (serum total cholesterol, serum triglycerides, serum high density lipoproteins, serum very low density lipoproteins, serum low density lipoproteins) and HbA1c levels [4].

Data was entered in excel sheet and analysed using the Statistical Package for the Social Sciences 20 (SPSS Inc. Chicago). Results were presented in tabular and graphical forms. Mean, median, standard deviation and ranges were calculated for quantitative data. The Chi square analysis was used in testing for significant differences between proportions and frequencies. The T-test was used in testing for significant differences between two means. The confidence interval was set at 95% limit, with level of significance, $p \leq 0.05$.

Results

50 patients were enrolled in the study. The age distribution of the subjects was between 19 to 72 years with mean age of patients 54.8±15.75 years. 31 (62%) were males and 19 (38%) were females. It was established that 58% of our study subjects were hypertensive, 76% were smokers, 32% were alcoholics and 34% had family history of CVA (Table 1).

Analysis of lipid profile among both the groups of stroke, i.e., hemorrhagic and ischemic stroke, revealed that the most common rate deranged in the ischemic group was decreased HDL, which is deranged in 54.1% of patients and the second most common rate deranged was increased VLDL, which was deranged in 40.5%. Among the hemorrhagic group the most common deranged rate was also decreased HDL, which was deranged in 46.1% of patients, and the second most common deranged rate was increased total cholesterol, which was deranged in 53.8% of patients (Table 2).

Our study concluded a significant association between serum total cholesterol, triglyceride, LDL level, VLDL level and risk of stroke. High levels of total cholesterol, triglycerides, LDL cholesterol were associated with higher risk of stroke. Lowered HDL cholesterol levels were not significantly associated with stroke. The ratio of HDL/LDL cholesterol, TC/HDL cholesterol for males and females was evaluated. However, the association with risk of stroke was not found (Table 3).

Discussion

Association of Total Cholesterol to the Non-Diabetics with Stroke

Our study involved 50 subjects and total cholesterol was elevated in the non-diabetics with stroke. The serum total cholesterol levels in cases of either ischaemic or haemorrhagic

Table 1. Risk factors of stroke among study participants

Variables		Hemorrhagic Stroke (n=13)	Ischemic Stroke (n=37)	Chi-square test
Gender	Male	9 (69.2)	22 (59.5)	0.4 p=0.5
	Female	4 (13.8)	15 (40.5)	
Age in years	<30	1 (7.6)	2 (5.4)	1.4 p=0.5
	30-60	6 (46.2)	24 (64.9)	
	>60	6 (46.2)	11 (29.7)	
Hypertension	Present	9 (69.2)	20 (54.1)	0.9 p=0.3
	Absent	4 (30.8)	17 (45.9)	
Smoking	Yes	8 (61.5)	30 (81.1)	2.01 p=0.2
	No	5 (38.5)	7 (18.9)	
Alcoholic	Yes	5 (38.5)	11 (29.7)	0.34 p=0.6
	No	8 (61.5)	26 (70.3)	
Family h/o CVA	Present	5 (38.5)	12 (32.4)	0.16 p=0.7
	Absent	8 (61.5)	25 (67.6)	

Table 2. Lipid profile among cases and controls

Variables		Hemorrhagic Stroke (n=13)	Ischemic Stroke (n=37)	Chi-square test
Total Cholesterol	<200	7 (53.8)	24 (64.8)	0.6 p=0.5*
	200-240	5 (38.5)	9 (24.3)	
	>240	1 (7.7)	4 (10.9)	
Serum Triglyceride	<150	6 (46.1)	21 (56.8)	0.7 p=0.5*
	150-199	4 (30.8)	11 (29.7)	
	200-499	3 (23.1)	5 (13.5)	
LDL cholesterol	<100	7 (53.8)	17 (45.9)	0.5 p=0.5*
	100-130	1 (7.7)	9 (24.4)	
	131-160	3 (23.1)	5 (13.5)	
	>160	2 (15.4)	6 (16.2)	
HDL cholesterol	<40	6 (46.1)	20 (54.1)	0.24 p=0.6
	≥40	7 (53.8)	17 (45.9)	
VLDL cholesterol	≤30	6 (46.1)	22 (59.5)	0.7 p=0.4
	>30	7 (53.8)	15 (40.5)	

*Note. * – Fisher's Exact Test.*

Table 3. Comparison of lipid profile between types of stroke

Lipid Component	Mean Value in Ischemic Stroke	Mean Value in Hemorrhagic Stroke	P Value
TC	171.5	214.7	0.001
Serum TG	138.2	163.7	0.018
HDL	43.4	45.8	0.822
LDL	100.5	136.1	0.004
VLDL	27.6	32.7	0.174

stroke were high (10%) (total cholesterol >240 mg% according to the Adult Treatment Panel (ATP) – the 3rd guidelines of National Cholesterol Education Program (NCEP)).

Similar studies by Sreedhar K et al. [5] Benfante et al. [6], Di Mascio et al. [7] showed both ischemic and hemorrhagic stroke were associated with increased cholesterol levels.

Contrary to our study, Iso et al. [8] emphasized an inverse association between serum cholesterol level and hemorrhagic stroke. There was no correlation between serum cholesterol and risk of stroke in a study by Harmsen et al. [9] Rastenyte et al. [10] and Hart CL et al. [11]

Association of Triglycerides to the Non-Diabetics with Stroke

It was established that serum triglycerides were high in 23.1% of hemorrhagic stroke patients and 13.5% of ischemic stroke patients (>200 mg% according to ATP – 3rd guidelines). Sreedhar K et al. [5] in his study showed 80% of non-diabetic stroke patients with S.triglyceride >200mg/dl had ischemic stroke and the other 20% had hemorrhagic stroke. Tilvis R.S et al. [12] in his study showed serum triglyceride was higher in ischemic stroke. Farid et al. [13] also had similar results in his study in 1972. Hachinski et al. [14] showed a positive association of triglycerides in patients with atherothrombotic stroke and transient ischemic attacks compare to the control subjects. Albucher J.K et al. [15] 2000 showed serum triglycerides in normal range in the study on stroke.

Association of Serum HDL Cholesterol to the Non-Diabetics with Stroke

In the study of HDL cholesterol in stroke patients, it was found out that the ischemic group (54.1%) patients had greater abnormal levels (<40mg% according to ATP – 3rd guidelines) than the haemorrhagic group (46.1%). Simons et al. [16] study revealed that HDL cholesterol had protective effect on ischemic stroke. Alok Mohankar et al. [17] in 1993 showed that increased LDL levels and low HDL levels were associated with atherosclerosis. Albucher et al. [15] study clearly indicated HDL – cholesterol as the only lipid associated with stroke risk. It emphasised the need for management of low HDL cholesterol in young patients regardless of atherosclerosis.

Association of Serum LDL Cholesterol to the Non-Diabetics with Stroke

Among ischemic stroke cases, 16.2% had high LDL cholesterol and this was 15.4% among haemorrhagic stroke cases. Sreedhar K et al. [5] showed that high levels of serum LDL cholesterol had significant risk of ischemic stroke in the non-diabetics. Botet et al. [18] and Hachinski et al. [14] showed positive correlation between LDL cholesterol levels and risk of stroke. Kurth T et al. [19] 2007 showed remarkable increase in serum LDL levels in ischemic stroke patients.

Association of Serum VLDL Cholesterol to the Non-Diabetics with Stroke

In our study, among ischemic stroke cases 40.5% had increased VLDL cholesterol and this was 53.8% among haemorrhagic stroke cases. Bidyadhar et al. [20] 1984 showed that VLDL was increased in their study on stroke. Sreedhar K et al. [5] in the study showed that high VLDL was not associated with risk of stroke in non-diabetic patients.

Conclusions

According to our study the conclusion can be drawn that the most common type of lipid abnormality were abnormal triglycerides, abnormal VLDL, abnormal LDL. So, these parameters should be considered while predicting the risk of stroke in a dyslipidemic patient. Stroke patients with dyslipidemia need a comprehensive health care approach involving dietician, physician and good bio chemistry back up. In Indian scenario, where majority of the patients belong to the low socio-economic status, life style modification plays a more important role in prevention and management of stroke and dyslipidemia in contrast to high cost of lipid lowering agents.

Conflict of Interests

Authors declare no conflict of interest.

Author's Contributions

Dr. Vijeth S.B., Dr. Vijayalaxmi Mangasuli, Dr. Amrutha A.M., Dr. Bhoovanchandra N., Dr. Bhagyalaxmi Sidenur – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Dr. Vijeth S.B., Dr. Vijayalaxmi Mangasuli, Dr. Amrutha A.M.* – investigation, formal analysis.

ПОПЕРЕЧНЕ ДОСЛІДЖЕННЯ ПОКАЗНИКІВ ЛІПІДНОГО ПРОФІЛЮ У ПАЦІЄНТІВ-НЕДІАБЕТИКІВ, КОТРІ ПЕРЕНЕСЛИ ІНСУЛЬТ, МІСТА ЧИТРАДУРГА

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Вступ. *Зв'язок між рівнем сироваткових ліпідів, ліпопротеїнів та частотою розвитку порушень мозкового кровообігу не є достатньо доведеним. Оскільки рівень атерогенності діабетиків та недіабетиків є різним, тому в дослідження були включені пацієнти без діабету.*

Мета. *Вивчення порушень ліпідного обміну у пацієнтів з недіабетичним інсультом у місцевій лікарні.*

Методи. *Дослідження проводилося у General Medicine Department, BMCH, Chitradurga протягом періоду з червня 2020 року по грудень 2020 року. Вивчався ліпідний профіль та показники глікемії натще у 50 пацієнтів з інсультом без діабету. Визначалися вміст глюкози крові натще (FBG), загального холестерину (TC), тригліцеридів (TG), ліпопротеїдів низької щільності (LDL) та ліпопротеїдів високої щільності (HDL) за допомогою стандартних біохімічних методів.*

Результати. *Віковий розподіл пацієнтів складав від 19 до 72 років із середнім віком 54,8±15,75 років. Чоловіки становили 62% (31), жінки – 38% (19). Серед досліджуваних 58% мали гіпертонічну хворобу, 76% були курцями, 32% – алкоголіки, а 34% мали сімейний анамнез із порушенням мозкового кровообігу. Серед групи ішемічного інсульту найчастіше спостерігалося зниження ліпопротеїдів високої щільності (54,1%), другим найпоширенішим відхиленням було збільшення ліпопротеїдів дуже низької щільності – у 40,5%. Серед групи з геморагічним інсультом найчастіше спостерігалося також зниження ліпопротеїдів високої щільності, яке виявлялося у 46,1% пацієнтів, другим найпоширенішим відхиленням було збільшення загального холестерину, яке спостерігалося у 53,8% пацієнтів.*

Висновки. *Під час прогнозування ризику інсульту слід враховувати ліпідний профіль.*

КЛЮЧОВІ СЛОВА: ліпідний профіль; дисліпідемія; недіабетичний інсульт; сироватковий холестерин.

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ADULT-ONSET CYSTIC HYGROMA IN AXILLA: A RARE CASE REPORT FROM INDIA AND LITERATURE REVIEW (case report)

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Background. *Cystic hygroma (CH), occurs in 1/6000 live births and in 90% of cases develops in age less than 2 years old. They are mainly located in cervicofacial region. Adult-onset CH is very rare.*

Objective. *The aim of this study is to review literature to discuss the clinical presentation, diagnosis, and treatment of CH in adults through a case report of unilocular CH in the axillary region in an adult male from India.*

Methods. *A first case report of unilocular CH in axillary region in an adult male from India is being investigated.*

Results. *Here we report a case of unilocular CH in the axillary region in a 49-year-old male with a 14x16x8 cm cystic swelling in left axilla with a history of aspiration failure. Contrast-enhanced MRI (CEMRI) showed well-defined thin walled, unilocular cystic lesion which appeared hyperintense on T2 & STIR and hypointense on T1W1 and showed thin peripheral rim of enhancement on post contrast images. The patient underwent surgical excision and the diagnosis of a pathological CH was established. His postoperative recovery was uneventful and had no evidence of recurrence.*

Conclusion. *Due to rarity of adult-onset unilocular CH in axilla, its evaluation for prompt diagnosis and definitive treatment to prevent recurrence and complications is urgent. Furthermore, this is the first reported case from India which has been successfully managed at a peripheral hospital in Northeast-India and our report of this case contributes to the evidences supporting the role of CH in a differential diagnosis for masses in the adult axilla, especially in acute phase with no predisposing factors.*

KEYWORDS: cystic hygroma; cystic lymphangioma; adult-onset; axilla.

Introduction

Cystic hygroma (CH), also known as cystic lymphangioma, first described by Redenbacker in 1828, is a congenital malformation of the lymphatic system, occurring either due to sequestration or obstruction of developing lymph vessels [1–3]. The incidence of CH is 1/6000 live births [4]. 90% of CH develops in the age of less than 2 years and accounts for one-tenth of all paediatric soft tissue tumours [2]. The five main locations are cervicofacial, axillary, inguinal, retroperitoneal, and thoracic regions; orbit, shoulder, breast, mediastinum, pancreas, liver, ovary and fallopian tubes are other uncommon sites [1, 3].

Adult-onset CH is very rare; only less than 150 cases have been reported [4]. Its occurrence in axilla is extremely rare [4–7]. At the Indian subcontinent, only three case reports are available in the literature [8–11]. To the best of our knowledge, this is the first reported case of unilocular CH in the axillary region in an adult male from India. In view of extremely rarity of the disease in adults we review the literature to discuss clinical presentation, diagnosis, and treatment of CH.

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Case Report

A 49-year-old male, with no known co-morbidities, noticed a spontaneous onset of palpable mass in the left axillary area in Jan 2021 and had initially reported to a medical centre. The lesion was soft, movable, non-tender with smooth skin surface at onset. Initial ultra-sonography (USG) of left axilla revealed a 64×42×46 mm unilocular cystic lesion with no calcifications within. Fine needle aspiration cytology (FNAC) proved a benign cystic lesion with no evidence of malignancy. The patient underwent USG guided percutaneous aspiration on Jan 16, 2021 and straw-coloured fluid was aspirated. However, the mass recurred in one week after aspiration. It kept growing slowly

and became tense in four months. On examination at our centre, there was a 14×16×8 cm cystic swelling in left axilla, extending to lateral chest wall, not attached to underlying muscles or overlying skin as shown in Fig. 1. The swelling was non-compressible and non-pulsatile. There were no other palpable axillary masses grossly. The patient denied any history of trauma at the affected area, any recent infections and there was no loss of weight or appetite.

USG axilla at our centre found an 8×10.5×11 cm unilocular cystic lesion in left axilla. No calcification/internal vascularity or breach in deep fascia was noted. The contrast-enhanced MRI (CEMRI) of left axillary region showed well-defined thin walled, unilocular cystic lesion, 10.5×9.5×11.5 cm in size extending inferiorly along the lateral chest wall. It was hyperintense on T2 & STIR and hypointense on T1W1 as shown in Fig. 2. The lesion showed thin peripheral rim of enhancement on post contrast images. Neither invasion of underlying muscles or lateral chest wall nor locoregional lymphadenopathy was seen.

In view of failure of image-guided percutaneous aspiration at the previous centre, decision of surgical excision was taken. Tumour

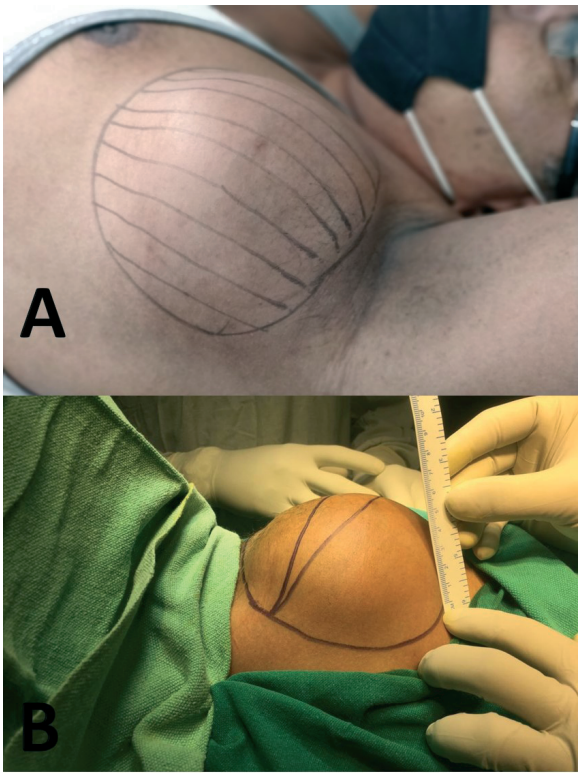

Fig. 1 *(A, B). Preoperative image showing 14×16×8 cm cystic swelling in left axilla, extending to lateral chest wall, not attached to underlying muscles or overlying skin.*

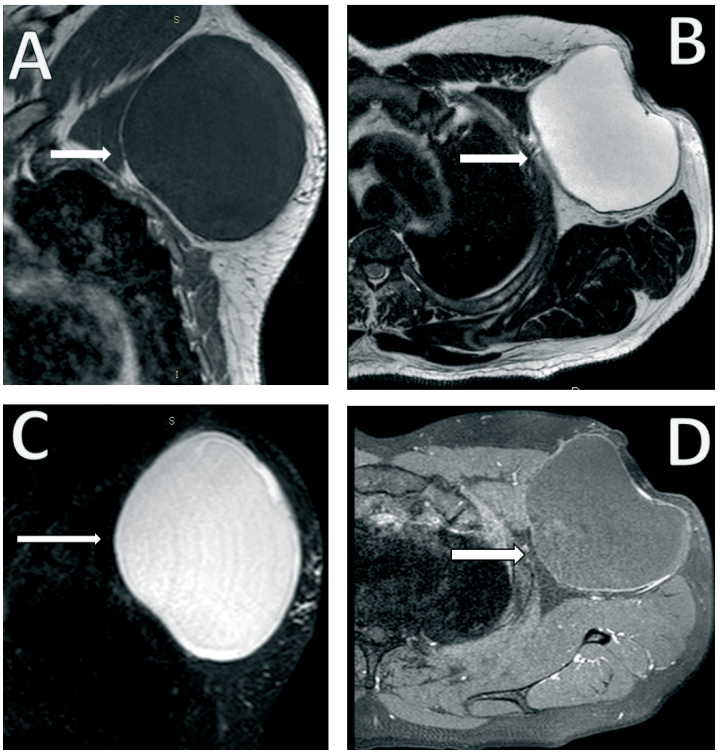

Fig. 2. *(A) T1WI sagittal section showing a large, well-defined, thin walled, unilocular cystic lesion, size 10.5×9.5×11.5 cm (AP×TR×CC) was evidenced in the left axillary region extending inferiorly along the lateral chest wall. The lesion was hypointense on T1WI. (B) T2WI axial section, showing hyperintense lesion with no internal septae/calcifications/mural nodules. (C) STIR coronal section image showing lesion as hyperintense. (D) Post contrast axial image of lesion with thin peripheral rim of enhancement.*

excision was performed under general anaesthesia. Intraoperative findings were 15×15 cm cystic swelling in left axilla extending from pectoralis major to latissimus dorsi anteroposteriorly and from axillary vein to angular vein craniocaudally. The intraoperative image and excised specimen are shown in Fig. 3 and 4 respectively. Fluid filled cyst with intact cyst wall was completely excised. The wound was primarily closed over suction drain. The drain was removed on postoperative day five. The patient was discharged after suture removal on postoperative day eight and was under regular follow-up via video-consultation due to the imminent threat of COVID-19 pandemic.

The tumour grossly appeared brownish and elastic, and the pathological diagnosis was CH. Under microscopy, the specimen was composed of thin-walled lymphatic spaces lined by flat epithelium with a collagenous background and accompanying lymphocytic infiltrates in the surrounding fibroadiposal stroma as shown in Fig. 5.

Discussion

CH is a benign and painless malformation of lymphatic system either due to sequestration or proliferation of lymphatics. It comprises 6% of all paediatric soft tissue tumours and usually presents in infants prior to the age of 1 year, and about 90% of the lesions occur in children younger than 2 years [12]. The most common sites of occurrence are the posterior triangle of the neck (75%), axilla (20%), and mediastinum. However, it may also involve groin, retroperitoneal space and pelvis [3].

CH is rare in adult and very few cases have been reported in literature [4, 5, 7, 10–21]. Development of CH in adulthood is caused by delayed proliferation of cell rests with infection,

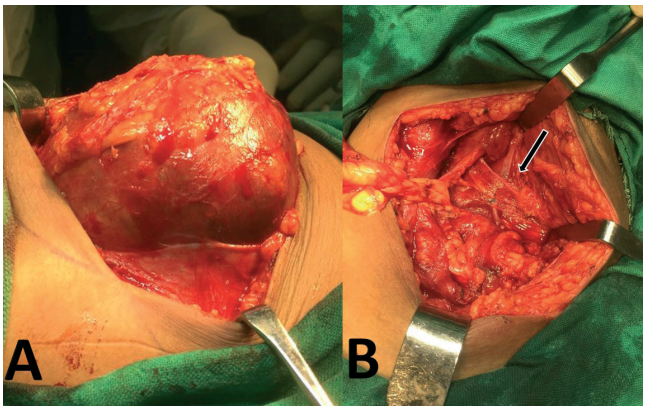

Fig. 3. *(A) – Intraoperative image showing well encapsulated unilocular macrocystic lesion. (B) – Intraoperative image, surgical bed with completely excised lesion and arrow pointing at the neurovascular pedicle lying in close proximity.*

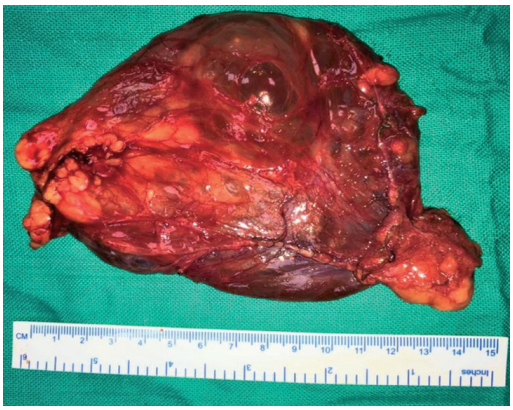

Fig. 4. *Gross image of the excised specimen showing a transparent well circumscribed unilocular macrocystic lesion with straw-coloured fluid within it.*

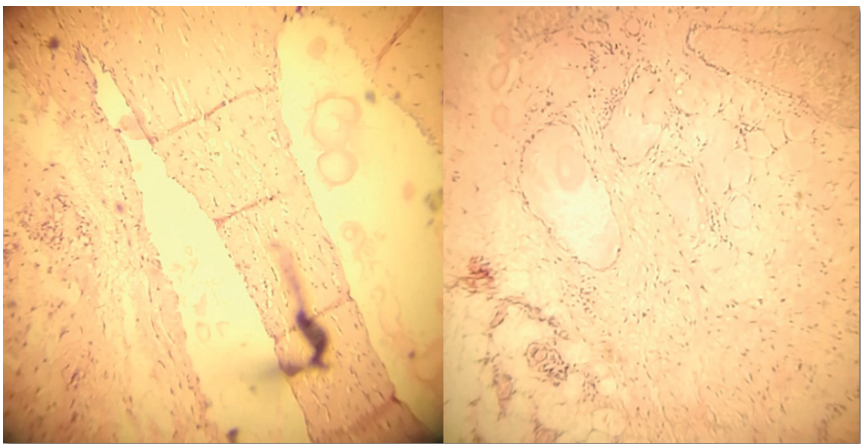

Fig. 5. *Microscopic image, 40× magnification, in hematoxylin and eosin (H&E) stain, showing components of thin-walled varying sized dilated lymphatic spaces lined by flattened epithelium with chronic inflammatory cells in fibrous stroma, compatible with cystic hygroma.*

trauma, or iatrogenic stimuli as being the predisposing factors. Michail et al. reported a cystic hygroma in a female patient which developed rapidly in the axillary region in the absence of any predisposing factors [7].

Axillary CH in adults is usually asymptomatic. On physical examination, it appears as painless, mobile cystic swelling with well-defined margins, is fluctuant and compressible. The clinical course is usually progressive. Rapid progression is due to lymph accumulation or on-going haemorrhage inside that may lead to rupture of the cyst. Pain and erythema over the swelling indicates cellulitis or lymphangitis which may progress to sepsis. Most common organisms responsible for infection in CH are staphylococcus and streptococcus.

Differential diagnosis of CH includes lymphadenopathy, abscess, hematoma and soft tissue sarcoma. Diagnosis can be made with adequate history and its characteristic appearance on clinical examination. USG, computed tomography and MRI are all helpful imaging tools to confirm the diagnosis [5, 6]. MRI is superior to computed tomography as it offers detailed information about the soft tissue mass and it also provides appropriate preoperative staging and exact anatomical delineation for surgical planning. In the described case, lesion appeared hyperintense on T2 & STIR and hypointense on T1W1 as shown in Fig. 2. The lesion showed thin peripheral rim of enhancement on post contrast images. Role of Fine needle aspiration cytology (FNAC) in diagnostic workup of CH is criticised due to risk of infection and bleeding associated with the procedure. Walter Colangeli et al reported in their study that FNAC had a low diagnostic value and it did not modify the treatment approach [14, 22]. In the described case, FNAC was performed as initial workup before the patient reported at our centre and hence it was not repeated due to its limited yield in diagnosis. Grossly CH may appear as unilocular or multilocular cystic lesions with varying size and is classified as microcystic (size of the cyst less than two cm), macrocystic (size of the cyst more than two cm) and mixed variety [14]. Microscopically CH on H&E staining appears as varying sized dilated lymphatic channels with chronic inflammatory cells in fibrous stroma [3, 4, 23]. In our case, unilocular macrocystic CH was present in axilla and diagnosis was confirmed on final histopathology.

Indications of treatment for CH are cosmesis, any associated complications like lymph leakage, repeated inflammation and pressure effect due to cyst. Treatment options for adult-onset CH range from minimally invasive procedures to surgical excision [24]. Minimally invasive modalities include image guided aspiration and intralesional sclerotherapy (using bleomycin, OK-432, doxycycline, acetic acid, alcohol, hypertonic saline) and serial laser therapy [25–26]. Sandeep K Rahul et al have advocated successful use of bleomycin sclerotherapy for a series of CH at unusual sites [27]. Although aspiration followed by sclerotherapy is the preferred modality in infants, however few studies have also shown its inefficacy [15, 28, 29]. Copley at al reported a case of CH in the axilla, which was managed by ultrasound guided aspiration but it was unsuccessful due to recurrence, which was then managed successfully by total excision [30]. Failure and complications of sclerotherapy due to immune response to OK-432 in multiloculated, microcystic and mixed variety of CH have also been frequently reported [31].

Minimally invasive procedures might have been the standard of care for infantile CH but surgical excision is still the mainstay of treatment for adult onset CH as the lesion is well circumscribed [16, 21, 23]. Few centres have advocated a combined method of conservative excision with deroofing of a cyst plus intralesional sclerotherapy, in cases where complete excision is not possible and damage to vital structures are predicted [16]. Risk of rupture of cyst per operatively, inadequate partial excision due to its proximity to vital structures and recurrence are the drawbacks with surgical therapy [32]. In the described case, decision for surgical excision was taken in view of failure of image-guided aspiration, well encapsulated unilocular macrocystic uncomplicated adult-onset CH in axilla with minimal neurovascular sacrifice.

Long-term follow up is suggested as very few cases of adult onset CH is available in literature [17]. Güner et al [5] managed a case of axillary cystic hygroma in an 83-year-old male and did not find any recurrence during five months' follow-up period. McCaffrey et al. [4] reported a case of cystic hygroma in a 58-year-old male in right upper flank extending up to axilla and there was no recurrence on follow-up in one year after surgery. The patient in our described case is regularly being followed up via video-consultation and has had no recurrence or complications till now.

Conclusion

Due to rarity of adult-onset unilocular CH occurring in axilla, its evaluation for prompt diagnosis and definitive treatment to prevent recurrence and complications is a topical issue. Furthermore, this becomes the first reported case from India which has been successfully managed at a peripheral hospital in Northeast-India and our reporting of this case contributes to the evidences supporting the role of CH in a differential diagnosis for masses in the adult axilla, especially in acute stage with no predisposing factors. CH presents as a soft, painless, and movable cystic mass. Contrast enhanced MRI is the imaging modality of choice as it helps in confirming the diagnosis as well as preoperative planning. Although a gamut of treatment options is available for treating this benign condition, surgical excision is still the procedure of choice for adult-onset CH. Furthermore, management of adult-onset CH in the axilla with proximity to important neurovascular structures is contributed by our description of this rare case.

Conflict of Interests

Authors declare no conflict of interest.

Authors' Contributions

Rahul Pandey, Ranjan Kumar – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Sandip Maheshwari, Thongam Sachin Singh* – data curation, writing – reviewing and editing; *Sandeep Bhalla, Inam Danish Khan* – investigation, formal analysis.

КІСТОЗНА ГІГРОМА ПАХВИ У ДОРОСЛИХ: РІДКІСНИЙ ВИПАДОК З ІНДІЇ ТА ОГЛЯД ЛІТЕРАТУРИ (клінічний випадок)

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Вступ. *Кістозна гігрома (КГ) зустрічається у 1/6000 новонароджених і в 90% випадків розвивається у віці до 2 років. Зазвичай КГ локалізується в шийно-лицевій діянці. КГ у дорослих зустрічається надзвичайно рідко.*

Мета. *Завданням цього дослідження є огляд літератури та обговорення клінічної картини, діагностики та лікування КГ у дорослих з описом випадку однокамерної КГ пахви у дорослого чоловіка з Індії.*

Методи. *Повідомляється про перший випадок однокамерної КГ пахви у дорослого чоловіка з Індії.*

Результати. *Описаний випадок однокамерної КГ в пахвовій ділянці у 49-річного чоловіка з кістозною пухлиною 14×16×8 см в лівій пахвовій западині з невдалою аспірацією в анамнезі. МРТ з контрастом (CEMRI) продемонструвала чітко виражений тонкостінний однокамерний кістозний утвір, який виявився гіперінтенсивним на T2 та STIR і гіпоінтенсивним на T1W1 та мав тонкий підсилений периферичний обідок на постконтрастних зображеннях. Пацієнту проведено хірургічне висічення утвору та встановлено післяопераційний діагноз КГ. Післяопераційний період в пацієнта проходив без ознак рецидиву.*

Висновок. *Однокамерна КГ пахви є рідкісним явищем, тому її правильна діагностика та вибір оптимальної тактики лікування для запобігання рецидивам і ускладненням має першочергове значення. Крім того, даний випадок є першим зареєстрованим в Індії, успішне лікування було проведене в умовах периферичної лікарні на північному сході Індії. Даний випадок і повідомлення про нього підтверджують роль КГ у диференціальній діагностиці новоутворів пахви у дорослих, особливо тих, що виникають гостро і без передумов.*

КЛЮЧОВІ СЛОВА: кістозна гігрома; кістозна лімфангіома; захворювання дорослих; пахва.

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INFLUENCE OF THICK EXTRACT FROM MAITAKE MUSHROOMS ON SIGNS OF INFLAMMATORY PROCESS IN EXPERIMENTAL TOXIC HEPATITIS

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Background. *The priority of the contemporary pharmaceutical industry is to create effective, safe and inexpensive drugs to ensure the highest quality of care and optimal use of available raw materials.*

Objective. *The aim of our study was to investigate anti-inflammatory properties of the Maitake mushrooms thick extract in the experiment on rats with paracetamol(acetaminophen)-induced hepatitis.*

Methods. *60 white male rats, weighing 180-210 g, randomized into 10 groups of 6 animals in each, were used for the experiment. Paracetamol hepatitis was simulated by acetaminophen intragastric administering in a dose of 1250 mg/kg 1 time per day (for 2 days) as a suspension in 2% starch gel solution. Maitake mushrooms thick extract, which was administered intragastrically 2 hours before the administration of acetaminophen and daily after the lesion in a dose of 150 mg/kg of the animal's body weight, was used for the toxic lesion correction. "Silibor" was selected as the comparison drug, which was administered according to the same scheme as the investigated extract in a dose of 20 mg/kg of the animal's body weight. Euthanasia was conducted on the 3rd, 7th and 10th day of the experiment with sodium barbamyl. Liver homogenate and animal serum were used for the studies. The development of inflammatory processes was studied by the content of pro-inflammatory and anti-inflammatory cytokines, as well as C-reactive protein in the serum of rats with toxic hepatitis and after the application of Maitake mushroom extract and the comparison drug.*

Results. *It was found that the introduction of acetaminophen to animals for the acute hepatitis simulation is accompanied by changes in the cytokine profile, i.e. an increase in the level of IL-6 and a decrease in the level of IL-4 in the serum of rats. Inflammatory development is evidenced by the content of C-reactive protein increase in the blood of the affected animals. The application of Maitake mushroom extract facilitated bringing the studied indicators almost to the level of intact control.*

Conclusions. *Reduction of inflammation signs in rats with the simulated paracetamol hepatitis under the influence of Maitake mushrooms thick extract confirms its anti-inflammatory properties.*

KEYWORDS: maitake mushrooms, paracetamol, acute hepatitis, inflammation.

Introduction

Due to the serious consequences of hepatitis, there is a need for its earliest diagnosis and appropriate pharmacotherapy. The search for effective hepatoprotectors, which can influence the initial stages of initiation and development of inflammatory processes in the liver without side effects, is an important task of contemporary medicine [1, 2]

Maitake mushroom has properties for which the Japanese have valued it for centuries: the ability to lose weight, to reduce discomfort and problems associated with menopause in women, to gently eliminate the unpleasant sensations of premenstrual syndrome, to lower blood sugar level, to reduce the effects of inflammation, to increase immunity due to B polysaccharides, available in the composition of the fungus [3, 4, 5, 6, 7, 8, 9].

The aim of our study was to investigate the anti-inflammatory properties of the Maitake mushrooms thick extract in the experiment on rats with acute hepatitis induced by paracetamol (acetaminophen).

Methods

The material for the experimental work was a thick extract of Maitake mushrooms (TEOMM), obtained by scientists from the Department of Chemistry of Natural Compounds of the National University of Pharmacy.

The experiments were performed on white outbred male rats, weighing 180-210 g, kept on the standard diet of the vivarium of I. Horbachevsky Ternopil National Medical University. All studies were conducted in compliance with the rules of bioethics in accordance with the

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"European Convention for the protection of vertebrate animals, which are used for experimental and other scientific purposes" [10].

Intoxication with acetaminophen, which was administered intragastrically in a dose of 1250 mg/kg 1 time per day for 2 days as a suspension in 2% starch gel solution, was a model of toxic lesions in rats [11, 12]. TEOMM was administered intragastrically 2 hours before administration of the toxic agent and daily after the lesion in a dose of 150 mg/kg of the animal's body weight, which, in our previous studies, was found to be conditionally therapeutic for this extract [5]. "Silibor" (the active basis is silymarin) was chosen as the comparison drug. It was administered according to the same scheme as the investigated extract in a dose of 20 mg/kg (in terms of silymarin) of the animal's body weight. The value of the dose of the comparison drug was chosen according to the instructions for its application and using the coefficients of species sensitivity by Rybolovlev Yu. R. and his method of converting the dose for humans to the dose for rats [12, 13]. The animals were randomized into 10 groups, 6 rats each: the 1st group – the animals of intact control; the 2nd, 3rd and 4th – the animals affected by acetaminophen on the 3rd, 7th and 10th days of the study, respectively; the 5th, 6th and 7th groups – the rats, which were injected with an extract of Maitake mushrooms in a dose of 150 mg/kg of body weight on the 3rd, 7th and 10th days of the experiment, respectively; the 8th, 9th and 10th groups of animals that were intragastrally administered with the drug silibor on the 3rd, 7th and 10th days of the study, respectively. The rats were removed from the experiment by euthanasia under sodium barbamyl anesthesia. Euthanasia was conducted on the 3rd, 7th and 10th days of the experiment. The study was subjected to liver homogenate and animal serum. Blood was taken from the heart of the animals.

The concentration of pro-inflammatory and anti-inflammatory interleukins in the serum of rats was determined by enzyme-linked immunosorbent assay using commercial kits (GEHealthcare: Amersham, UK) [14]. The content of C-reactive protein (CRP) was determined by immunoturbidimetric method [15, 16].

The obtained data were statistically processed by the program STATISTICA 12. The significance of intergroup differences was determined using the criterion of rank sums of the Wilcoxon and the Mann-Whitney tests. p values lower than 0.05 were considered to be statistically significant [17, 18].

Results

C-reactive protein is a non-glycosylated protein with a pentameric structure, which belongs to β-globulins. Due to its affinity to phosphorylcholine, which is a component of the cell walls of some bacteria and unicellular fungi, CRP is able to bind the relevant microbial cells and opsonize them for phagocytosis or lysis with complement [16, 19]. CRP acts as a pro-inflammatory "trigger" that stimulates monocytic synthesis of cytokines such as tumour necrosis factor-α, interleukin-1 and interleukin-6. Thus, CRP performs an immunoregulatory function: it stimulates protective reactions and activates immunity [14].

CRP is determined in almost all pathological processes and diseases regarding tissue damage. The increased levels of C-reactive protein are one of the earliest laboratory signs of inflammation or tissue damage. Increased protein production by the liver begins 6 hours after the onset of inflammation [20].

We found out that in rats with acute hepatitis induced by acetaminophen, the serum CRP increased in 1.9, 2.4 and 2.7 times on the 3rd, 7th and 10th days of the study, respectively, according to the group of intact control. After correction with TEOMM there was a probable decrease in the content of CRP in the serum of rats in 1.2, 1.5 and 2.1 times on the 3rd, 7th and 10th days of the experiment, respectively, in the animals of control pathology. When using silibor, the studied indicator probably ($p \leq 0.05$) decreased in 1.4 and 1.9 times on the 7th and 10th days of the experiment, respectively, for the animals with toxic hepatitis (Tab. 1).

The obtained results are a confirmation of the inflammatory processes development in the body of rats with acute hepatitis, which is caused by the action of acetaminophen.

Cytokines, a group of hormone-like proteins, peptides and mediators of inflammation, play a significant role in the pathogenesis of development and course of inflammatory processes. Imbalance in the cytokine system can have a significant effect on the course of inflammatory reaction [21].

Hepatocytes are very sensitive to the action of cytokines, as they contain a number of specific receptors on their surface, through which the regulation of protein synthesis, proliferation, differentiation, specialized functioning and apoptosis of liver cells is carried out. Pro-

Table 1. The content of C-reactive protein in the serum (mg/l) of the rats affected by acetaminophen, and after application of the Maitake mushrooms extract (M±m; n=60)

Groups of animals	Indicators		
	3rd day	7th day	10th day
IC	3.51±0.28	3.51±0.28	3.51±0.28
CP	6.83±0.23*	8.47±0.24*	9.54±0.32*
CP+silibor	6.10±0.20	5.93±0.29**	5.08±0.23**
CP+TEOMM	5.71±0.18**	5.63±0.28**	4.49±0.3**

*Notes. Here and in the following tables * – probable changes between the rate of the control and paracetamol-affected animals, ** – probable changes between the rates of the paracetamol-affected and treated animals.*

inflammatory cytokines: IL-1, IL-6, IL-8, IL-12, TNF-, IFN-γ are involved in the specific immune response triggering, while anti-inflammatory cytokines: IL-4, IL-10, IL-13, TGF are involved in the development of reactions of the anti-inflammatory process and inhibit the synthesis of pro-inflammatory interleukins [14, 15, 20, 21].

Our further research was to study the content of pro-inflammatory and anti-inflammatory cytokines, IL-4 and IL-6, in the blood serum of rats with simulated acetaminophen hepatitis, as well as to study the corrective effect of TEOMM and silibor on them.

Numerous studies confirm that violation of normal proportions of pro-inflammatory and anti-inflammatory cytokines synthesis can lead to disruption of regulation and development of vital immune reactions and, above all, inflammatory reactions. In the case of violations of local protective reactions, inflammation spreads, cytokine synthesis increases, then they enter the bloodstream and have their effect on the systemic level, i.e. have their effect on almost all organs and systems of the body [19, 20, 22].

It was experimentally established that during the formation of acute hepatitis in rats on the 3rd day of the study, the content of IL-6 increased by 82% compare to the intact animals. On the 7th and 10th days of the experiment, the content of the studied indicator increased by 126% and 150%, respectively, compare to the intact control animals (Tab. 2).

TEOMM application for correction the detected disorders in toxic hepatitis led to a probable decrease in the content of IL-6 on the 7th day of the study compare to control. The comparison drug Silibor had a positive effect on this indicator, although slightly inferior to our studied extract.

Local inflammatory process, after introduction of a pro-inflammatory agent, is important for healing and protection of the body from infection. However, excessive accumulation of pro-inflammatory cytokines in the blood leads to generalized sepsis and multiorgan failure. It is anti-inflammatory interleukins, which include IL-4 and IL-10, are able to reduce inflammation and cause a cessation of the inflammatory response [14, 15].

Hence, it was advisable to study the content of anti-inflammatory cytokines, in particular IL-4, in the serum of rats with toxic lesion.

After affection of animals with acetaminophen in the serum, the IL-4 content decrease in 1.4, 1.5 and 1.6 times was observed on the 3rd, 7th and 10th days of the experiment, respectively, compare to the group of intact control animals (Tab. 3).

TEOMM caused a probable increase in the IL-4 content by 30% and 46% on the 7th and 10th days of the study, respectively, in the animals with toxic hepatitis. When using the comparison drug, a probable ($p \leq 0.05$) increase in the cytokine content on the 7th day in 1.3 times and in 1.4 times on the 10th day of the study compare to the control pathology group was noted.

The obtained results suggest that the use of TEOMM in rats at a dose of 150 mg/kg for 10

Table 2. The content of IL-6 in the serum (pg/l) of the rats affected by paracetamol, and after application of the Maitake mushrooms extract (M±m; n=60)

Groups of animals	Indicators		
	3rd day	7th day	10th day
IC	2.96±0.22	2.96±0.22	2.96±0.22
CP	5.38±0.30*	6.69±0.34*	7.39±0.27*
CP+silibor	4.93±0.21	4.51±0.26**	4.35±0.29**
CP+TEOMM	4.85±0.16	4.22±0.22**	3.98±0.26**

Table 3. The content of IL-4 in the serum (pg/l) of the rats affected by paracetamol, and after application of the Maitake mushrooms extract (M±m; n=60)

Groups of animals	Indicators		
	3rd day	7th day	10th day
IC	1.57±0.08	1.57±0.08	1.57±0.08
CP	1.14±0.05*	1.03±0.07*	0.96±0.09*
CP+silibor	1.22±0.07	1.29±0.08**	1.34±0.06**
CP+TEOMM	1.29±0.05	1.34±0.06**	1.40±0.08**

days has a positive effect on the initial stages of the inflammatory reaction.

Discussion

The inflammatory process caused by immune mechanisms is significant in the pathogenesis of diseases. CRP is a marker of systemic inflammation. Therefore, the experimentally revealed increase in its level in the serum of animals with toxic lesion indicates an inflammatory process in the body. CRP is one of the activators of the complement system – a compound of complex proteins involved in the formation of the body's immune response [16].

Cytokines are a major factor in the interaction between immune cells and somatic cells. Determination of their concentration in the blood provides information about the functional activity of different types of immunocompetent cells, severity of the inflammatory process, its transition to the systemic level, prognosis of the disease. Excessive production of cytokines and other mediators of inflammation disrupts regulatory function of the immune system, their uncontrolled release takes place, as well as the imbalance between pro-inflammatory and anti-inflammatory cytokines with a predominance of pro-inflammatory. As a result, the mediators of inflammation from the factors that protect the body become damaging for it [15].

The study of the content of the pro-inflammatory cytokine IL-6 showed a probable increase throughout the experiment. At the same time, the serum content of the anti-inflammatory cytokine IL-4 decreased. This indicates an imbalance of pro-inflammatory and anti-inflammatory cytokines caused by toxic liver damage by acetaminophen.

We established an anti-inflammatory effect of the studied extract, which consists in changing the cytokine profile, in particular in reducing the dynamics of the pro-inflammatory IL-6 content and increase in the anti-inflammatory cytokine IL-4 content compare to the group of intact control rats.

Conclusions

It was established that in cases of acute hepatitis in the rats induced by acetaminophen, the concentration of CRP increased in the blood serum, the content of pro-inflammatory IL-6 probably increased and the content of anti-inflammatory cytokine IL-4 decreased, which indicated the development of inflammatory processes in the affected animals.

The application of a Maitake mushrooms thick extract had a positive effect on the content of C-reactive protein and cytokines in the serum of the animals with acute hepatitis, which indicated its anti-inflammatory properties and the relevance of further study to creation of effective drugs.

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Conflict of interest

The authors declare no conflict of interests in this study.

Author Contributions

Herasymets I.I. – conceptualization, data curation, formal analysis, investigation, visualization, article writing; *Fira L.S.* – conceptualization, supervision, validation, methodology; *Medvid I.I.* – formal analysis, investigation, software.

ВПЛИВ ГУСТОГО ЕКСТРАКТУ З ГРИБІВ МАЙТАКЕ НА ОЗНАКИ ЗАПАЛЬНОГО ПРОЦЕСУ ЗА ЕКСПЕРИМЕНТАЛЬНОГО ТОКСИЧНОГО ГЕПАТИТУ

І.І. Герасимець, Л.С. Фіра, І.І. Медвідь
ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І.Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Пріоритетним завданням сучасної фармацевтичної галузі є створення ефективних, безпечних та доступних лікарських засобів, щоб забезпечити максимально високу якість надання медичної допомоги та оптимального використання наявних сировинних ресурсів.*

Мета роботи. *Метою нашого дослідження було вивчити протизапальні властивості густого екстракту з грибів майтаке в експерименті на щурах із гепатитом, індукованим парацетамолом (ацетамінофеном).*

Методи. *Для проведення експерименту було використано 60 білих щурів-самців, масою 180-210 г, рандомізованих на 10 груп по 6 тварин у кожній. Парацетамоловий гепатит моделювали шляхом введення ацетамінофену інтрагастрально у дозі 1250 мг/кг 1 раз на добу у вигляді суспензії в 2 % розчині крохмального гелю протягом 2 діб. Для корекції токсичного ураження використовували густий екстракт грибів майтаке, який вводили інтрагастрально за 2 години до введення ацетамінофену та щоденно після ураження в дозі 150 мг/кг маси тіла тварини. Як препарат порівняння обрали "Силібор" (виробник – ТОВ «Фармацевтична компанія «Здоров'я»), який вводили за тією ж схемою, що і екстракт майтаке в дозі 20 мг/кг маси тіла тварини. На 3-тю, 7-му та 10-ту добу експерименту здійснювали евтаназію щурів із використанням барбамілу натрію. Для досліджень брали гомогенат печінки та сироватку крові. Розвиток запальних процесів вивчали за вмістом про- та протизапальних цитокінів, а також С-реактивного протеїну у сироватці крові щурів із токсичним гепатитом та після застосування екстракту грибів майтаке та препарату порівняння.*

Результати. *Встановлено, що введення тваринам ацетамінофену для моделювання гострого гепатиту супроводжується змінами цитокінового профілю, а саме, зростанням рівня IL-6 та зменшенням рівня IL-4 у сироватці крові щурів. Про розвиток запального процесу свідчить підвищення вмісту С-реактивного протеїну в крові уражених тварин.Застосування екстракту грибів майтаке сприяло наближенню досліджуваних показників до рівня інтактного контролю.*

Висновки. *Зменшення ознак запального процесу у щурів при модельованому парацетамоловому гепатиті під впливом густого екстракту грибів майтаке підтверджує його протизапальні властивості.*

КЛЮЧОВІ СЛОВА: гриби майтаке, парацетамол, гострий гепатит, запалення.

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DISTRIBUTION AND QUANTITATIVE CHANGES OF MAST CELLS IN GUINEA PIGS LUNG IN OVALBUMIN-INDUCED ALLERGIC INFLAMMATION

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Background. *One of the most important cells in local immunity in lung are mast cells. They are involved in both innate and adaptive immune responses to inhaled allergens. The question of distribution of these both cell types in guinea pig lung in case of experimental allergic inflammation in most aspects remains open.*

Objective. *The aim of this research is to study the distribution and quantitative changes of mast cells in lung of guinea pigs in ovalbumin-induced allergic inflammation.*

Methods. *the lungs of 48 male guinea pigs have been studied using histological, morphometric and statistical methods in cases of experimental ovalbumin-induced allergic inflammation. The total number of mucosa related must cells and perivascular mast cells in guinea pig lungs were counted.*

Results. *It has been established that mucosa related mast cells are normally more abundant in guinea pigs lung than perivascular ones. Maximum increase in a number of mucosa related mast cells was revealed in the early period of allergic inflammation, as evidenced by maximum increase coefficient of 1.4 in the 1st experimental group, compare to the control ($P^{*/**}<0.05$). However, maximum increase in number of perivascular mast cells in 5 times was found during the late period of allergic inflammation in the 4th experimental group ($P^{*/**}<0.05$).*

Conclusion. *Experimental sensitization and challenge with ovalbumin leads to statistically significant increase in average number of both types of mast cells but predominantly the latter ones. It has been proved that cells dynamics is multidirectional.*

KEYWORDS: mucosa related must cell, perivascular mast cell, lung, guinea pig, allergic inflammation.

Introduction

Mast cells are present in vascularized tissues of almost all organs except the central nervous system and the retina. Derived from pluripotent red bone marrow stem cells, they differentiate in connective tissue from their progenitor cells under the influence of c-Kit ligand (CD117) in the presence of growth factors and various cytokines realized by the connective tissue microenvironment of the organs in which they are located and function [1, 2]. The cytoplasm of mature mast cells contains 50-200 granules of inflammatory mediators, such as histamine, heparin, numerous cytokines, chondroitin sulphate, and neutral proteases (chymase and tryptase), provided mechanisms to increase the permeability of the microvessel wall and perivascular connective tissue, angiogenesis [3]. They regulate the inflammatory process in the connective tissue, influencing the permeability of the vascular wall and the amorphous component of the intercellular substance. In addition, they are involved in implementation of allergic reactions due to the presence of FcεRI receptors on immunoglobulins type E on their plasmalemma. On the other hand, microvessel endothelial cells, secreting adhesion molecules VCAM-1, ICAM-1 and ELAM-1, initiate migration of mast cells precursors from the peripheral bloodstream into the tissue where the inflammatory process takes place [4].

It is established that in lung of human and BALB/c mice in normal physiological conditions the number of mast cell progenitors is insignificant, but in cases of antigen-induced inflammation under the influence of α4β7 integrins, VCAM-1 and CXCR2 they actively migrate to lung tissue [5]. Mast cells, located in different parts of the lungs and respiratory tract, have excellent histochemical properties and express different mediators. Two phenotypes of mast cells have been studied in lung of human and small mammals: mucosa related mast cells (synthesize only tryptase) and perivascular mast cells (synthesize tryptase, chymase, and carboxypep-

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tidase), with the predominance of the latter. Previous studies have postulated hyperplasia of both mast cell phenotypes in the human lung with development of allergic inflammatory process. In contrast to human, BALB/c mice during the allergic inflammatory process showed the increase in the number of mucosa related mast cells only, without affecting the dynamics and differentiation of resident perivascular mast cells [4, 5]. Very little is known about mast cells and their distribution in guinea pigs lung in case of experimental antigen sensitization, although the high sensitivity and susceptibility of their respiratory system to allergic and infectious diseases makes them a useful experimental model of pathological conditions such as bronchial asthma and tuberculosis [6].

Therefore, the aim of the research is to study distribution and quantitative changes of mast cells in lung of guinea pigs in ovalbumin-induced allergic inflammation.

Methods

The object of the experimental study was lung removed from 48 sexually mature male guinea pigs, kept in standard conditions of the vivarium of the Zaporizhzhya State Medical University. Experiments on animals were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council Directives 86/609 / EEC (1986), Laws of Ukraine № 3447-IV "On the protection of animals from cruel treatment, general ethical principles of animal experiments", approved by the First National Congress of Ukraine on Bioethics (2001). Induction of allergic airway inflammation was carried out by subcutaneous sensitization and subsequent aeroallergization with ovalbumin (OVA) [7]. On days 1, 7, 14 of the experiment, guinea pigs were sensitized: subcutaneous injection into the interscapular region of 0.5 mg OVA (Sigma Chemical Co., USA) together with an adjuvant – aluminium hydroxide, 10 mg (AlumVax Hydroxide vaccine adjuvant, OZ Biosciences, France) diluted in 1 ml of saline. From 21 to 28 days of the experiment, the animals were aeroallergized OVA at the dose of 10 mg/ml of saline for 15 min/day using an LD-211C compressor inhaler (Little Doctor International, Singapore) in an inhalation chamber. The animals were divided into 6 groups (8 animals in each group) for investigations. The first four groups were animals, sensitized and challenged OVA, withdrawn from the experiment, respectively, on the 23rd, 30th, 36th and 44th days after its start; the 5th group – control, the animals were injected subcutaneously with 1 ml of saline and inhaled with saline; the 6th – intact group. For the purpose of rational presentation of the obtained data and their interpretation, we conditionally distinguish the early (23rd, 30th days of the experiment) and late (36th and 44th days after the start of the experiment) periods of development of allergic inflammatory process in lung.

The animals were withdrawn from the experiment by overdose of thiopental anesthesia according to the established terms (on the 23rd, 30th, 36th and 44th days of the experiment). Pieces of lung were fixed in 10% neutral buffered formalin solution (pH 7.2-7.4). Histological sections were stained with alcyan blue with a critical concentration of MgCl2 0.2M to determine the dynamics of mast cell distribution and their morphometric features [8]. A complex of morphometric studies was carried out on a Carl Zeiss Primo Star microscope using the ZEISS ZEN 2011 software. The total number of mucosa related mast cells and perivascular mast cells per unit area of 5000 μm^2 was counted, using a microscope with oil immersion technique (×1000).

The research results were processed by current statistical methods of analysis on a personal computer using the standard software package Microsoft Office 2010 (Microsoft Excel) and STATISTICA® for Windows 6.0 (StatSoft Inc., USA, license 46 No. AXXR712D833214FAN5) based on the Windows 10 operating system. Hypothesis for normal distribution of the studied parameters was checked using the Shapiro-Wilk test and the Kolmogorov-Smirnov test of consistency. The arithmetic means (M) and standard errors of the mean (± m) were calculated. The statistical significance of intergroup differences according to the data obtained was established using the parametric Student"s t-test (p *) and the nonparametric Whitney-Mann U-test (p **). The obtained data was compared between the median and interquartile range Me (Q1; Q3). Differences between the compared values at the level of 95% ($p<0.05$) were considered statistically significant.

Results

The morphometric examination of mast cells in the intrapulmonary airways and lung parenchyma of intact guinea pigs has shown that the average number of mucosa related

mast cells is 2.62±0.05, perivascular mast cells 1.38±0.07 in the field of view. Normally, in guinea pigs lung, mucosa related mast cells are more abundant, than perivascular mast cells, as evidenced by the increase factor 1.6. We have analysed the number of mast cells in the connective tissue of guinea pigs lung and have found that there was no statistically significant difference between the animals in the intact and control groups that proves that the procedure itself does not affect changes in the number of must cells. Therefore, we compare the results of the experimental and control groups.

OVA – sensitization and challenge leads to quantitative changes in the dynamics of mast cells number of lung connective tissue. The increase in the number of mast cells of both phenotypes is determined in animals of the 1st experimental group from the 23rd day of observation in the early period of development of experimental allergic inflammation, but more significant OVA – challenge effects the number of perivascular mast cells. In addition, further dynamics of changes in their number is different in different experimental groups (Fig. 1).

The maximum average number of mucosa related mast cells, observed in the 1st experimental group, is statistically significantly higher in 1.4 times compare to the animals of the control group ($P^{*/**}<0.05$). Further investigation has shown that starting from the 30th day of the experiment, there is a tendency for their gradual recovery to the indicators of the control group, reaching the latter on the 44th day of observation.

The average number of perivascular mast cells in animals of the 1st experimental group is by 1.6 times higher compare to the number of mucosa related mast cells, and statistically significantly higher in 3.6 times compare to the control group ($P^{*/**}<0.05$). There is a tendency to increase in the number of perivascular mast cells with the maximum rate on the 44th day of observation, starting from the 36th day of the experiment (Fig. 2).

On the 30th day of observation the average number of mucosa related mast cells is 3.62±0.05 in the field of view, which is in 1.3 times more than the same indicator in the control group ($P^{*/**}<0.05$). However, the average number of perivascular mast cells in animals of the 2nd experimental group on the 30th day of observation is in 1.5 times higher than the number of mucosa related mast cells, and statistically significantly higher in 3.5 times compare to the same point in the control group ($P^{*/**}<0.05$) (Fig. 2).

We have established changes in the dynamics of mast cells of both phenotypes during the late period of development of experimental allergic inflammation in guinea pigs lung. The average number of mucosa related mast cells in the animals of the 3rd experimental group is 3.5±0.05 in the field of view, which is statistically significantly in 1.3 times higher ($p^{**}<0.05$), compare to the same indicator of the control group (Fig. 1). The average number of mucosa related mast cells acquires the control indicator on the 44th day of the experiment. The average number of perivascular mast cells in the animals of the 3rd experimental group on the 36th day of observation is in 2 times higher compare to the number of mucosa related mast cells, and statistically significantly higher in 4.5 times compare to the same point in the control

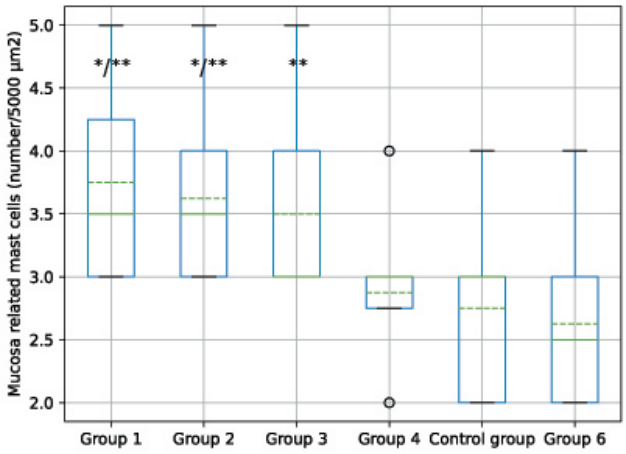

Fig. 1. *Morphometric changes in the number of mucosa related mast cells of guinea pigs lung.*
*Note: * – P<0.05 (Student's t-test); ** – P<0.05 (Whitney–Mann U-test) compare to the control group. Me (Q1; Q3). The median (Me) is shown by the green line. M±m (n=8). The arithmetic mean (M) is shown by the dotted green line.*

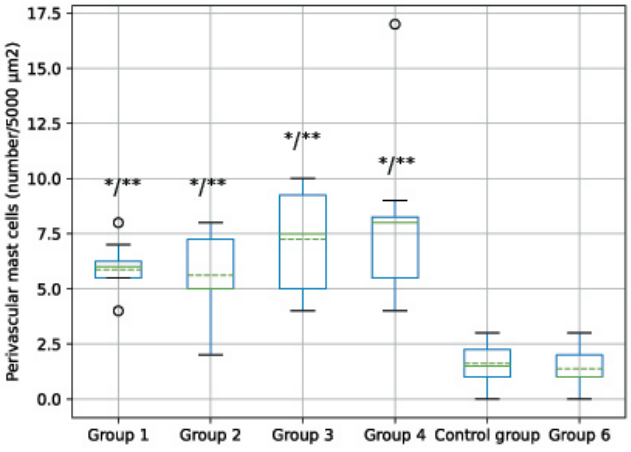

Fig. 2. *Morphometric changes in the number of perivascular mast cells in connective tissue of guinea pigs lung parenchyma. Note: * – P<0.05 (Student's t-test); ** – P<0.05 (Whitney–Mann U-test) compare to the control group. Me (Q1; Q3). The median (Me) – the green line. M±m (n=8). The arithmetic mean (M) – the dotted green line.*

group ($P^{*/**}$<0.05). The hyperplasia of perivascular mast cells (Fig. 3) is found in animals of the 4th experimental group on the 44th day after the start of the experiment (8.0±0.29 in the field of view), which is statistically significantly in 5 times higher than in the control group ($P^{*/**}$<0.05).

Discussion

Thus it has been established that in contrast to human and other small mammals (mice), mucosa related must cells are normally more abundant in guinea pigs lung than perivascular ones. Nevertheless, in contrast to BALB/c mice, a common feature of guinea pigs lung connective tissue and human lung in cases of experimental allergic inflammation due to sensitization and challenge with OVA is a statistically significant increase of both mucosa related and perivascular mast cells. However, we have demonstrated that the mast cells number changes of different phenotypes in guinea pigs lung have different nature. Our results revealed the maximum increase in the number of mucosa related mast cells in the early period of development of experimental allergic inflammation, as evidenced by the maximum increase coefficient of 1.4 in the 1st experimental group, compare to the control. It should be noted that the degranulation of mucosa related mast cells promotes the release of heparin, increasing permeability of capillaries and improves trophic of respiratory mucosa. However, the maximum increase in the number of perivascular mast cells in 5 times was evidenced during the late period of allergic inflammatory process development in lung of animals of the 4th experimental group.

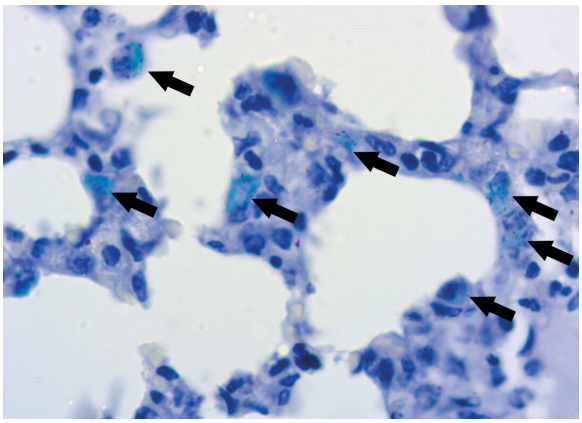

Fig. 3. *Perivascular must cells hyperplasia (arrows show) in connective tissue of the pulmonary interstitium of guinea pigs after sensitization and challenge with ovalbumin on the 44th day of the experiment. Staining: alcian blue. ×1000.*

Thus, the increase of perivascular mast cells is predominantly greater than mucosa related mast cells in OVA – sensitization. This should be decisive for morphological and histochemical changes of the microcirculatory bed, lymphoid tissue cells in connective tissues of pulmonary interstitium, which were previously described [7, 9]. For instance, mast cells together with respiratory endocrinocytes help maintain homeostasis of the local lung immune system [10-13]. Moreover, mast cells are involved in both innate and adaptive immune responses to allergens. Due to the presence of heparin secreted by perivascular mast cells into the intercellular substance of connective tissue the permeability of microvessels increases; in allergic inflammation it causes migration of lymphocytes and plasma cells into the perivascular intercellular substance [14-16]. In addition, mast cells contribute to maintenance

of chronic allergic airway inflammation and are crucial in initiating immune response to the allergen, during which they transmit signals that stimulate IgE synthesis by plasma cells and differentiation of Th2 lymphocytes [17].

Conclusion

Normally mucosa related mast cells are the predominant mast cell type in guinea pigs lung. OVA - sensitization and challenge leads to the statistically significant increase in the number of both mast cells types: mucosa related and perivascular mast cells.

The dynamics of increase in the number of mast cells of different phenotypes in guinea pigs lung is of a different nature in case of OVA – sensitization. More significant is increase in the number of perivascular mast cells in 5 times during the late period of allergic inflammation development in the 4th experimental group. Despite this the maximum increase in the number of mucosa related mast cells in 1.4 times is established during the early period of experimental allergic inflammation in the 1st experimental group, compare to the control.

Conflict of Interests

Authors declare no conflict of interest.

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Author's Contributions

Svitlana S. Popko – conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – reviewing and editing; *Valentina M. Yevtushenko* – data curation, writing – reviewing and editing.

ОСОБЛИВОСТІ РОЗПОДІЛУ І КІЛЬКІСНИХ ЗМІН МАСТОЦИТІВ В ЛЕГЕНЯХ МОРСЬКИХ СВИНОК НА ТЛІ ОВАЛЬБУМІН-ІНДУКОВАНОГО АЛЕРГІЧНОГО ЗАПАЛЕННЯ

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ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ. ЗАПОРІЖЖЯ, УКРАЇНА

Вступ. *Одними з найважливіших ключових клітин місцевого імунітету в легенях є мастоцити. Вони беруть участь як у реакціях вродженого, так і адаптивного імунітету у відповідь на інгаляційні алергени. Питання про розподіл обох фенотипів мастоцитів у легенях морської свинки при експериментальному аллергічному запаленні у більшості випадків залишається відкритим.*

Мета. *Вивчити розподіл та кількісні зміни мастоцитів у легенях морської свинки в умовах овальбумін-індукованого алергічного запалення.*

Методи. *За допомогою гістологічного, морфометричного, статистичного методів вивчили легені 48 самців морської свинки в умовах експериментального овальбумін-індукованого алергічного запалення. Визначали середню кількість мастоцитів слизових оболонок та навколосудинних мастоцитів у легенях морської свинки.*

Результати. *Доведено, що в легенях морської свинки в нормі за кількістю переважають мастоцити слизових оболонок, ніж периваскулярні мастоцити. У роботі продемонстрована динаміка зростання вмісту мастоцитів різних фенотипів у легенях морської свинки, яка має різнонаправлений характер в умовах сенсибілізації овальбуміном. Більш суттєвим є приріст саме навколосудинних мастоцитів у 5 азів протягом пізнього періоду розвитку алергічного запального процесу в легенях тварин 4-ої експериментальної групи ($P^{*/**}<0.05$), водночас максимальний приріст кількості мастоцитів слизової оболонки дихальних шляхів виявляється протягом раннього періоду розвитку експериментального алергічного запалення, про що свідчить максимальний коефіцієнт збільшення 1,4 в 1-ій експериментальній групі, порівняно з контролем ($P^{*/**}<0.05$).*

Висновок. *Сенсибілізація та аероалергізація овальбуміном призводить до статистично значимого зростання кількості як мастоцитів слизової оболонки дихальних шляхів, так і навколосудинних мастоцитів, з переважанням останніх. В умовах сенсибілізації овальбуміном динаміка зростання вмісту мастоцитів різних фенотипів у легенях морської свинки має різнонаправлений характер.*

КЛЮЧОВІ СЛОВА: мастоцити слизових оболонок, периваскулярні мастоцити, легені, морська свинка, алергічне запалення.

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