Melanoma Incidence in Czech Republic, the Relation between Histology, Body Site of Melanoma, and Duration of Lesions

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ABSTRACT

Aim: To evaluate the occurrence of melanoma in the period 1996–2017 in East Bohemia region in the Czech Republic. Method: We studied the incidence of melanoma and the age of diagnosis (adjusted calculation) and the parameters such as histology, body site of lesions, the length of the duration of lesions in 2810 patients. Results and conclusion: No change in the occurrence of melanoma and in age of melanoma during this period was found. The difference between men and women was not confirmed in histology, but the difference between men and women was confirmed in the body site of lesion and in the length of duration of lesion. No relation between the length of duration of lesions from which melanoma had originated and its histology was confirmed. The relation was confirmed between histology and body site of melanoma. The relation between the body site and the length of duration of previous lesions was confirmed also. The increasing occurrence of melanoma on the trunk according to the duration of the previous lesions was confirmed.

KEYWORDS

incidence of melanoma; histology of melanoma; body site of melanoma

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KEY POINTS

Question: What is the incidence of melanoma and is there any relation between the histology of melanoma, body site of melanoma, and the duration of lesion?

Findings: 2810 patients with a new diagnosis of melanoma were examined in the period of 1996–2017. The change in the occurrence of new melanoma and the age of melanoma was not confirmed. The relations between the followed parameters are shown in the study.

Meanings: The increase in the occurrence of melanoma on the trunk according to the duration of the previous lesions was confirmed; women suffer significantly more often from melanoma on lower limbs and on upper limbs, men suffer significantly more from melanoma on the trunk.

INTRODUCTION

Melanoma is one of the most malignant skin tumors with constantly rising incidence worldwide, especially in fair-skinned populations (1–3). Historically, melanoma was a rare cancer, but in the last 50 years its incidence has risen faster than almost any other cancer. Skin cancer (the majority attributed to melanoma) was the cause of almost 2000 deaths in Australia in 2010 and it is currently the most common cancer in young Australians aged 15 to 39 years (4–7). In the United States alone, 87,110 individuals were predicted to be diagnosed with melanoma in 2017 (2, 5). If melanoma is diagnosed in its early stages, resection of the lesion is associated with favourable survival rates (3, 8). Once melanoma is advanced, surgery is no longer sufficient and the disease becomes more difficult to treat (3, 8–10). However, more recently developed immunotherapeutic treatments combined with radiation can improve survival further to several years (9).

As the incidence of melanoma steadily increases in both sexes, further improvement in primary prevention and early detection strategies is crucial (11). Melanoma arises through multiple various causal pathways and reflects a dynamic interdependence between environmental factors and genetic alterations. Epidemiological data support two major pathways in the pathogenesis of cutaneous melanoma: one by cumulative sun exposure to the site of the future melanoma in sun sensitive people and other by early sun exposure and nevus proneness, promoted by host factors, intermittent sun exposure, or both (12–18).

THE AIM OF THE STUDY IS TO EVALUATE:

1) The occurrence of new melanoma in years 1996–2017 in men and in women.
2) If there is some difference in the occurrence of new melanoma from the year 2002 to the year 2017 and if there is some difference in age of diagnosis (the incidence).
3) If there is a difference between men and women in parameters such as histology, body site of lesions, the length of the duration of lesions.
4) The relation between the histology and the body site of melanoma.
5) The relation between the length of the duration of lesion and the body site of melanoma.
6) The relation between the histology and the length of the duration of lesions from which the melanoma had arisen.

PATIENTS AND METHODS

This is an epidemiological study based on the examination of patients with a new histopathologically confirmed primary melanoma (lentigo maligna, melanoma in situ, invasive cutaneous superficial or nodular, mucosal melanoma or unknown primary melanoma) in the period 1996–2017. All these patients were examined at the Department of Dermatology, Faculty Hospital, Hradec Králové, Charles University, Czech Republic. The diagnosis of melanoma was made according to its histology. Patients' information and degree of spread of the melanoma was obtained during the examination of patients at out-patient department in the Department of Dermatology, Faculty Hospital Hradec, Králové, Charles University, Czech Republic. We evaluated these data: 1) The occurrence of new melanoma in years 1996–2017 in men and in women. 2) If there is some difference in the occurrences of new melanoma from the year 2002 to the year 2017 and if there is some difference in age of diagnosis (the incidence). 3) For how long the patient had observed the skin lesion from which the melanoma was confirmed – since childhood, 0–4 years, 5–9 years, more than 9 years. 4) The body site of lesion – face (including neck, scalp, ears), trunk, upper limbs (including hand, axillae), lower limbs (including feet plantar, subungual, heel, metatarsus and dorsum). 5) Histology of lesion – we distinguished nodular melanoma, superficial melanoma, melanoma in situ, lentigo maligna. Cases of the four main histological subtypes were evaluated in this study. The cases of mucosal, desmoplastic melanoma and melanoma of unknown origin are also included to the whole number of melanomas examined in this period.

This study was approved by Ethics commitee of Faculty Hospital Hradec Králové, Charles University, Czech Republic. There is no conflict of interest. Consolidated Standards of Reporting Trials and Strengthening the Reporting of Observational Studies in Epidemiology guidelines were followed.

STATISTICAL ANALYSIS

We evaluated 1) The occurrence of new melanoma in years 1996–2017 in men and in women 2) If there is some change in the occurrence of new melanoma (the incidence) from the year 2002 to the year 2017 and if there is some difference in age of diagnosis.

The age distribution changes year after year. This aging of the society is caused mainly by the post war baby boom repeated one generation later. This is the reason why we have to use standardization to be able to compare the number of patients in various years. We used the year 2017 as a standard.

In each year we formed age groups five years wide, that is, 0–4, 5–9, 10–14, etc. In each year we counted the number of patients with a certain type of melanoma for each group and divided it by the number of inhabitants in that group in the given year. That gave us the age-specific incidence. When we multiplied it by the number of inhabitants in the age group in the standard year, we obtained what we called an adjusted number of patients in the age group for the given year. We added the adjusted numbers over all the age groups in the given year to obtain an adjusted number of patients in that year.

To calculate the average age of patients in a given year, we followed the idea that is used when we calculate the mean when only a histogram is presented. We took the midpoint of each group in a given year, multiplied it by the adjusted number of patients in the group and summed the products up over all the groups in the given year. When the sum of products obtained in this manner was divided by the adjusted number of patients in that year, it gave us the adjusted average age in that year.

Unfortunately, major administrative changes were made as to the division of the country into smaller regions during the year 2001. These changes made the distributions of ages incomparable and intractable. This was the reason why we could make adjustment to numbers of patients and calculations of adjusted ages only beginning with the year 2002. Since the year 2017 was the last one in which the patients' data were recorded, sixteen years of adjusted numbers and ages of patients were available. Regarding the evaluation of the relation between other parameters (histology of melanoma and body site of melanoma; histology of melanoma and the length of the duration of lesion; body site of melanoma and duration of lesions), we included the patients from the period 1996–2012. Pairs of these classifications were entered in the contingency tables and the chi-square test for independence of these classifications was perfomed with the level of significance set to 1%.

Tab. 1 Number of patients with new diagnosis of melanoma in the years 1996–2017.

	No. of patients			No. of patients in %		
Year	**Men**	**Women**	**Total**	**Men**	**Women**	**Total %**
1996	48	48	96	50.0	50.0	100.0
1997	45	50	95	47.4	52.6	100.0
1998	37	42	79	46.8	53.2	100.0
1999	48	63	111	43.2	56.8	100.0
2000	48	57	105	45.7	54.3	100.0
2001	41	53	94	43.6	56.4	100.0
2002	54	67	121	44.6	55.4	100.0
2003	49	58	107	45.8	54.2	100.0
2004	71	73	144	49.3	50.7	100.0
2005	72	67	139	51.8	48.2	100.0
2006	53	64	117	45.3	54.7	100.0
2007	68	62	130	52.3	47.7	100.0
2008	71	71	142	50.0	50.0	100.0
2009	85	69	154	55.2	44.8	100.0
2010	56	57	113	49.6	50.4	100.0
2011	59	74	133	44.4	55.6	100.0
2012	82	88	170	48.2	51.8	100.0
2013	63	73	136	46.3	53.7	100.0
2014	79	73	152	52.0	48.0	100.0
2015	76	69	145	52.4	47.6	100.0
2016	82	94	176	46.6	53.4	100.0
2017	82	69	151	54.3	45.7	100.0
total	**1369**	**1441**	**2810**			
p-value	0.887					

The statistical difference between men and women was not confirmed (*p-value* = 0.887).

RESULTS

1) THE OCCURRENCE OF NEW MELANOMA IN YEARS 1996–2017 IN MEN AND IN WOMEN.

In the period 1996–2017, altogether 2810 patients with new a diagnosis of melanoma were examined, 1369 men (54.3%) and 1441 women (45.7%), 70 of them suffered from multiple melanoma. The cases of mucosal, desmoplastic melanoma, and melanoma of unknown origin are also included in the whole number of melanoma examined in this period. The number of patients with new a diagnosis of melanoma in every year of this period is shown in Table 1. The difference in the occurrence of melanoma between men and women was not confirmed.

Supplement to Table 1. The statistical evaluation of the difference of the occurrence of new melanoma and the age of the diagnosis in period 2002–2017 (adjusted number of patients with melanoma and adjusted average age of diagnosis). The difference in the occurrence of melanoma in the period 2002–2017 was not confirmed. The statistical difference in age of melanoma diagnosis was not confirmed either.

The calculation of the difference in the occurrence of melanoma and the difference of age		
Year	**Adjusted number of patients**	**Average adjusted age of diagnosis**
2002	140.9	62.1
2003	122.0	59.3
2004	162.9	59.5
2005	158.5	62.9
2006	131.7	60.2
2007	141.8	60.2
2008	157.2	63.3
2009	167.7	61.3
2010	117.7	60.2
2011	134.8	59.8
2012	178.9	59.2
2013	143.8	60.5
2014	155.9	60.4
2015	145.9	60.7
2016	177.4	60.2
2017	151.0	61.3
p-value	0.23	0.617

2) IF THERE IS SOME DIFFERENCE IN THE OCCURRENCE OF NEW MELANOMA FROM THE YEAR 2002 TO THE YEAR 2017 AND IF THERE IS SOME DIFFERENCE IN AGE OF DIAGNOSIS (THE INCIDENCE).

We studied, if there is an increase in the occurrence of new melanomas and if there is some difference in age of melanoma diagnosis from the year 2002 to the year 2017. The calculations were done with respect to the number of inhabitants in the region and to average age of inhabitants in this region (the adjusted calculation). The statistical evaluation of the difference of the occurrence of new melanoma and the age of the diagnosis in period 2002–2017 was performed; it is shown in the Supplement to Table 1. The difference in the occurrence of melanoma in the period 2002–2017 was not confirmed. The statistical difference in age of melanoma diagnosis was not confirmed either.

3) IF THERE IS A DIFFERENCE BETWEEN MEN AND WOMEN IN PARAMETERS SUCH AS HISTOLOGY, BODY SITE OF LESIONS, THE LENGTH OF THE DURATION OF LESIONS.

Regarding the **histology**, lentigo maligna was confirmed in 217 patients (109 men – 8% and 108 women – 7.5%), melanoma in situ in 300 patients (140 men – 10.2% and 160 women – 11.1%), melanoma nodulare in 423 patients (225 men – 16.4% and 198 women – 13.7%) and melanoma superficiale in 1870 patients (895 men – 65.4% and 975 women – 67.7%). We have not confirmed the statistical difference in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale, or melanoma nodulare between men and women (*p-value* = 0.200). Number of patients (men and women, including the number in%) with lentigo maligna, melanoma in situ, melanoma superficiale, melanoma nodulare is shown in the Table 2. The whole number of patients is 2810. Regarding **the body site** – 47 patients suffered from melanoma of unknown primary origin (24 men – 1.8% and 23 women – 1.6%), 615 patients suffered from melanoma on lower limbs (157 men – 11.5% and 458 women – 31.8%), 522 patients on upper limbs (217 men – 15.9% and 305 women – 21.2%), 355 patients on a face (167 men – 12.2% and 188 women – 13%), 1250 patients on a trunk (794 men – 58% and 456 women – 31.6%), 21 patients suffered from mucosal melanoma (10 men – 0.7% and 11 women – 0.8%). We have confirmed the difference between men and women regarding the body site (*p-value* = 0.000). Our study shows, that women suffer significantly more often from melanoma on lower limbs and on upper limbs, on the other hand, men suffer significantly more from melanoma on the trunk. Number of patients (men and women, including the number in %) with different body site of melanoma (unknown origin, mucosal, upper limbs, lower limbs, face, trunk) is shown in Table 3. Regarding the **duration of lesions**, 2256 patients could

Tab. 2 Number of patients (men, women) with lentigo maligna, melanoma in situ, melanoma superficiale, melanoma nodulare. The whole number of patients is 2810. The difference in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale and melanoma nodulare between men and women was not confirmed (*p-value* = 0.200).

	Histology				
	LM	M in situ	nod	sup	total patients
men	109	140	225	895	1369
women	108	160	198	975	1441
total patients	217	300	423	1870	2810

	LM	M in situ	nod	sup	total %
men	8.0%	10.2%	16.4%	65.4%	100.0%
women	7.5%	11.1%	13.7%	67.7%	100.0%
p-value	0.200				

Explanation: LM – lentigo maligna, M in situ – melanoma in situ, nod – melanoma nodulare, sup – melanoma superficiale

Tab. 3 Number of patients (men, women) with various body site of melanoma (unknown origin, mucosal, upper limbs, lower limbs, face, trunk). The whole number of patients is 2810. The difference between men and women regarding the body site was confirmed (*p-value* = 0.000).

	body site of melanoma						
	unknown	lower	upper	mucosal	face	trunk	total patients
men	24	157	217	10	167	794	1369
women	23	458	305	11	188	456	1441
total patients	47	615	522	21	355	1250	2810

	body site of melanoma						
	unknown	lower	upper	mucosal	face	trunk	total %
men	1.8%	11.5%	15.9%	0.7%	12.2%	58.0 %	100.0%
women	1.6%	31.8%	21.2%	0.8%	13.0%	31.6 %	100.0%
p-value	0.000						

Explanation: unknown – melanoma of unknown origin, lower – lower limbs, upper – upper limbs

Tab. 4 Number of patients (men, women) with different duration of previous lesions, from which melanoma had originated (0–4 years, 5–9 years, over 9 year, from childhood). The whole number of patients is 2256. The difference between men and women regarding the duration of lesions was confirmed (*p-value* = 0.002). Patients, that could not determine the duration of lesion, are not included in this statistical evaluation.

	duration of lesions				
	0–4 y	5–9 y	over 9 y	childhood	total patients
men	486	100	193	267	1046
women	660	103	178	269	1210
total patients	1146	203	371	536	2256

	duration of lesions				
	0–4 y	5–9 y	over 9 y	childhood	total %
men	46.5%	9.6%	18.5%	25.5%	100.0%
women	54.5%	8.5%	14.7%	22.2%	100.0%
p-value	0.002				

determine the duration of lesions: 1146 patients had observed the lesion in the duration of 0–4 years (486 men – 46.5% and 660 women – 54.5%), 203 patients had observed the lesions for 5–9 years (100 men – 9.6% and 103 women – 8.5%), 371 patients had observed the lesion for more than 9 years (193 men – 18.5% and 178 women – 14.7%), 536 patients had observed the pigmenal nevus since childhood (267 men – 25.2% and 269 women – 22.2%), 554 patients (25%) could not state for how long they had observed the previous lesions from which melanoma had originated. These patients are not included in this statistical evaluation. We confirmed the statistical difference between men and women regarding the duration of lesions (*p-value* = 0.002). Women suffer significantly more from melanoma in the duration of 0–4 years; the study shows, that the duration of lesion of 0-4 years was confirmed in 54.5% of women, but only in 46.5% of men (Table 4).

4) THE RELATION BETWEEN HISTOLOGY AND BODY SITE OF MELANOMA.

We evaluated the relation between the histology (lentigo maligna, melanoma in situ, nodular melanoma, superficial melanoma) and the body site of melanoma (lower limbs, upper limbs, face, trunk). The occurrence of lentigo maligna was recorded in 9.6% on lower limbs, in 13.5% on upper limbs, in 29.3% on a face and in 47.6% on a trunk. Melanoma in situ was recorded in 20.4% on lower limbs, in 25.2% on upper limbs, in 7.2% on a face, and in 47.2% on a trunk. Melanoma nodulare was recorded in 19.5% patients on lower limbs, in 21.6% on upper limbs, in 12.7% on a face, and in 46.2% on a trunk. Melanoma superficiale was recorded in 25% on lower limbs, in 18.1% on upper limbs, in 11.7% on a face, and in 45.2% on a trunk. The dependence between the histology and body site of lesions was confirmed, *p-value* = 0.000. The total number of patients was 2826. This relation is shown in Table 5. Our study shows, that lentigo maligna, melanoma in situ, melanoma nodulare and melanoma superficiale are found from 45.2% to 47.6% on a trunk, lentigo maligna is found on a face in 29.3%, and in 9.6% of patients on lower limbs.

Tab. 5 The relation between the histology and the body site of melanoma (lower limbs, upper limbs, face, trunk). The relation was confirmed, (*p-value* = 0.000). Total number of patients is 2826.

	body site				
histology	lower limbs	upper limbs	face	trunk	total patients
LM	22	31	67	109	229
M in situ	65	80	23	150	318
nod	83	92	54	197	426
sup	463	335	217	838	1853
total patients	633	538	361	1294	2826

	body site				
histology	lower limbs	upper limbs	face	trunk	total %
LM	9.6%	13.5%	29.3%	47.6%	100.0%
M in situ	20.4%	25.2%	7.2%	47.2%	100.0%
nod	19.5%	21.6%	12.7%	46.2%	100.0%
sup	25.0%	18.1%	11.7%	45.2%	100.0%
p-value	0.000*				

Explanation: LM – lentigo maligna, M in situ – melanoma in situ, nod – melanoma nodulare, sup – melanoma superficiale

5) THE RELATION BETWEEN THE LENGTH OF THE DURATION OF LESION AND THE BODY SITE OF MELANOMA.

We evaluated the relation between the duration of lesions (0–4 years, 5–9 years, over 9 years, from childhood) and the body site of melanoma (lower limbs, upper limbs, face, trunk). The occurrence of melanoma in a duration of 0–4 years was recorded in 27.4% on lower limbs, in 22.3% on upper limbs, in 16.7% on a face, and in 33.6% on a trunk. The occurrence of melanoma in the duration of 5–9 years was recorded in 16.9% on lower limbs, in 20.9% on upper limbs, in 20.9% on a face, and in 41.3% on a trunk. The occurrence of melanoma in the duration over 9 years was recorded in 22% patients on lower limbs, in 16.9% on upper limbs, in 16.6% on a face, and in 44.5% on a trunk. The occurrence of melanoma in a duration since childhood was recorded in 25.1% on lower limbs, in 18.6% on upper limbs, in 5.6% on face, and in 50.7% on trunk. The relation between the duration of lesions and body site of lesions was confirmed, *p-value* = 0.000. The total number of patients was 2232. The relation is shown in Table 6. Our study shows that melanoma on the trunk was confirmed by the lesions since childhood in 50. 7% but on the face from the lesions since childhood only in 5.6%. We can observe the increase in the occurrence of melanoma on the trunk according to the duration of the previous lesions –

Tab. 6 The relation between the duration of lesions (0–4 years, 5–9 years, over 9 years, from childhood) and the body site of melanoma (lower limbs, upper limbs, face, trunk). The relation was confirmed (*p-value* = 0.000). Total number of patients is 2232.

	body site				
duration of lesion	lower limbs	upper limbs	face	trunk	total patients
0–4 y	307	250	187	377	1121
5–9 y	34	42	42	83	201
over 9 y	82	63	62	166	373
childhood	135	100	30	272	537
total patients	558	455	321	898	2232

	body site				
duration of lesion	lower limbs	upper limbs	face	trunk	total %
0–4 y	27.4%	22.3%	16.7%	33.6%	100.0%
5–9 y	16.9%	20.9%	20.9%	41.3%	100.0%
over 9 y	22.0%	16.9%	16.6%	44.5%	100.0%
childhood	25.1%	18.6%	5.6%	**50.7%**	100.0%
p-value	0.000*				

lesions in the duration of 0–4 years appear on the trunk in 33.6%, in the duration of 5–9 years in 41.3%, in the duration over 9 years in 44.5%, and since childhood in 50.7%.

6) THE RELATION BETWEEN THE HISTOLOGY AND THE LENGTH OF THE DURATION OF LESION FROM WHICH THE MELANOMA HAD ARISEN.

We evaluated the relation between the duration of lesions (0–4 years, 5–9 years, 10–19 years, over 19 years, since childhood) and the histology (lentigo maligna, melanoma in situ, nodular melanoma, superficial melanoma). From lesions in the duration of 0–4 years, lentigo maligna was confirmed in 7.8%, melanoma in situ in 9.8%, melanoma nodulare in 17% , and melanoma superficiale in 65.4%. From lesions in the duration of 5–9 years, lentigo maligna was confirmed in 8.3%, melanoma in situ in 11.7%, melanoma nodulare in 11.2% and melanoma superficiale in 68.8%. From lesions in the duration of 10–19 years, lentigo maligna was confirmed in 7.4%, melanoma in situ in 13.2%, melanoma nodulare in 11.9%, and melanoma superficiale in 67.5%. From lesions in the duration over 19 years, lentigo maligna was confirmed in 11.1%, melanoma in situ in 9.5%, melanoma nodulare in 15.9%, and melanoma superficiale in 63.5%. From pigmented nevus since childhood, lentigo maligna was confirmed in 8.1%, melanoma in situ in 8.9%, melanoma nodulare in 15.2%, and melanoma superficiale in 67.8%. The relation between the duration of lesions and histology was not confirmed (*p-value* = 0.390). The total number of patients was 2271. The relation is shown in Table 7.

Tab. 7 The relation between the duration of lesions (0–4 years, 5–9 years, 10–19 years, over 19 years, from childhood) and the histology. The relation was not confirmed (*p-value* = 0.390). Total number of patients is 2271.

	histology				
duration of lesions	LM	M in situ	nod	sup	total patients
0–4 y	90	113	196	753	1152
5–9 y	17	24	23	141	205
10–19 y	23	41	37	210	311
over 19 y	7	6	10	40	63
childhood	44	48	82	366	540
total patients	181	232	348	1510	2271

	histology				
duration of lesions	LM	M in situ	nod	sup	total %
0–4 y	7.8%	9.8%	17.0%	65.4%	100.0%
5–9 y	8.3%	11.7%	11.2%	68.8%	100.0%
10–19 y	7.4%	13.2%	11.9%	67.5%	100.0%
over 19 y	11.1%	9.5%	15.9%	63.5%	100.0%
childhood	8.1%	8.9%	15.2%	67.8%	100.0%
p-value	0.390				

Explanation: LM – lentigo maligna, M in situ – melanoma in situ, nod – melanoma nodulare, sup – melanoma superficiale

DISCUSSION

There have been many interesting papers regarding the epidemiology and incidence of melanoma to come out in recent years. According to the literature, further work is needed to understand fully the issues raised by several studies (20). In this study, we evaluated as the incidence both several parameters in epidemiology of melanoma in the period from the year 1996 to the year 2017 in East Bohemia region in the Czech Republic in middle Europe. There are 551 thousands inhabitants and the area of this region is 4,759 square km.

The advantage of our study is that all patients included in this study were personaly examined and were followed at the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech Republic. According to the adjusted calculation, we did not confirm the statistical important difference in the occurrence of new melanomas in the period from 2002 to 2017, neither between men and women; nor did we confirm the difference in age of melanoma diagnosis – the age of melanoma diagnosis is 59–62 years in this period. Also, we did not confirmed the difference in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale, and melanoma nodulare between men and women. On the other hand, we confirmed the statistical difference between men and women in the body site of melanoma and the length of the duration of lesions. Our study shows that women suffer significantly more often from melanoma on lower limbs (31.8% women, 11.5% men) and on upper limbs (21.2% women, 15.9% men). On the other hand, men suffer significantly more from melanoma on the trunk (58% of men versus 31.6% of women). The duration of lesion of 0–4 years was confirmed in 54.5% of women but only in 46.5% of men.

We also evaluated the relation between the parameters, such as the histology, the length of the duration of lesion from which the melanoma had arisen, and the body site of melanoma. We confirmed that there is a significant relation of body site of melanoma to its histology and to the length of the duration of lesions which melanoma had arisen from. No relation was confirmed between the length of the duration of lesions and its histology. Our study shows that lentigo maligna, melanoma in situ, melanoma nodulare, and melanoma superficiale are found from 45.2% to 47.6% on a trunk but lentigo maligna is found more often on a face (29.3%) and less often on lower limbs (9.6%), melanoma in situ only in 7.2% on a face but in 20.4% on lower limbs. Melanoma from pigmented nevus from childhood was confirmed on the trunk in 50.7%, but only in 5.6% on the face. We can observe the increasing occurrence of melanoma on a trunk according to the duration of the previous lesions – melanoma had arisen on the trunk from lesions in the duration of 0–4 years in 33.6%, in the duration of 5–9 years in 41.3%, in the duration over 9 years in 44.5%, and since childhood in 50.7% as mentioned above. Regarding the duration of lesions since childhood, we confirmed that 540 patients (19%, 2810 patients = 100%) suffered from nevus pigmentosus from childhood.

Some of our results are in contrast to other studies. There is a universal agreement that the incidence of mel-

anoma diagnoses is increasing and a similar trend has been observed in Europe (21, 22). Multiple studies using the US Surveillance, Epidemiology and End Results (SEER) Program and National Program of Cancer Registries have consistently reported increasing melanoma incidence between 1973 and 1997 (23–25) More recent studies (1992–2006) reported that melanoma incidence increased 3% to 4% per year across most demographic groups (1, 26). However, a recent study of the Centers for Disease Control and Prevention database suggests that incidence in New England states may be decreasing (27).

Finally, it has been suggested that the observed increased melanoma incidence may be an artifact of underreporting in earlier decades (28). Most of the studies cited above relied on SEER and National Program of Cancer Registries data to compare melanoma rates at different time points (29). Many previous epidemiologic studies were missing data on tumor thickness, and many registries did not capture in situ lesions (30, 31). These factors could account for an underrepresentation of thicker melanomas and overestimation of mortality from thin melanomas.

According to some studies, males are approximately 1.5-times more likely to develop a melanoma than females but the different prevalence in both sexes must be analyzed in relation to age: the incidence rate of melanoma is greater in women than in men until they reach the age of 40 years, however, by 75 years of age, the incidence is almost 3-times as high in men than in women (32–34). According to other studies, higher melanoma rates have been mostly observed in elderly or male populations, whereas the female sex seems to represent an independent risk factor for early onset melanoma for women younger than 45 years (35–37). According to recent data, the rising melanoma trends mostly affect the older age groups, whereas the incidence seems to stabilize in the youngest age groups (24–44 years) (38). However, melanoma still affects mostly younger patients, with a median age diagnosis of 57–64 worldwide (38). This is in agreement with our study, the average age of new melanoma is 59–62 years according to our results.

Regarding the body site of melanoma, our study shows, that women suffer significantly more often from melanoma on lower limbs and on upper limbs, men suffer significantly more from melanoma on the trunk. According to some studies, the anatomical location of melanoma also varies according to gender. Males tend to have worse clinical and histological characteristics at primary diagnosis; melanomas in men are more often located on the head, neck, and trunk, commonly ulcerated and have a higher Breslow thickness (39, 40). Males are more likely to report greater exposure to the sun, mainly due to greater participation in outdoor work and leisure activities, compared to females (41). Females are likely to be more knowledgeable about skin cancer than males (42). However, the higher knowledge and use of sun protective measures among women conflicts with findings that women have a greater desire for a tan and their increased perception that a tan is healthy compared with men (31, 43). Two pathways have been hypothesized for the development of cutaneous melanoma: one typically affects the head and neck, a site with chronic sun damage, and the other affects the trunk, which is less exposed to the sun. These results appear to support the hypothesis of divergent pathways to melanoma and that recreational sun exposure and indoor tanning are associated with melanoma on the lower limbs, the most common site of melanoma in women. These findings appear to have important preventive implications (44, 45). This is in agreement with our results, that women suffer significantly more often from melanoma on lower limbs (31.8% of women versus 11.5% of men), men suffer significantly more often from melanoma on the trunk (58% of men versus 31.6% of women).

In our study we confirmed that melanoma had originated from nevus pigmentosus from childhood in 540 patients (19%) – on the trunk in 50.7% of patients, in 5.6% of patients on the face. According to the literature, approximately 25–33% of cutaneous melanomas derive from a benign, melanocytic nevus, whereas this percentage may be as high as 50% in patients with numerous nevi (17, 46–48). Transformation of nevi to melanoma occurs most commonly in non-chronically sun-damaged skin. Nevus-prone patients with an increased number of melanocytic nevi tend to develop melanomas at a younger age and on axial locations. On the other hand, *nevus resistant* patients with fewer nevi tend to develop de novo melanomas on habitually sun-exposed skin or at older ages (49, 50). There is a strong evidence that an intermittent pattern of sun exposure increases the melanoma risk. Chronic sun exposure shows no association or a weak inverse association with melanoma risk – it can explain the rare occurrence of melanoma from pigmented nevus from childhood on the face observed in our study. Episodic, intermittent, high-intensity exposure to sunlight has been linked to the development of melanoma in Australia (5, 6). Total lifetime sun exposure is positively associated with melanoma risk, but the relationship is weaker than that for intermittent sun exposure (32, 33, 51, 52). *Sunburn* is a marker of an intermittent pattern of sun exposure and there is a tendency for greater consistency of positive associations for sunburn than for intermittent exposure (32–35, 51, 52). Furthermore it may explain our results, as we suppose, that melanoma resulting from the nevus pigmentosus from childhood could be sunburned on the trunk, but there is chronic sun exposure on the face. Melanoma risk differs not only by a pattern of sun exposure but also by *body site, age,* and *phenotype of a patient* (36, 49). According to some studies, head and neck melanomas have been linked to chronic sun exposure with older age of diagnosis and melanoma on the trunk and limbs to younger ages and intermittent exposure. According to another study, sun exposure can cause melanoma on all body sites, but risks tend to be higher for usually sun-exposed sites than occasionally exposed sites (37, 53). For sunburn, strong positive associations have been found at all body sites (head/neck, trunk, arms, and legs) and with no significant site-specific differences in a recent meta-analyses and pooled analyses (37, 38, 54). In our study, we confirmed a significant relation between the histology and body site of melanoma. Our study shows that lentigo maligna, melanoma in situ, melanoma nodulare, and melanoma superficiale are found to be from 45.2% to 47.6% on the trunk, but lentigo maligna is found more often on

a face (29.3%) and less often on lower limbs (9.6%), melanoma in situ only in 7.2% on a face, but in 20.4% on lower limbs. Melanoma nodulare was confirmed only in 12.7% of patients on the face, but in 19.5% and 21.6% of patients on upper and lower limbs respectively and in 46.2% of patients on the trunk. According to the literature, in contrast to cutaneous superficial spreading melanoma, the occurrence of nodular melanoma and mucosal melanoma seems to be independent of UV exposure. Specifically, in the case of nodular melanoma, the influence of UV is controversially discussed in the literature. Some studies reported a higher prevalence of nodular melanoma on sun-exposed skin such as the lower limbs, head, and neck. However, nodular melanoma can also affect non-chronically sun-exposed body areas such as the trunk in fair- but also dark-skinned patients (48, 55–57).

Individuals with large or giant congenital melanocytic nevi (CMNs) at birth are at higher risk of melanoma development which increases according to the size of CMN and is the highest in those nevi traditionally designated as garment nevi (58–60). Also, personal history of a prior melanoma is a strong predictor for the development of a subsequent melanoma, with approximately tenfold increased risk (61). Additionally, melanoma seems to appear more commonly in immunosuppressed patients, including patients with prior organ transplantation, hematologic malignancies, or human immunodeficiency virus infection, as well as patients taking immunosuppressive medication (62).

The epidemiologic, genomic, and anatomic profiles of melanoma significantly differ across the world and mostly depend on a constellation of environmental and (epi) genetic factors (11).The purpose of our study was to contribute to the evaluation of the incidence of melanoma in East Bohemia region in the Czech Republic in middle Europe and to evaluate the relation between the followed parameters.

CONCLUSION

No statistical difference in the occurrence of new melanomas during the period 2002–2017 was found, furthermore, no difference in the age of patients with melanoma; also there is no difference in the occurrence between men and women. The difference between men and women was not confirmed in histology, but the difference between men and women was confirmed in the body site of lesion and in the length of duration of lesion. Women suffer significantly more often from melanoma on lower limbs and on upper limbs, men suffer significantly more from melanoma on the trunk. The length of the duration of lesion of 0–4 years to the diagnosis of melanoma was confirmed in 54.5% of women, but only in 46.5% of men. No relation between the length of duration of lesions from which melanoma had originated and its histology was confirmed. The relation was confirmed between histology and body site of melanoma. The increasing occurrence of melanoma on the trunk according to the duration of the previous lesions was confirmed.

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Epidemiology of Melanoma in the Czech Republic in East Bohemia in the Period 2002–2017 and the Effect of the Annual Sunshine Exposure

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ABSTRACT

Aim: The evaluation of the trend in the occurrence of melanoma nodulare, melanoma superficiale, lentigo maligna and melanoma in situ in the period of 2002–2017 in East Bohemia region in the Czech Republic. We examine if the annual numbers of hours of sunshine could affect the number of patients with melanoma. Method: In the peridod of 2002–2017, altogether 2230 patients with new diagnosis of melanoma were examined. We studied 1) If there is some trend in the occurrence of lentigo maligna and melanoma in situ, melanoma superficiale, and melanoma nodulare and if there is a difference in the age of patients with this diagnosis (adjusted calculation of specific kind of melanomas and adjusted calculation of age). 2) If the annual numbers of hours of sunshine affect the trend in the occurrence of melanoma and if the annual numbers of hours of sunshine affect the body site of melanoma. Results and conclusion: Our study confirmed that the number of patients with lentigo maligna and melanoma in situ had increased in East Bohemia region in the period of 2002–2017. The number of melanomas of nodular and superficial type does not increase. The total number of melanomas in this period does not increase either. No difference of the age of patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ was confirmed. We confirmed no relation of the annual numbers of hours of sunshine to the number of melanoma and to the body site of melanoma.

KEYWORDS

melanoma; sunshine exposure; lentigo maligna; melanoma in situ; adjusted calculation

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INTRODUCTION

There is a universal agreement that the incidence of melanoma diagnoses increases and a similar trend has been observed in Europe, but the higher melanoma incidence has not been fully explained (1–5). Multiple studies using the US Surveillance, Epidemiology and End Results (SEER) Program and National Program of Cancer Registries have consistently reported increasing melanoma incidence between 1973 and 1997 (6–8). More recent studies (1992–2006) reported that melanoma incidence increased by 3% to 4% per year across most demographic groups (1, 9). Melanoma arises through multiple different causal pathways and reflects a dynamic interdependence between environmental factors and genetic alterations. Several factors have been identified that significantly influence the incidence and the clinical and oncogenic characteristics of this disease. These factors mainly comprise increased UV exposure, tanning bed use, family and personal history of melanoma, and certain phenotypical characteristics, such as fair skin and hair color. Epidemiological data support two major pathways in the pathogenesis of cutaneous melanoma: one by cumulative sun exposure to the site of the future melanoma in sun sensitive people and other by early sun exposure and nevus proneness, promoted by host factors, intermittent sun exposure, or both (11–18).

THE AIM OF THIS STUDY IS TO EVALUATE:

1) If there is some change in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale and melanoma nodulare from the year 2002 to the year 2017 (the incidence) and if there is some change in age of melanoma diagnosis.
2) If the annual numbers of hours of sunshine could affect the number of patients suffering from melanoma nodulare, superficiale, lentigo maligna and melanoma in situ.
3) If there is some relation between the annual numbers of hours of sunshine and the body site of melanoma (lentigo maligna, melanoma in situ, melanoma superficiale and melanoma nodulare).

PATIENTS AND METHODS

All patients included in this study were examined at the Department of Dermatology, Faculty Hospital, Hradec Králové, Charles University, Czech Republic. The diagnosis of melanoma was made according to its histology. Patients' information and degree of spread of the melanoma was obtained during the examination at out-patient department at the Department of Dermatology, Faculty Hospital, Hradec Králové, Charles University, Czech Republic.

WE EVALUATED THE FOLLOWING DATA:

1) If there are some changes in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale and melanoma nodulare from the year 2002 to the year 2017 (the incidence) and if there is some change in age of diagnosis.
2) If the annual number of hours of sunshine could affect the number of patients suffering from melanoma nodulare, superficiale, lentigo maligna and melanoma in situ.
3) If there is some relation between the annual numbers of hours of sunshine and the body site of melanoma (lentigo maligna, melanoma in situ, melanoma superficiale, and melanoma nodulare).

The data about the annual numbers of hours of sunshine were obtained from the Hydro-metereological Institute in Hradec Králové.

STATISTICAL ANALYSIS

We evaluated

1) If there is some trend in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale and melanoma nodulare from the year 2002 to the year 2017 and if there is some difference in age of diagnosis (the incidence). W**e** studied the dependence of the adjusted number of patients on the year of the first occurrence/diagnosis.
2) If the annual numbers of hours of sunshine could affect the number of patients suffering from melanoma nodulare, superficiale, lentigo maligna and melanoma in situ.
3) If there is some relation between the annual numbers of hours of sunshine and the body site of melanoma (lentigo maligna, melanoma in situ, melanoma superficiale, and melanoma nodulare). The data about annual number of hours of sunshine were obtained from the Hydro – metereological Institute in Hradec Králové. To analyse the data we used the multiple regression model. We also included the number of hours from the previous two years as a lag variable because it could effect the formation of melanoma in the following year.

The age distribution changes year after year. This is the reason why we have to use standardization to be able to compare the number of patients in various years. We used the year 2017 as a standard. In each year we formed age groups five years wide, that is, 0–4, 5–9, 10–14, etc. In each year we counted the number of patients with a certain type of melanoma for each group and divided it by the number of inhabitants in that group in the given year. That gave us the age-specific incidence. When we multiplied it by the number of inhabitants in the age group in the standard year, we obtained what we called an adjusted number of patients in the age group for the given year. We added the adjusted numbers over all the age groups in the given year to obtain an adjusted number of patients in that year.

To calculate the average age of patients in a given year, we followed the idea that is used when we calculate the mean when only a histogram is presented. We took the midpoint of each group in a given year, multiplied it by the adjusted number of patients in the group and summed the products up over all the groups in the given year. When the sum of products obtained in this manner was divided

by the adjusted number of patients in that year, it gave us the adjusted average age in that year.

Unfortunately, major administrative changes were made as to the division of the country into smaller regions during the year 2001. These changes made the distributions of ages incomparable and intractable. This was the reason why we could make adjustment to numbers of patients and calculations of adjusted ages only beginning with the year 2002. Since the year 2017 was the last one in which the patients' data were recorded, sixteen years of adjusted numbers and ages of patients were available.

RESULTS

In the period 2002–2017, altogether 2230 patients with new diagnosis of melanoma were examined, 1102 men (49.4%) and 1128 women (51.6%). The cases of mucosal, desmoplastic melanoma and melanoma of unknown origin are also included in the whole number of melanomas examined in this period. The total number of patients (men, women) with new diagnosis of melanoma in every year of this period is shown in Table 1.

The statistical evaluation of the trend in the occurrence of new melanoma (total number of all kinds of melanomas) and the age of the diagnosis in the period of 2002–2017 was performed (adjusted number of patients with melanoma and adjusted average age of diagnosis) – Table 2. The difference in the occurrence of the total number of melanoma in the period 2002–2017 was not confirmed – Graph to Table 2. The change in age of melanoma diagnosis was not confirmed either. Regarding the histology, lentigo maligna and melanoma in situ was confirmed in 441 patients, melanoma nodulare in 388 patients and melanoma superficiale in 1404 patients, 55 of them suffered from multiple melanomas.

1) The evaluation, if there is some change in the occurrence of lentigo maligna, melanoma in situ, melanoma superficiale, and melanoma nodulare from the year 2002 to the year 2017 and if there is some change in age of diagnosis.

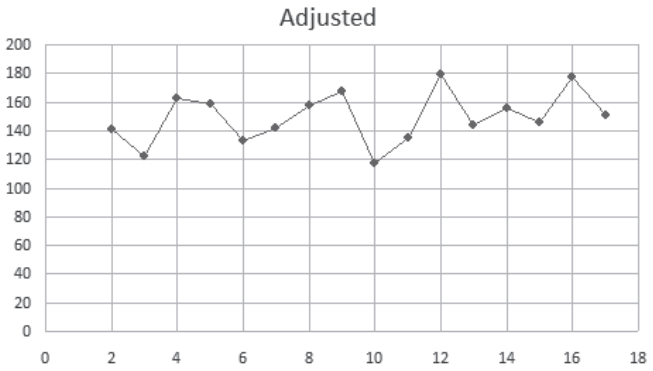
We studied the dependence of the adjusted number of patients on the year of the first occurrence/diagnosis. The statistical evaluation of the difference of the occurrence of melanoma nodulare, superficiale, lentigo malig-

Tab. 1 Number of patients with new diagnosis of melanoma in the years 2002–2017 (2230 patients = 100%).

Year	Men	%	Women	%	Total patients
2002	54	44.6	67	55.4	121
2003	49	45.8	58	54.2	107
2004	71	49.3	73	50.7	144
2005	72	51.8	67	48.2	139
2006	53	45.3	64	54.7	117
2007	68	52.3	62	47.7	130
2008	71	50.0	71	50.0	142
2009	85	55.2	69	44.8	154
2010	56	49.6	57	50.4	113
2011	59	44.4	74	55.6	133
2012	82	48.2	88	51.8	170
2013	63	46.3	73	53.7	136
2014	79	52.0	73	48.0	152
2015	76	52.4	69	47.6	145
2016	82	46.6	94	53.4	176
2017	82	54.3	69	45.7	151
Total patients	**1102**		**1128**		**2230**
p-value	0.887				

Tab. 2 The trend in the occurrence of new melanoma and the age of diagnosis in the period of 2002–2017 was performed (adjusted number of patients with melanoma and adjusted average age of diagnosis). The change in the occurrence of melanoma in the period 2002–2017 was not confirmed. The change in age of melanoma diagnosis was not confirmed either.

Year	Adjusted number of patients	Adjusted age of patients
2002	140.9	62.1
2003	122.0	59.3
2004	162.9	59.5
2005	158.5	62.9
2006	131.7	60.2
2007	141.8	60.2
2008	157.2	63.3
2009	167.7	61.3
2010	117.7	60.2
2011	134.8	59.8
2012	178.9	59.2
2013	143.8	60.5
2014	155.9	60.4
2015	145.9	60.7
2016	177.4	60.2
2017	151.0	61.3
p-value	0.230	0.617

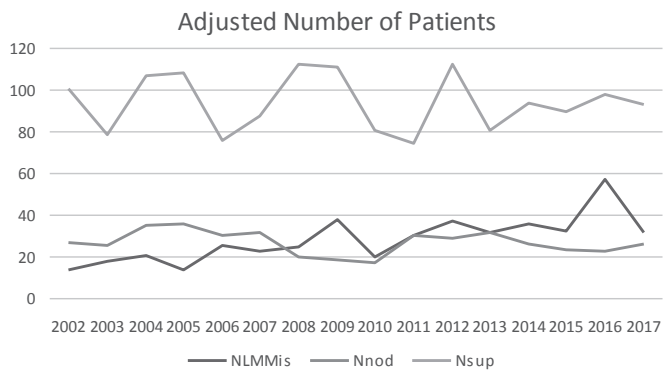


Graph to Table 2 The changes in the occurrence of new melanoma (total number of melanoma) in the period of 2002–2017 (adjusted number of patients with melanoma was calculated). The increase in the occurrence of melanoma in the period 2002–2017 was not confirmed, (x – axis: year, y – axis: the adjusted number of patients with all kinds of melanomas).

na and melanoma in situ in the period of 2002–2017 was performed (adjusted number of patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ). The difference in the occurrence of melanoma nodulare and superficiale was not confirmed (*p-value* = 0.248, *p-value* = 0.753), the difference in the occurrence of lentigo maligna and melanoma in situ in the period 2002–2017 was confirmed (*p-value* = 0.00037) – Table 3, Graph to Table 3.

Tab. 3 The changes in the occurrence of melanoma nodulare, superficiale, lentigo maligna and melanoma in situ in the period of 2002–2017 was performed (adjusted number of patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ). The difference in the occurrence of melanoma nodulare and superficiale was not confirmed (*p-value* = 0.248, *p-value* = 0.753), the changes in the occurrence of lentigo maligna and melanoma in situ in the period 2002–2017 was confirmed (*p-value* = 0.0004*).

	Adjusted number of patients		
Year	Lentigo maligna, melanoma in situ	Melanoma nodulare	Melanoma superficiale
2002	13.7	26.7	100.5
2003	18.1	25.7	78.3
2004	20.4	35.4	107.1
2005	14.1	36.0	108.3
2006	25.5	30.3	75.9
2007	23.0	31.4	87.4
2008	24.6	20.3	112.4
2009	38.0	18.9	110.7
2010	20.2	17.0	80.5
2011	30.3	30.2	74.4
2012	37.5	28.9	112.5
2013	31.4	32.0	80.4
2014	36.0	26.2	93.7
2015	32.4	23.6	90.0
2016	57.3	22.4	97.7
2017	32	26	93
p-value	0.0004*	0.248	0.753



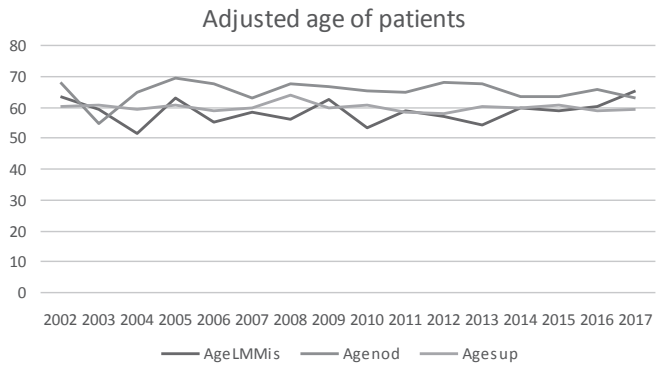
Graph to Table 3 The trend in the occurrence of melanoma nodulare, superficiale, lentigo maligna and melanoma in situ in period 2002–2017 was performed (adjusted number of patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ), (axis x – year, axis y – the adjusted number of patients with specific kind of melanoma, blue – lentigo maligna a and melanoma in situ, black – melanoma superficiale, red – melanoma nodulare).

The statistical evaluation of the change in the age of the diagnosis in patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ (adjusted average age of diagnosis) was performed. The difference in the age of patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ in the period of 2002–2017 was not confirmed – Table 4, Graph to Table 4.

2) The evaluation if the annual numbers of hours of sunshine could affect the number of patients suffering from melanoma nodulare, superficiale, lentigo maligna and melanoma in situ.

Tab. 4 The change in the age of the diagnosis in patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ (adjusted average age of diagnosis). The difference of the age in patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ in the period 2002–2017 was not confirmed.

	Adjusted average age of diagnosis		
Year	Lentigo maligna, melanoma in situ	Melanoma nodulare	Melanoma superficiale
2002	63.4	67.8	60.3
2003	59.4	54.6	60.8
2004	51.5	64.8	59.2
2005	63.1	69.4	60.9
2006	55.2	67.7	58.9
2007	58.6	63.0	59.6
2008	56.2	67.8	64.0
2009	62.3	66.8	59.9
2010	53.4	65.4	60.8
2011	58.7	64.8	58.2
2012	57.2	67.9	57.7
2013	54.3	67.5	60.2
2014	59.7	63.2	59.8
2015	58.8	63.3	60.8
2016	60.0	65.7	59.0
2017	65.3	63.0	59.5
p-value	0.632	0.933	0.405



Graph to Table 4 The trend of the age of the diagnosis in patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ with (adjusted average age of diagnosis (x – axis: year, y – axis: the adjusted age, blue – lentigo maligna a and melanoma in situ, black – melanoma superficiale, red – melanoma nodulare).

It turns out that the number of patients was independent of the number of hours of sunshine. All the *p-values* regarding the regression coefficients associated with the number of hours of sunshine were greater than 0.05 indicating the acceptance of the null hypothesis that these coefficients are zero which means independence. The length of sunshine in hours in every year in the period 2002–2017 and the total number of melanomas, number of melanoma nodulare, superficiale, lentigo maligna and melanoma in situ is shown in Table 5.

3) The evaluation if there is some relation between the annual numbers of hours of sunshine and the body site of melanoma (lentigo maligna, melanoma in situ, melanoma superficiale, and melanoma nodulare.

We also examined the effect of the number of hours of sunshine per year on numbers of melanoma with various locations on the body. Upper, lower limbs, and face were put together as they form a location exposed to sun. We used a regression model to determine whether there is any dependence of the number of melanomas on hours of sunshine. The relation between the number of melanomas (lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale) in body site exposed to sun (face, upper limbs, lower limbs) and the annual length of sunshine in hours is shown in Table 6. In all the cases the *p-values* were larger than 0.05, which means no dependence was confirmed. The relation between number of melanomas on face (lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale) and the annual length of sunshine in hours was not confirmed either – Table 7. In all the cases the *p-values* were larger than 0.05, which means no dependence.We also included the number of hours from the previous one year and two years as a lag variable because it could affect the formation of melanoma in the following years – the relation was not confirmed either.

DISCUSSION

According to the literature, the incidence of melanoma steadily increases in both sexes and further improvement in primary prevention and early detection strategies is crucial (19). Yet the epidemiologic, genomic, and anatomic profiles of the disease significantly differ across the world and mostly depend on a constellation of environmental and (epi) genetic factors. In this study, we evaluated the changes in epidemiology of melanoma in the period from the year 2002 to the year 2017 in Eastern Bohemia region in the Czech Republic in central Europe. There are 551 thausand inhabitants in the region and its area is 4759 square km. The advantage of our study is that all patients included in this study were personaly examined and were followed up at the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech Republic. We studied the dependence of the adjusted number of patients on the year of the first occurrence/diagnosis. The statistically important changes in the occurrence of melanoma nodulare and superficiale were not confirmed, but the increase in the occurrence lentigo

Tab. 5 The annual length of the sunshine in hours in the period 2002–2017 and the number of melanomas. The relation between total number of melanomas, lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale and annual length of sunshine in hours was not confirmed. We also included the number of hours from the previous one year* and two years* as a lag variable because it could effect the formation of melanoma in the following years – the relation was not confirmed.

		Adjusted number of patients			
Year	Annual sunshine hours	Lentigo maligna, melanoma in situ	Melanoma nodulare	Melanoma superficiale	Total number of melanoma
2002	1795	13.7	26.7	100.5	140.9
2003	2283	18.1	25.7	78.3	122.0
2004	1791	20.4	35.4	107.1	162.9
2005	2005	14.1	36.0	108.3	158.5
2006	1939	25.5	30.3	75.9	131.7
2007	1876	23.0	31.4	87.4	141.8
2008	1784	24.6	20.3	112.4	157.2
2009	1715	38.0	18.9	110.7	167.7
2010	1700	20.2	17.0	80.5	117.7
2011	1981	30.3	30.2	74.4	134.8
2012	1931	37.5	28.9	112.5	178.9
2013	1902	31.4	32.0	80.4	143.8
2014	1729	36.0	26.2	93.7	155.9
2015	1903	32.4	23.6	90.0	145.9
2016	1710	57.3	22.4	97.7	177.4
2017	1690	32	26	93	151.0
p-value		0.673	0.343	0.529	0.073
*p-value**		0.191	0.113	0.524	0.076

maligna and melanoma in situ in the period 2002–2017 was confirmed. We would like to make a comment on the fact, that it is not true, that the number of melanomas of nodular and superficial type increases. The total number of melanomas according to the adjusted calculation does not increase either. Nor did we confirm the difference in age of melanoma diagnosis (melanoma superficiale, melanoma nodulare, lentigo maligna and malenoma in situ) – the age of melanoma diagnosis is approximately 60 years. Also, we did not confirme the differnece of the occurrence of melanoma between men and women. These results are in contrast to other studies. Data on melanoma from the majority of countries show a rapid increase of the incidence of this type of cancer with a slowing of the rate of incidence in the period of 1990–2000. Males are approximately 1.5 times more likely to develop a melanoma than females while, according to other studies, the different prevalence in both sexes must be analyzed in relation to age: the incidence rate of melanoma is grater in women than in men until they reach the age of 40 years, however, by 75 years of age, the incidence is almost 3-times as high in men versus women (20). On the other hand, a recent study of the Centers for Disease Control and Prevention database suggests that incidence in New England states may be decreasing (10). At this study, melanoma death and incidence rates per state during 2003 and 2013 were recorded. Rates were per 100 000 persons and were age-adjusted to the 2000 standard population of the US Census Bureau's population projections series (10). Additional factors may have contributed to the observed increased melanoma incidence. Melanoma in situ (stage 0) lesions represent an increasingly larger percentage of the overall increase in melanoma incidence. For example, while there have been on average 2.6% annual increases in all US melanoma diagnoses in recent years, melanoma in situ diagnoses increased at an annual rate of 9.5%. Similar trends have been noted in Europe and Australia (21, 22). These results are in agreement with the results of our study. The increased proportion of early-stage lesions suggests that factors related to overdiagnosis, screening, an increased number of biopsy specimens, and incomplete reporting may have contributed to the increased incidence of melanoma. One explanation for increased melanoma incidence with stable mortality is the misclassification by pathologists of biologically benign melanocytic lesions as melanoma. Although the histologic criteria for melanoma have been well-defined, it is not possible to predict the biologic behavior of lesions that share features overlapping with nevi and melanoma (23–26). On the other hand, it has been suggested that the melanoma "epidemic" is primarily the result of previous underdiagnosis rather than current overdiagnosis, resulting from improved

Tab. 6 The relation between number of melanomas (lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale) in body **site exposed to sun** (face, upper limbs, lower limbs) and the annual the length of sunshine in hours was not confirmed. We also included the number of hours from the previous one year* and two years as a lag variable because it could effect the formation of melanoma in the following years – the relation was not confirmed.

		Adjusted number of melanomas		
Year	Annual sunshine hours	Lentigo maligna, melanoma in situ exposed	Melanoma nodulare exposed	Melanoma superficiale exposed
2002	1795	5.8	16.9	51.6
2003	2283	10.9	10.4	48.4
2004	1791	11.1	15.7	49.9
2005	2005	5.9	21.5	58.7
2006	1939	15.5	15.3	49.6
2007	1876	11.5	15.9	38.8
2008	1784	13.1	13.1	64.4
2009	1715	18.9	10.3	51.9
2010	1700	10.9	9.8	37.9
2011	1981	15.5	16.8	30.1
2012	1931	19.7	20.7	64.3
2013	1902	19.3	14.1	41.6
2014	1729	17.7	9.5	51.1
2015	1903	14.4	15.3	42.8
2016	1710	33.1	6.1	52.9
2017	1690	18	12	61
p-value		0.145	0.185	0.852
*p-value**		0.114	0.413	0.969

Tab. 7 The relation between number of melanomas on the **face** (lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale) and the annual length of sunshine in hours was not confirmed. We also included the number of hours from the previous one year* and two years as a lag variable because it could effect the formation of melanoma in the following years – the relation was not confirmed.

		Adjusted number of melanomas on the face		
Year	Annual sunshine hours	Lentigo maligna, melanoma in situ	Melanoma nodulare	Melanoma superficiale
2002	1795	3.6	4.1	8.2
2003	2283	6.3	3.4	10.3
2004	1791	3.5	1.1	12.8
2005	2005	2.8	4.7	17.5
2006	1939	5.5	3.5	6.5
2007	1876	4.6	4.9	7.1
2008	1784	2.5	7.1	16.8
2009	1715	5.8	2.5	15.1
2010	1700	1.5	1.8	10.6
2011	1981	4.1	2.6	6.7
2012	1931	5.3	2.7	14.3
2013	1902	3.1	2.1	8.8
2014	1729	4.9	2.1	11.1
2015	1903	2.1	5.2	5.3
2016	1710	9.1	1.1	13.1
2017	1690	7	2	18
p-value		0.818	0.191	0.468
*p-value**		0.321	0.232	0.706

histologic diagnostic criteria that allow melanomas to be recognized more accurately and at earlier stages (27). There is also an important role of screening and increased biopsies. Skin cancer screenings sponsored by the American Academy of Dermatology began in 1985 and since that time increased melanoma awareness has resulted in an increasing fraction of the population being screened for melanoma (28, 29). Several studies have documented a correlation of increasing melanoma incidence with biopsy (30). Although the majority of increased melanoma diagnoses are represented by thin lesions, diagnosis of thicker lesions has also increased over the past decades (31). Melanoma incidence has increased without regard to socioeconomic status, which is a surrogate marker for access to care and screening, (32).

We evaluated also the relation between the length of sunshine in hours in every year in the period 2002–2017 and the total number of melanomas. It turns out that the number of patients was independent of the number of hours of sunshine. We also examined the effect of the number of hours of sunshine per year on numbers of melanoma with various locations on the body. The relation between the number of melanomas (lentigo maligna, melanoma in situ, melanoma nodulare, melanoma superficiale) in body site exposed to sun (face, upper limbs and lower limbs) and annual length of sunshine in hours was not confirmed. We also included the number of hours from the previous one year and two years as a lag variable because it could effect the formation of melanoma in the following years – the effect was not confirmed. According to the literature, there is a strong evidence that an intermittent pattern of sun exposure increases melanoma risk. Chronic sun exposure shows no association, or a weak inverse association with melanoma risk. Total lifetime sun exposure is positively associated with melanoma risk, but the relationship is weaker than that for intermittent sun exposure. *Sunburn* is a marker of an intermittent pattern of sun exposure and there is a tendency for greater consistency of positive associations for sunburn than for intermittent exposure; significantly higher risk was found for intermittent than chronic exposure among studies that published results for both exposures (33–42).The role of ultraviolet radiation exposure as a leading environmental cause of melanoma is supported by a wealth of descriptive evidence in the past, including a high prevalence of melanoma in populations that migrated from a low to a high ambient ultraviolet radiation environment, a higher incidence in fair skinned compared with darker skinned individuals and a latitude dependent rise in melanoma rates among white populations with proximity to the equator (43, 44). However, differences in rates between indoor and outdoor workers and variations in the anatomical distribution of the tumour suggest a complex association of melanoma with ultraviolet radiation that does not confirm a straightforward dose relationship model. A history of intermittent exposure to excess ultraviolet radiation doses and of painful sunburns, as a marker of host sensitivity, were a consistent finding in the majority of case–control studies and were confirmed in recent systematic reviews (42, 45).

According to the study by Swerdlow, the incidence of malignant melanoma of the skin has risen rapidly in England and Wales, especially in women. Mean incidences in the 14 English health regions and Wales correlated negatively with latitude and positively with hours of sunshine, suggesting that exposure to sunshine was an important causal factor. Male and female incidences within a region tended to show similar yearly fluctuations, implying a common factor affecting the incidence in both men and women with a short latent period of action. This factor may be exposure to sunshine, which may cause melanoma after an induction period of about two years; for women the incidence of melanoma in the regions of England and Wales correlated positively with hours of sunshine two years earlier (46).

Apart from environmental risk factors, phenotypic and genetic characteristics also have been consistently associated with an increased risk of melanoma development. Additionally, melanoma seems to appear more commonly in immunosuppressed patients, including patients with prior organ transplantation, hematologic malignancies, or human immunodeficiency virus infection, as well as patients taking immunosuppressive medication (47–59).

CONCLUSION

The number of patients with lentigo maligna and melanoma in situ increased. The number of melanomas of nodular and superficial type does not increase and the total number of melanomas does not increase either. No difference of the age in patients with melanoma nodulare, superficiale, lentigo maligna and melanoma in situ was confirmed. No relation of the annual numbers of hours of sunshine to the number of patients with new diagnosis of melanoma and to the body site of melanoma was confirmed either.

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Experimental Evaluation of the Impact of Gadolinium Orthovanadate GdVO4:Eu3+ Nanoparticles on the Carrageenan-Induced Intestinal Inflammation

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ABSTRACT

Aim: To evaluate the effects of orally administered gadolinium orthovanadate $GdVO_4:Eu^{3+}$ nanoparticles (VNPs) on the course of chronic carrageenan-induced intestinal inflammation.

Methods: Samples of small intestinal tissue were collected from four groups of rats (intact, after administration of VNPs, with carrageenan-induced intestinal inflammation, with carrageenan-induced intestinal inflammation orally exposed to VNPs) to assess the intestinal morphology and HSP90α expression. Levels of seromucoid, C-reactive protein, TNF-α, IL-1β and IL-10 were determined in blood serum.

Results: Oral exposure to VNPs was associated with neither elevation of inflammation markers in blood serum nor HSP90α overexpression in the small intestine, i.e. no toxic effects of VNPs were observed. Carrageenan-induced intestinal inflammation was accompanied by higher levels of TNF-α and IL-1β, as well as HSP90α upregulation in the intestinal mucosa, compared with controls. Administration of VNPs to rats with enteritis did not lead to statistically significant changes in concentrations of circulating pro-inflammatory cytokines with the trend towards their increase.

Conclusion: No adverse effects were observed in rats orally exposed to VNPs at a dose of 20 μg/kg during two weeks. Using the experimental model of carrageenan-induced enteritis, it was demonstrated that VNPs at the dose used in our study did not affect the course of intestinal inflammation.

KEYWORDS

nanoparticles; intestinal inflammation; HSP90α; rats; carrageenan

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INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by a chronic intestinal inflammation and includes two subtypes: Crohn's disease (CD) and ulcerative colitis (UC) (1). In CD, the inflammation is transmural and may affect the entire gut, whereas in UC it is mainly limited to the mucosal layer of the large intestine (2). IBD is a multifaceted disease whose development is associated with complex interactions between genetic and environmental factors, including features of intestinal microbiota, abnormalities of the innate immune system, dietary habits, etc. (3). Its conventional treatment includes 5-aminosalicylate, glucocorticoid drugs, methotraxate, azathioprine, and anti-tumor necrosis factor (TNF) agents such as infliximab, adalimumab, golimumab. Furthermore, IL-12/23 antagonists (ustekinumab), inhibitors of intestinal lymphocyte trafficking (vedolizumab, a monoclonal antibody to the α4β7 integrin), and small molecule inhibitors of Janus kinases, including tofacitinib, are currently available in the market (4–8). Nevertheless, the development of novel therapeutic agents for the treatment of IBD remains of huge importance, since the current first-line anti-TNF treatment may be ineffective or the intolerance to anti-TNF agents can emerge in patients (9).

There is strong evidence that IBD is accompanied by the excessive generation of reactive oxygen species (ROS) and subsequent development of oxidative stress (10, 11). Overproduction of ROS in IBD results in oxidative damage to macromolecules (lipid peroxidation and protein oxidative modification), loss of cell membrane integrity, low ATP production by mitochondria, apoptosis, etc. (10, 12). This fact substantiates the search for novel, effective antioxidant-based agents for the treatment of IBD (13). In particular, the therapeutic potential of nanoparticles with antioxidant properties has been studied for decades. Converging lines of evidence indicate that they act as ROS scavengers (14, 15). It has been reported that gadolinium orthovanadate $GdVO_4:Eu^{3+}$ nanoparticles (VNPs) can scavenge free radicals *in vitro* (16). However, little is known about the therapeutic action of VNPs *in vivo*. To assess the therapeutic potential of VNPs, we have chosen the already characterized experimental model of carrageenan-induced intestinal inflammation (17–19).

The aim of our research was to study the impact of orally administered VNPs on the course of carrageenan-induced intestinal inflammation.

MATERIALS AND METHODS

1. DESIGN OF THE STUDY, CHARACTERISTICS OF ANIMALS AND GROUPS

Fifty female WAG rats weighing 160–190 g were provided by the vivarium of Kharkiv National Medical University. They were randomly subdivided into five equal groups (n = 10). Carrageenan-induced intestinal inflammation was induced in the rats from groups A and B. The animals from group B were orally administered a water solution of VNPs at a dose of 20 μg/kg of weight against the background of intestinal inflammation. Group C included the intact animals fed on a standard diet and obtained a solution of VNPs at a dose of 20 μg/kg of weight. Groups D_1 and D_2 served as controls and consisted of intact rats. The rats were housed in cages. They were maintained in standard laboratory conditions at room temperature (24 ± 2 °C). Access to food was free. All the animals were sacrificed. Blood samples were collected to prepare serum for evaluating the systemic levels of inflammation markers. Furthermore, fragments of small intestine were sampled for immunohistochemical studies.

2. CHARACTERISTICS OF NANOPARTICLES

The synthesis of $GdVO_4:Eu^{3+}$ nanoparticle water colloidal solution was carried out in accordance with the method reported earlier (21). Briefly, 10 mL of aqueous solution of rare-earth chlorides (0.01 mol/L) was mixed with 8 mL of ethylenediaminetetraacetic acid disodium salt (EDTA 2 Na) solution (0.01 mol/L). It was followed by the addition of 8 mL Na_3VO_4 (0.01 mol/L) to the solution obtained dropwise (pH = 13). The mixture was intensively stirred by a magnetic stirrer until yellowish transparent solution was formed.

The colorless transparent solution obtained as a result scattered light under the side illumination (Tindal cone). The solution was cooled and dialyzed against water for 24 h to remove the excess of ions. A dialysis membrane with a molecular weight cutoff of 12 kDa with a pore size of approximately 2.5 nm was used. The composition of spindle-like nanoparticles – $Gd_{(0,9)}Eu_{(0,1)}VO_4$ – with average size of 8 × 25 nm was formed (Figure 1).

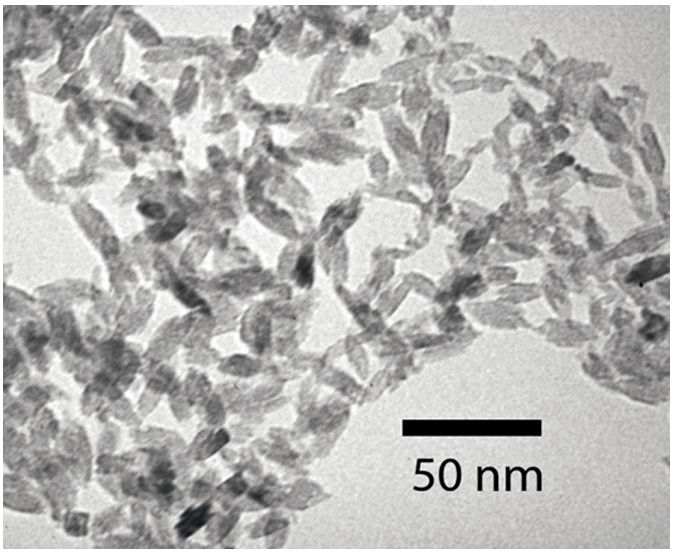

Fig. 1 TEM images of nanoparticles in colloidal solutions. VNPs with an average size of 8 × 25 nm used in this study are shown.

3. CARRAGEENAN-INDUCED INTESTINAL INFLAMMATION MODEL

Intestinal inflammation in the rats from group A and group B was induced by the daily oral administration of k-carrageenan-containing 1% processed *Eucheuma* seaweed (PES) in drinking water (140 mg per kg of weight) during 4 months. In addition to carrageenan, PES contained less

than 15% of algal cellulose. The solution was prepared at least 24 h prior to its administration and stored at low temperature (2 °C).

Development of intestinal inflammation was confirmed in each animal from groups A and B using routine histological staining techniques (hematoxylin and eosin staining, PAS reaction, and hallocyanine-chrome alum Einarsson's stain).

4. DETERMINATION OF SYSTEMIC LEVELS OF INFLAMMATORY AND ANTI-INFLAMMATORY BIOMARKERS

Systemic levels of pro-inflammatory cytokines TNF-α and IL-1β were assessed by commercially available ELISA kits purchased from *eBioScience* (Austria). The procedures were done strictly in accordance with manufacturers' instructions. Concentrations of TNF-α, IL-1β and IL-10 in blood serum were expressed in pg/ml. ELISA method was also used to assess the levels of anti-inflammatory cytokine IL-10 in blood serum of animals (eBioScience ELISA kit).

Furthermore, the levels of inflammatory markers such as seromucoid and C-reactive protein were determined in blood serum of rats from groups C and D_2 by routine techniques. Seromucoid and C-reactive protein levels were assessed using commercially available kits manufactured by *Filicit-Diagnostika* (Ukraine). Seromucoid levels were expressed in units of the Shank-Hoagland scale (SH units), whereas the content of C-reactive protein in blood serum was represented in mg/L. In addition, the content of middle molecules was determined in blood serum of animals from groups C and D_2 by the Gabrielyan's method to evaluate the severity of endogenous intoxication (22). Trichloroacetic acid was added to serum. Then the mixture was centrifuged during 20 minutes at 3000 rpm. After centrifugation the samples were 10-fold diluted with distilled water. After stirring, the measurement was performed at λ = 254 nm and at λ = 280 nm. The 280 nm / 254 nm absorbance ratio was calculated. Concentrations of middle molecules were expressed in standard units.

5. IMMUNOHISTOCHEMICAL EVALUATION OF HSP90α EXPRESSION IN THE SMALL INTESTINE

Tissue samples of small intestine were fixed in a 10% formalin solution. Then paraffin-embedded tissues were used to obtain 4-μm-thick sections, which were immunostained using commercially available mouse monoclonal antibodies to HSP90α purchased from *Thermo Fischer Scientific* (USA). After incubation with the primary antibodies, the microslides were treated with an anti-(mouse IgG)–horseradish peroxidase conjugate. Visualization was carried out using 3,3′-diaminobenzidine (DAB) staining. The presence of brown coloration indicated the positive reaction.

6. BIOETHICS

All the experimental procedures were performed following the guidelines of EU Directive 2010/63/EU on the protection of animals used for scientific purposes, which is based on the Council of Europe Convection for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123).

7. STATISTICAL ANALYSIS

Numerical data were analyzed using the Kruskal-Wallis ANOVA test if three independent parameters were compared. It was followed by the Dunn's multiple comparison post-hoc test. Two independent groups of variables were compared by a non-parametric Mann-Whitney U test. It was selected based on the outcome of the Shapiro-Wilk and Kolmogorov-Smirnov normality tests. Differences were considered statistically significant at $p < 0.05$. The data obtained in our research were analyzed with GraphPad Prism 5.0 (GraphPad software, USA).

RESULTS

To assess the toxicity and pro-inflammatory potential of VNPs, we determined the levels IL-1β, middle molecules, C-reactive protein, and seromucoid in blood serum of rats from group C and compared them with the corresponding parameters of animals from the control group D_2. The concentrations of pro-inflammatory IL-1β, middle molecules and acute phase proteins (seromucoid and C-reactive protein) in blood serum of rats orally exposed to the solution of VNPs were statistically insignificantly ($p > 0.05$) higher than in the animals from the control group D_2 (Table 1).

Morphological studies of small intestine in rats from both control groups demonstrated that the epithelial layer of villi was intact. Epithelial cells at the top of intestinal villi regenerated well. No significant leukocyte infiltrate was found (Figures 2, 3).

Immunolabelling allowed us to find out that HSP90α was primarily expressed in the cytosol. HSP90α-positive cells were detected both in the epithelial lining and intestinal glands. Immunostaining was also observed in the lami-

Tab. 1 Evaluation of orally administered $GdVO_4$ nanoparticle toxicity (Me (IQR)).

Groups of animals / Blood serum parameters (units)	Group D_2 (intact animals, n = 10)	Group C (rats orally exposed to VNPs, n = 10)	p value
IL-1β (pg/ml)	62.32 (47.23; 69.89)	64.21 (55.36; 75.54)	0.529
C-reactive protein (mg/L)	1.12 (0.97; 1.18)	1.20 (1.09; 1.30)	0.059
Seromucoid (SH units)	3.00 (2.88; 3.35)	3.45 (3.10; 3.68)	0.129
Middle molecules (standard units)	0.080 (0.074; 0.084)	0.083 (0.079; 0.088)	0.172

Note: Differences were considered statistically significant at $p < 0.05$

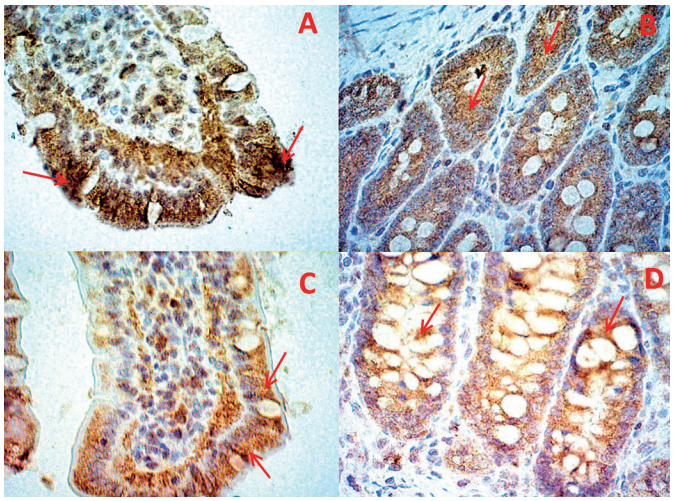

Fig. 2 Small intestinal mucosa immunostaining. A) Control group D_2. Positive HSP90α staining is found in the epithelial lining and the lamina propria (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×400. B) Control group D_2. HSP90α-positive cells are revealed in the intestinal glands (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×100. C). Group C. The HSP90α expression pattern after the oral administration of $GdVO_4$ nanoparticles does not differ from the control group D_2. HSP90α is moderately expressed in the lamina propria and epitheliocytes (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×400. D) Group C. HSP90α is expressed at the moderate level in the intestinal glands (marked with red arrows). No HSP90α overexpression was found compared with the control animals. Immunohistochemical reaction with antibodies to HSP90α. ×100.

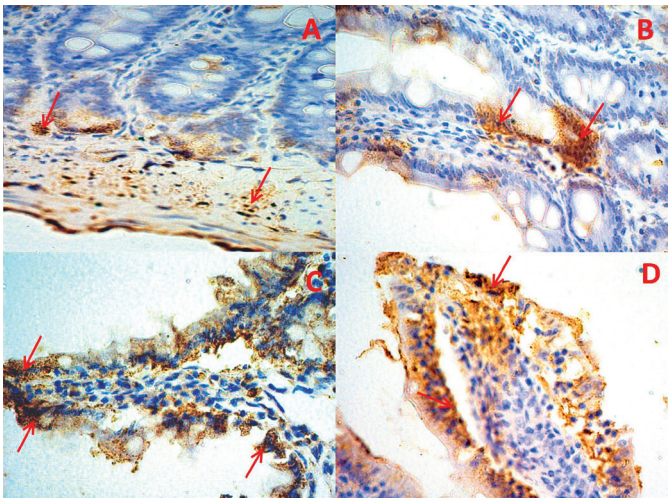

Fig. 3 Small intestinal mucosa immunostaining. A) Control group D_1. HSP90α staining is weak and the amount of HSP90α-labelled cells is limited. HSP90α-positive cells are found both in the lamina propria and glands (marked with red arrows). Immunohistochemical reaction with anti-bodies to HSP90α. ×100. B) Control group D_1. Many cells are HSP90α-negative. The foci of HSP90α positive immunostaining are shown (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×100. C). Group A. A more pronounced HSP90α expression is found in the lamina propria and epithelial cells against the background of intestinal inflammation compared with the control group (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×100. D) Group A. HSP90α overexpression is observed in the intestinal villi (marked with red arrows). The amount of HSP90α-positive cells is higher than in the control group. Immunohistochemical reaction with antibodies to HSP90α. ×100.

na propria. However, the amount of HSP90α-stained cells both in the lamina propria and intestinal epitheliocytes was moderate (Figures 2, 3).

Evaluation of the impact of VNPs on small intestine morphology and HSP90α expression showed that the oral exposure of rats to nanoparticles affected neither histological features of the small intestine nor the chaperone expression. No signs of intestinal inflammation were revealed in rats from group C. Epithelium and villi were not damaged. The leukocyte infiltrate was as non-abundant as in both control groups D_1 and D_2 (Figure 2).

In this study, we observed almost the same pattern of HSP90α expression in rats orally exposed to VNPs as in groups D_1 and D_2. Qualitative analysis indicated that HSP90α was moderately expressed in the lamina propria, epithelial cells, and glands (Figure 2).

We observed the statistically significant ($p < 0.0001$ and $p = 0.005$, respectively) 4.1-fold and 1.8-fold increase in the concentrations of circulating pro-inflammatory cytokines TNF-α and IL-1β in rats from group A compared with the control group D_1 (Table 2). The content of anti-inflammatory IL-10 did not differ from controls ($p > 0.05$). Levels of TNF-α and IL-1β in rats with carrageenan-induced inflammation treated with VNPs was higher than in group A. However, the difference was found to be statistically insignificant. Circulating IL-10 levels in animals from group B were statistically insignificantly ($p > 0.05$) higher than in both group D_1 and group A (Table 2).

Tab. 2 Levels of pro-inflammatory markers in untreated and treated rats with carrageenan-induced intestinal inflammation (Me (IQR)).

Blood serum parameters (units)	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-10 (pg/ml)
Groups of animals			
Group D_1 (intact animals, n = 10)	38.9 (33.2; 48.4)	52.01 (36.17; 67.04)	54.2 (48.8; 64.3)
Group A (rats with carrageenan-induced intestinal inflammation, n = 10)	161.2 (114.6; 236.8)	96.08 (80.89; 118.51)	53.0 (47.7; 59.8)
Group B (rats with carrageenan-induced intestinal inflammation treated with VNPs, n = 10)	223.7 (112.4; 360.4)	140.03 (99.91; 180.88)	57.6 (48.2; 72.6)
p value	$p_1 < 0.0001$* $p_2 > 0.05$	$p_1 = 0.0005$* $p_2 > 0.05$	$p = 0.719$

Note: Differences were considered statistically significant at $p < 0.05$ (* indicates the statistical significance of differences between two independent variables). p_1 is the difference between groups D_1 and A, while p_2 is the difference between groups A and B.

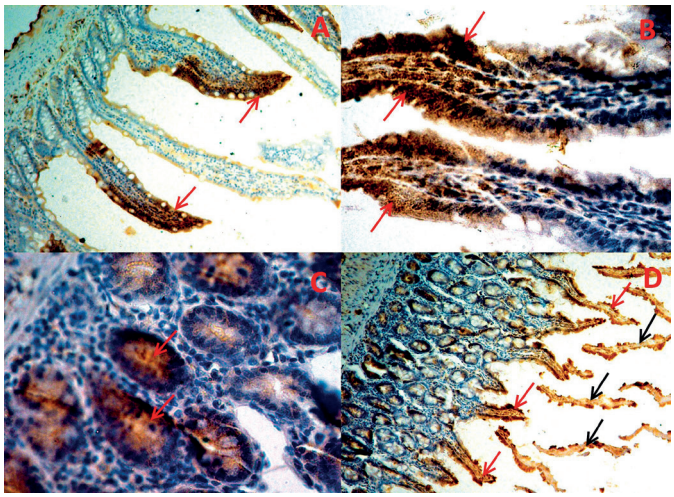

Fig. 4. Small intestinal mucosa immunostaining. Group B. Rats with carrageenan-induced intestinal inflammation treated with $GdVO_4$ nanoparticles A) Strong HSP90α immunostaining is observed at the top of intestinal villi (marked with red arrows). However, the epithelial layer below is preserved. Macrophage infiltration can be seen. Immunohistochemical reaction with antibodies to HSP90α. ×100. B) Very significant HSP90α labeling is found in the intestinal villi against the background of leukocyte infiltration. HSP90α-positive cells are marked with red arrows. Immunohistochemical reaction with antibodies to HSP90α. ×400. C). Strong HSP90α staining is revealed in the intestinal glands (marked with red arrows). Immunohistochemical reaction with antibodies to HSP90α. ×400. D) Fragments of the destroyed villi with strong HSP90α immunostaining are seen in the small intestinal lumen (marked with red arrows). Furthermore, the strongest HSP90α staining was found at the top of villi (marked with black arrows). Immunohistochemical reaction with antibodies to HSP90α. ×100.

We demonstrated that the oral intake of carrageenan-containing solution by animals from group A resulted in the development of intestinal inflammation, evidenced by the damage to the intestinal villi, especially at their top. Furthermore, the damaged intestinal villi lacked epithelial cells in some regions. The lamina propria both in the villi and at the level of glands was significantly infiltrated with macrophages (Figure 3).

Analysis of HSP90α immunostaining showed that the epithelial cells of villi were strongly labeled. Moreover, the significant HSP90α upregulation was detected in glandular epithelial cells, not only at the base of intestinal glands but also above. Both the number of HSP90α-labeled epithelial cells and the intensity of immunostaining were higher in group A compared with controls (Figure 3).

Administration of VNPs against the background of carrageenan-induced inflammation by rats from group B was associated with the leukocyte infiltration with the predominance of macrophages. The infiltration abundance in rats from group B did not differ significantly from group A. In addition, the villi with the undamaged epithelial lining were found. It is interesting to note that some regions of the intestinal wall contained villi with the destroyed tops, while the lower portions of villi were well epithelialized, indicating the rapid regeneration (Figure 4).

Strong HSP90α staining was primarily observed at the top of villi. However, some villi were either not or weakly immunostained. In some regions, the moderate HSP90α expression was revealed.

DISCUSSION

Nanotechnology has already shown its significant potential in the field of medicine. Biomedical application of nanoparticles seems to be promising therapeutic agents due to their relatively small size and unique characteristics (23, 24). Nevertheless, the possibility of administering nanoparticles as drugs raises concerns regarding their adverse effects and probable toxicity. Thus, we evaluated safety and oral exposure risks of VNPs. Our findings indicate that the oral consumption of VNPs during two weeks is not associated with the statistically significant changes in the content of circulating inflammatory markers such as IL-1β, seromucoid, and C-reactive proteins. Biochemical data are supported by the results of morphological studies. No morphological signs of intestinal inflammation were found in animals exposed to VNPs. Furthermore, the development of intoxication in response to VNPs oral consumption was not found, evidenced by the absence of middle molecules elevation in blood serum.

We also assessed expression of HSP90α, which is a molecular chaperone involved in the regulation of cellular proteostasis promoting protein folding and refolding in response to stress factors (25). It is worth mentioning that HSP90α is an isoform of the chaperone upregulated in stress conditions, while its β form is expressed constitutively (26). It has been reported that HSP90α is upregulated during inflammation (including the intestinal one) and in response to oxidative stress (27). No changes in its expression confirm the data of biochemical studies and indicate the absence of inflammation in the intestine after the oral consumption of VNPs.

Our biochemical and histological findings suggest that VNPs have no toxic effects when exposed orally at a dose of 20 μg/kg of weight during two weeks. Based on our findings, VNPs cannot be considered pro-inflammatory agents. Such conclusion is consistent with data of studies focused on elucidation of VNP properties and biological effects (28–31).

The next task of our research was to assess the therapeutic potential of VNPs in intestinal inflammation caused by oral consumption of a carrageenan-containing solution. Carrageenans are sulfated hydrocolloids of polysaccharide nature extracted from microalgae and used in food industry as thickeners, stabilizers, and emulsifiers (32). In addition, this food additive can trigger the development of intestinal inflammation as a result of its oral consumption by rats (17–20). The development of inflammation in the rats from group A was confirmed in this study histologically and biochemically. Changes in the blood serum cytokine profile observed in our study indicated the active inflammatory process in the intestine. We believe that elevation of circulating pro-inflammatory TNF-α and IL-1β is mediated, at least partially by ROS, whose overexpression is known to be stimulated by carrageenan (33). In our previous study, we linked HSP90α intestinal overexpression revealed in this study with the development of oxidative stress in carrageenan-induced enteritis as well (17). This overexpression seems to be protective and aim at providing re-folding of damaged protein to promote survival of enterocytes.

VNPs did not stimulate the synthesis of anti-inflammatory IL-10 and even worsened the imbalance between circulating pro-inflammatory and anti-inflammatory cytokines, albeit the difference was statistically insignificant. Thus, we believe that VNPs at the dose used in our study does not affect the course of inflammation. It is worth noting that that their oral consumption does not lead to the intensification of inflammatory response. Furthermore, the strongest HSP90α immunostaining in animals from group B is observed at the top of intestinal villi and seem to be compensatory. However, this was not sufficient to provide the cell survival and resulted in the reduced viability of cells and activation of cell death. In Figure 4 (D) we can notice such damaged villi alienated from the mucosa in the lumen of small intestine with strong HSP90α expression. In response to cell death, the regeneration should be activated. And we have managed to find the areas of extensive regeneration of enterocytes at the bottom of villi. We believe that such regeneration may be protective and can be associated with the action of VNPs. Such regions with so intense regeneration were not found in non-treated rats.

CONCLUSION

Oral exposure to VNPs at a dose of 20 μg/kg of weight by rats during two weeks showed no adverse effects. VNPs neither affect the level of circulating inflammatory markers nor influence the small intestinal morphology. Furthermore, their oral intake was not associated with overexpression of ROS-inducable chaperone HSP90α in the intestinal mucosa. Evaluation of VNP therapeutic potential using an experimental model of carrageenan-induced enteritis demonstrated no significant effects on the course of inflammation. However, HSP90α overexpression in rats with carrageenan-induced intestinal inflammation treated with VNPs prevailed at the top of villi in a combination with the active proliferation at the bottom.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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Comparison of Subthreshold 532 nm Diode Micropulse Laser with Conventional Laser Photocoagulation in the Treatment of Non-Centre Involved Clinically Significant Diabetic Macular Edema

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ABSTRACT

Background: The aim of the study was to investigate the effect of the 532 nm (green) diode subthreshold micropulse laser (SML) in the treatment of non-centre involved clinically significant macular edema (CSME) in comparison to the conventional laser photocoagulation (CLP).

Methods: A total of 60 eyes of patients diagnosed with non-centre involved CSME were randomly divided into two groups. SML photocoagulation was performed in the first group (G1), while CLP in the second one (G2). Central macular thickness (CMT) and best corrected visual acuity (BCVA) were measured prior to treatment and at 3 and 6 months after intervention.

Results: G1 participants had significantly better CMT at 6 months after laser application ($p = 0.04$) compared to G2. Additionally, CMT in both groups was significantly lower 6 months after laser application in comparison to baseline values (G1: $p < 0.001$, G2: $p = 0.002$). Moreover, significant improvement was detected 6 months after SML in G1 regarding BCVA compared to values before laser treatment ($p = 0.001$).

Conclusion: SML was more effective than CLP in reducing CMT and improving BCVA in patients with non-centre involved CSME. Therefore, it seems that SML can be a good substitute for CLP in DME treatment if confirmed in future studies.

KEYWORDS

micropulse laser; subthreshold laser; 532 nm; conventional laser photocoagulation; diabetic macular edema

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INTRODUCTION

Diabetic retinopathy (DR), the most common and severe ocular complication of diabetes mellitus (DM), remains the leading cause of preventable blindness in the working-age population in developed countries (1–3). Diabetic Macular Edema (DME), a frequent complication of DR, constitutes one of the main causes of visual impairment in DR patients (4–6). It is defined by the presence of retinal edema involving or threatening the fovea in patients with DM (7). According to epidemiologic studies, it is estimated that approximately one third of patients with DM have signs of DR, and one third of them suffer from vision threatening DR, including DME (8). The most severe spectrum of DME is clinically significant macular edema (CSME), which is defined as 1) retinal thickening (edema) at or within 500 μm of the center of the fovea or 2) hard exudates at or within 500 μm of the foveal center if associated with thickening of the adjacent retina and/or 3) zones of retinal thickening 1 disc area in size, at least part of which being within 1 disc diameter of the center (8).

Since the early treatment diabetic retinopathy study (ETDRS) (9, 10) showed that laser photocoagulation reduces the risk of visual acuity decrease by 50% in eyes with CSME, continuous-wave laser photocoagulation has been the standard treatment of DME for many years. Depending on the type of edema, conventional laser photocoagulation (CLP) pattern varies: focal photocoagulation is used for localized leakage areas and microaneurysms in focal DME, while grid pattern for diffuse edema (7). However, these methods have numerous disadvantages; among them deterioration of contrast sensitivity, of colour vision and of visual field (11) as well as potential complications, such as epiretinal fibrosis, subretinal scarring, choroidal neovascularization (CNV) and progressive enlargement of laser scars leading to foveal atrophy (12–14). These side effects have been associated with the spread of thermal energy from the single laser burns which contribute to collateral damage to the neighboring sensory retina and the choroid when continuous-wave mode is used (15).

To address potential collateral damage, micropulse lasers have been introduced. These lasers allow the management of DME. No scar or burn can be visualized with the subthreshold micropulse laser (SML) treatment (15). The subthreshold micropulse diode laser is available in different wavelenghts: 532 nm, 577 nm, or 810 nm. With micropulse mode, the laser energy is delivered in many repetitive short impulses [measured in microseconds (μs) – "micropulses"], within an "ON" cycle and an "OFF" cycle. The "ON" time, which is the duration of each micropulse, typically has a length of 100 to 300 μs, and the "OFF" time, which is the time between the pulses, has a duration of 1700 to 1900 μs (15).

The longer "OFF" interval plays a significant role in the protection of the overlying neural retina because it enables the tissues to "cool down". As a result, the diffusion of heat into the surrounding tissues is minimized and thus scarring is avoided. Former histological reports confirmed that the energy of SML affects almost selectively the melanocytes within the retinal pigment epithelium (PRE) with a minimum damage to the neural retina and choroidal layers (16). Laser power is set at a low level, so that the laser impact does not leave any visible lesion on the retina. In consequence, only a limited thermal impact is applied on the tissue, without exceeding the protein denaturation threshold of neural retina and without having any lethal effect (17). According to recent studies, still-viable RPE cells surrounding the burned areas appear a healing response to thermal injury by activating a therapeutic cellular cascade (18). In this way, vascular endothelial growth factor (VEGF) and neovascularization is suppressed, pigment epithelium-derived factor (PEDF) is up-regulated, the expression of other cytokines is modified, as well (19), resulting in the improvement of the retinal function, stabilizing visual acuity and decreasing macular edema (18, 20).

Therefore, the SML application can reduce the aforementioned complications induced by the laser heat associated with continuous-wave CLP and can lead to less negative impact on visual function. However, taking into account that SML uses smaller amount of energy per treatment, it may be possible that micropulse mode may not be as effective as continuous-wave CLP mode in the reduction of DME and therefore in the decrease of central macular thickness (CMT) (15).

To the best of our knowledge, there has been no clinical trial comparing the outcomes of 532 nm SML versus CLP in patients with non-centre involved CSME. Within this context, primary objective of this study was to investigate the efficacy of SML in the treatment of the non-centre involved CSME.

MATERIAL AND METHODS

SETTING

This is a prospective, comparative, randomized trial. Study protocol adhered to the tenets of the Declaration of Helsinki and written informed consent was provided by all participants. The institutional review board of Democritus University of Thrace approved the study protocol. The study was conducted at the Department of Ophthalmology in the University Hospital of Alexandroupolis, Greece, between January 2017 and June 2017.

PARTICIPANTS

Participants were enrolled from the Medical Retina Service of the hospital in a consecutive-if-eligible basis. Eligibility criteria included diagnosis of non-centre involved CSME. Patients populated randomly two distinct groups for the purposes of this study: 1) G1 group: patients that underwent SML, 2) G2 group: patients that underwent conventional focal laser photocoagulation. Exclusion criteria for all study groups included: 1) Former laser application and intravitreal anti-VEGF therapy, 2) eye conditions or other co-morbidities that could affect the disease status or the response to the treatment, 3) missing patient data, incomplete treatment protocol or incomplete patient monitoring.

EXAMINATION – LASER APPLICATION

In order to evaluate the efficacy of SML in the treatment of the non-centre involved CSME properly, we compared the results of the SML with those of the focal laser photocoagulation, the application of which has proven to be an effective and appropriate treatment for this particular condition. More specifically, we examined the change in best corrected visual acuity (BCVA) and the central macular thickness (CMT) after the aforementioned laser treatments.

At the initial visit, a detailed individual and family history was recorded for all patients. BCVA (Greek version of ETDRS chart) (21), CMT estimation using a spectral domain optical coherence tomography (SD-OCT) / scanning laser ophthalmolscopy (SLO) (Spectral OCT SLO, OPKO/OTI, Miami, FL) intraocular pressure (IOP) measurement using a Goldmann applanation tonometer, slit lamp examination and fundoscopy, as well as measurement of hemoglobin A1c (HbA1C) levels, were performed in all patients at the initial and at the 3 and 6 month-post-intervention visits.

The laser application (wavelength of 532 nm, green) was performed with Supra Scan 532 nm laser (Quantel Medical, Cedex, France) in all eyes by the same ophthalmologist as follows:

Laser treatment was performed using 532 nm micropulse laser with an Area-Centralis lens (Volk Optical Inc, Mentor, Ohio, USA). The micropulse laser power was derived from a test burn. The test burn was performed in the continuous-wave mode using a 100 μm spot diameter and a 200 ms duration in the nasal side outside the vascular arcade with the power titrated from 50 mW upward until a burn became barely visible. To perform the laser treatment, the laser was switched from continuous-wave emission mode to micropulse emission mode at 15% duty cycles and the power was doubled (100 mW) with a 100 ms exposure duration. The spot size was set at 50 to 100 μm and the number of spots varied according to the extension of DME. As regards conventional focal laser photocoagulation, a 50 μm spot diameter and a 100 ms duration was used. The power was adjusted according to each patients' needs.

STATISTICAL ANALYSIS

An a priori power analysis was performed. For an effect size of 0.8, 52 participants would be required, for the study to have a power of 0.8 at the significance level of 0.05. All data were collected in an Excel database and analysed statistically with the same software (Excel 2010, Microsoft Corp, Redmond, WA, USA).

The normality of measured data was evaluated using Kolmogorov-Smirnov test. Normal distribution data were assessed by Student's t-test. Non-parametric data were assessed with Mann–Whitney U test. All statistical tests were two-tailed. P-values less than 0.05 were considered statistically significant.

RESULTS

60 eyes from 60 patients (33 men, 27 women) diagnosed with non-centre involved CSME were included in this study. The mean age of the patients was 67.8 ± 8.05 years. Detailed demographic and clinical parameters are presented in Tables 1 and 2. Non-significant differences were detected with respect to age (p = 0.54), diabetes duration (p = 0.48), HbA1c (p = 0.72), and IOP (p = 0.87) No parameter demonstrated significant differences between the two groups before laser.

Tab. 1 Demographic and general characteristics of the two groups.

Variables		**G1**	**G2**	***p-value***
No.		30	30	
Sex	Male	17 (56.7%)	16 (53.3%)	0.86
	Female	13 (43.3%)	14 (46,7%)	
Mean ± SD				
Age (years)		67.6 ± 7.4	68 ± 8.7	0.54
Diabetes duration (years)		11.5 ± 10	12.5 ± 11	0.48
HbA1c (%)		7.2 ± 1.02	7.4 ± 1.04	0.72
IOP (mmHg)		17.98 ± 2.73	17.81 ± 2.89	0.87

G1: subthreshold micropulse laser Group, G2: conventional laser photocoagulation Group, HbA1c: Hemoglobin A1c, IOP: Intraocular Pressure, SD: Standard Deviation

Tab. 2 Group comparisons before laser.

Parameter (mean ± SD)	**G1**	**G2**	***p-value***
BCVA (ETDRS letters)	72.42 ± 14.50	71.25 ± 11.57	0.73
CMT (nm)	291.93 ± 67.24	303.5 ± 49.31	0.43

BCVA: best corrected visual acuity, CMT: central macular thickness, ETDRS: early treatment diabetic retinopathy study, G1: subthreshold micropulse laser Group, G2: conventional laser photocoagulation Group, SD: Standard Deviation

All comparisons after laser application are presented in Tables 3 and 4. Significant differences among groups' participants were not detected in the BCVA parameter at any timepoint. Indeed, in six months, the difference in BCVA was increased, but not at a significant level (p = 0.09). On the other hand, CMT in G1 was significantly lower 6 months after laser in comparison to G2 (p = 0.04), while no significant difference was detected for CMT in three months between G1 and G2 (p = 0.56).

Tab. 3 Group comparisons (3 months after laser).

Parameter (mean ± SD)	**G1**	**G2**	***p-value***
BCVA (ETDRS letters)	73.58 ± 11.84	70.25 ± 13.52	0.31
CMT (nm)	285.50 ± 87.52	298.67 ± 86.96	0.56

BCVA: best corrected visual acuity, CMT: central macular thickness, ETDRS: early treatment diabetic retinopathy study, G1: subthreshold micropulse laser Group, G2: conventional laser photocoagulation Group, SD: Standard Deviation

With respect to BCVA, participants in G1, treated with SML, demonstrated improved values at all follow-up timepoints, while participants in G2, treated with CLP, demonstrated a slight deterioration 3 months after laser. However, in 6 months, G2 showed a slight improvement in comparison to baseline value. Additionally, participants in

Tab. 4 Group comparisons (6 months after laser).

Parameter (mean ± SD)	G1	G2	*p-value*
BCVA (ETDRS letters)	77.50 ± 10.50†	72.42 ± 12.40	0.09
CMT (nm)	248.83 ± 56.33†	280.50 ± 59.41†	0.04*

BCVA: best corrected visual acuity, CMT: central macular thickness, ETDRS: early treatment diabetic retinopathy study, G1: subthreshold micropulse laser Group, G2: conventional laser photocoagulation Group, SD: Standard Deviation
* P < 0.05
† indicates significant difference with values before laser application

both groups demonstrated improved CMT values at each timepoint (Figures 1 and 2).

Three months after laser, both groups did not present significant differences in both parameters compared to baseline values (BCVA: G1: p = 0.52, G2: p = 0.67 / CMT: G1: p = 0.61, G2: p = 0.64). On the other hand, CMT in both groups was significantly lower 6 months after laser application in comparison to baseline values (G1: p < 0.001, G2: p = 0.002). Moreover, significant improvement was detected 6 months after micropulse laser in G1 regarding BCVA compared to values before laser treatment (p = 0.001), while no significant difference was found at the same timepoint in G2 after conventional focal laser photocoagulation (p = 0.30).

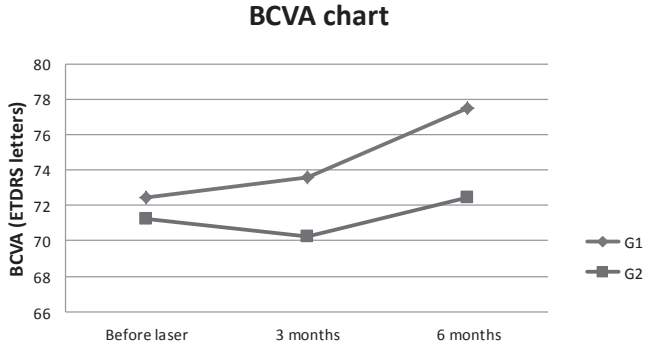
DISCUSSION

Nowadays, approximately 360 million people suffer from DM worldwide (22). By 2030, population with DM is estimated at a half billion (22). DR is a disease with an increasing prevalence in the general population, as average population age and dietary habits have changed. This disease now affects about 93 million people worldwide, of which 17 million suffer from Proliferative Diabetic Retinopathy (PDR) and 21 million from DME (23). Therefore, it is important to develop and apply treatments that are more efficient, accessible, less invasive and with the least possible side effects. Thus, more and more patients will comply with different treatment protocols that can prevent from significant visual loss.

Within this context, the evaluation of the relative efficacy of SML treatment versus CLP for the management of DME has become of major importance to retina specialists. In fact, several studies have dealt with the comparison of SML with CLP. Chen et al. (15) showed that the use of the SML results in slightly better visual acuity compared to the conventional laser, although the differences of the two groups are too small to be of clinical significance. However, according to them the two types of treatment appear to have a similar anatomical effect. Another study by Fazel et al. (24) showed that the SML was more effective than the CLP in reducing CMT and Central Macular Volume (CMV) as well as in improving visual acuity. Qiao et al. (25) reported that the SML results in an equal improvement in visual acuity, contrast sensitivity and reduction of the DME compared to the conventional ETDRS focal photocoagulation protocol, but clearly with less damage to the retina. In addition, other studies (18, 26, 27) showed minimal anatomical, clinically not visible, retinal changes using OCT, microperimetry and fluorescein angiography when a SML treatment was applied confirming the safety of this therapeutic method.

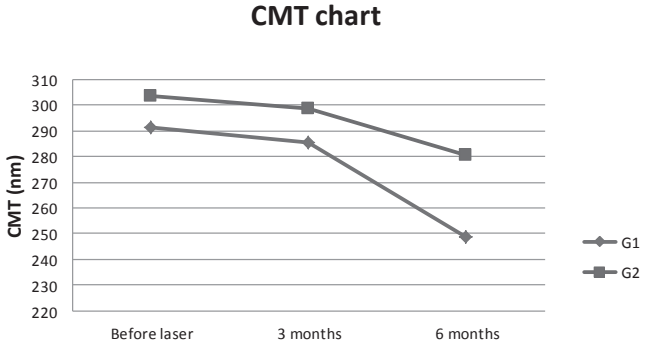
When attempting to interpret former published reports, certain caution should be applied regarding the laser wavelength used. The majority of former investigators have used either 577 nm (yellow) (27–29) or 810 nm (red) (24, 30–34). There are only few studies (16, 20, 35–37) that have used SML of 532 nm (green) for the treatment of DME. However, within the published studies that used 532 nm, three examined the frequency-doubled neodymium: YAG laser of 532 nm (20, 36, 37), while the study of Yu et al. (16), which compared subthreshold 810-nm and 532-nm diode micropulse laser on the retina by histologic examination and differential protein expression, used rabbits' eyes. Finally, Bhatnagar et al. (35) examined if SD-OCT could be used to detect subthreshold retinal burns created using the micropulse diode laser of 532 nm. Consequently, to our knowledge, the present study is the first comparative study that investigates the effect of subthreshold diode laser micropulse in comparison with continuous-wave CLP in the treatment of the non-centre involved CSME in a clinical setting.

Our study outcomes indicated non-inferiority of the SML when compared to continuous-wave CLP. In fact,



G1 = patients underwent subthreshold micropulse laser
G2 = patients underwent conventional focal laser photocoagulation
BCVA = best corrected visual acuity

Fig. 1 Best corrected visual acuity.



G1 = patients underwent subthreshold micropulse laser
G2 = patients underwent conventional focal laser photocoagulation
CMT = central macular thickness

Fig. 2 Central macular thickness.

a potential superiority of the SML has been detected both in the BCVA and CMT at the 6 month-examination point. Specifically, a) G1 participants, treated with SML, appeared a significant improvement of both BCVA and CMT at six months after the laser application, b) while G2 participants revealed a significant improvement at six months only in CMT, c) in fact, at six-month-follow-up, G1 participants had significantly lower CMT compared to patients treated with CLP.

Our promising results indicate the necessity of developing therapeutic guidelines regarding the laser energy, the shot size, the duration and the duty cycle of the SML for the treatment of the CSME. Former studies (38, 39) attempted to compare different laser settings at the same or different wavelengths, however, there is lack of published experience in order to address this significant lack of knowledge in SML treatment. Within this context, further studies and larger cohorts of patients are necessary to confirm our outcomes and contribute to the potential establishment of SML as a reliable treatment option of CSME.

CONCLUSIONS

In conclusion, our results revealed that SML was more effective than CLP in reducing CMT and improving BCVA in patients with non-centre involved CSME. Therefore, it seems that SML can be a good substitute for CLP in CSME treatment if confirmed in future studies, since it is an accessible technology, easy to use and without significant side effects. The use of the SML in an established therapeutic protocol will provide a safe and patient-friendly treatment option, in order to avoid significant visual loss.

FINANCIAL DISCLOSURE

No financial support was received for this study. None of the authors has any proprietary interests or conflicts of interest related to this submission. It is not simultaneously being considered for publication at any other journal.

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Association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: a Huge Meta-Analysis based on 44 Studies

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ABSTRACT

Background: the PAI-1 rs1799889 polymorphism has been reported to be associated with susceptibility to ischemic stroke. However, the results of previous studies have been inconsistent or controversial. Hence, we performed a systematic review and meta-analysis to evaluate the association of PAI-1 rs1799889 polymorphism with ischemic stroke risk. Methods: A comprehensive literature search was performed on PubMed, Web of Science, Scopus, SciELO, CNKI, and CBD databases up to November 05, 2019. Pooled odds ratio (OR) with 95% confidence interval (CI) were used to access the strength of this association in fixed- or random-effects model. Results: A total of 44 case-control studies with 8,620 cases and 10,260 controls were selected. Pooled data showed a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk in the overall populations (GG vs. AA: OR = 0.791, 95% CI 0.633–0.988, p = 0.039; GA vs. AA: OR = 0.807, 95% CI 0.683–0.953, p = 0.012; and GG+GA vs. AA: OR = 0.795, 95% CI 0.637–0.993, p = 0.043). Subgroup analysis by ethnicity revealed a significant association in Asian and Mixed populations, but not in Caucasians. Moreover, stratified analysis by country of origin revealed an increased risk of ischemic stroke in Chinese populations, but not among Dutch (Netherlands) and Swedish. Conclusions: This meta-analysis result suggested that PAI-1 rs1799889 polymorphism was associated with an increased risk of ischemic stroke, especially in Asian and Mixed populations.

KEYWORDS

ischemic stroke; cerebrovascular accident; PAI-1 gene; rs1799889; polymorphism; meta-analysis

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INTRODUCTION

Stroke is the second leading cause of death globally and leading cause of long-term disability worldwide (1). It poses a huge threat to public health and is the leading cause of death in developed and developing countries (2). It is estimated that approximately 70% of new strokes are ischemic in origin, 51% stroke death, and 58% of stroke disability-adjusted life years are because of ischemic stroke (3). The exact etiology of ischemic stroke is multifactorial and a complex interaction between modifiable and non-modifiable conventional risk and genetic factors could be behind the pathogenesis of this disease (4). Several variants at low-penetrance and high-penetrance genes have been identified as potential ischemic stroke susceptibility loci. Numerous studies have found that Plasminogen activator inhibitor-1 (PAI-1) also serpin E1 was involved in the pathogenesis of ischemic stroke (5). Therefore, PAI-1 gene polymorphisms and its circulating levels may be associated with the development of ischemic stroke (5, 6).

Human PAI-1 gene is located at chromosome 7q21.3–q22, contains 9 exons and spans 12.3 kb (7). PAI-1, a secreted single-chain glycoprotein, is one of the early inflammatory response genes, and its expression level changes dramatically in response to many stimuli, including growth factors and endotoxins (8, 9). Several polymorphisms within the PAI-1 gene have clearly been postulated to modulate the expression of PAI-1 (10, 11). Among SNPs of the PAI-1 gene, rs1799889 (4G/5G) polymorphism has been extensively studied in different disease (7, 12). PAI-1 rs1799889 is an inserted or deleted in the 4G sequence polymorphism in the PAI-1 promoter (4G/5G) at 675 bp upstream from the start of transcriptional start site in the promoter region. Studying the association of PAI-1 gene with different disease will help us to understand the mechanism of PAI-1 regulation and the role of PAI-1 in many physiological and pathological processes (12, 13).

Studies have shown that the 4G/4G genotype has been linked to higher PAI-1 level, compared with the 5G/5G genotype, with the heterozygous genotype associated with intermediate levels. In 2003, Chen et al., have reported that PAI-1 rs1799889 polymorphism alone is not associated with an increased risk of ischemic stroke. However, they revealed a significant contribution of PAI-1 4G/4G genotype with an increased triglyceride and decreased HDL cholesterol levels in the healthy group (14). There are several numbers of epidemiological studies have evaluated association between PAI-1 rs1799889 polymorphism and ischemic stroke risk, but their results were inconsistent or even contradictory. For example, Adamski et al., and Esparza-García et al., have reported that PAI-1 rs1799889 polymorphism was not associated with an increased risk of ischemic stroke in Polish and Mexican populations, receptively (15, 16). By contrast, Xu et al. results supported that PAI-1 rs1799889 polymorphism might be associated with an increased risk of ischemic stroke in Han Chinese (17). In recent years, some studies already studied potential associations PAI-1 rs1799889 polymorphism with risk of ischemic stroke. Nevertheless, the results of these studies were not always consistent and the sample size of each study was also statistically insufficient. Thus, we performed a meta-analysis to offer a more comprehensive estimation of the association between PAI-1 rs1799889 and ischemic stroke susceptibility in globally populations.

MATERIALS AND METHODS

SEARCH STRATEGY

We have performed a comprehensive literature search in PubMed, MEDLINE, EMBASE, Cochrane Library, Web of Science, Elsevier, SciELO, SID, WanFang, VIP, Chinese Biomedical Database (CBD) and Chinese National Knowledge Infrastructure (CNKI) to identify all eligible studies on PAI-1 4G/5G (rs1799889) polymorphism and risk of ischemic stroke up to November 05, 2019. The following keywords were adopted in the electronic searches: ("Ischemic Strok" OR "Atherothrombotic Cerebral Infarction") AND ("Plasminogen Activator Inhibitor-1 Gene" OR "PAI-1" OR "SERPINE1") AND ("insertion/deletion polymorphism" OR "4G/5G polymorphism" OR "4G/5G promoter polymorphism" OR "rs1799889" OR "–675 4G/5G") AND ("Gene" OR "Genotype" OR "Polymorphism SNP" OR "Mutation" OR "Variation" OR "Variant"). Publication language was restricted to English, Chinese, and Farsi. Also a manual search of the reference lists performed to retrieved articles for additional potential studies.

INCLUSION AND EXCLUDING CRITERIA

The inclusion criteria for the gene association studies in this meta-analysis were as follows: 1) studies with case-control or cohort design; 2) full-text published studies; 3) studies evaluated the association between PAI-1 rs1799889 polymorphism and ischemic stroke risk; and 4) provided the genotype distribution in both cases and controls for estimating an odds ratio (OR) with 95% confidence interval (CI). Additionally, studies were excluded if one of the following criteria was fulfilled: 1) studies without detailed raw data regarding PAI-1 rs1799889 polymorphism; 2) case only studies; 3) family-based, sibling, twins and linkage studies; 4) abstracts, review, letters, comments, conference editorials, presentations, case reports, case series previous meta-analyses; 5) duplicates or overlapping studies. If the authors published two or more studies using the same data (with overlapping data), the newest publication or the publication with the largest sample size was included. There was no any limitation by ethnicity, race, placed or geography area. Moreover, non-English publications were translated and included in the meta-analysis.

DATA EXTRACTION

Two authors (HN and MJA) systematically extracted data from all eligible studies using a standardized form. Then, they have checked the data extraction results and reached consensus. If different results were generated, the two authors carried out discussions until a consensus was reached or a third author was invited to resolve the disagreement and then a final decision were made by the

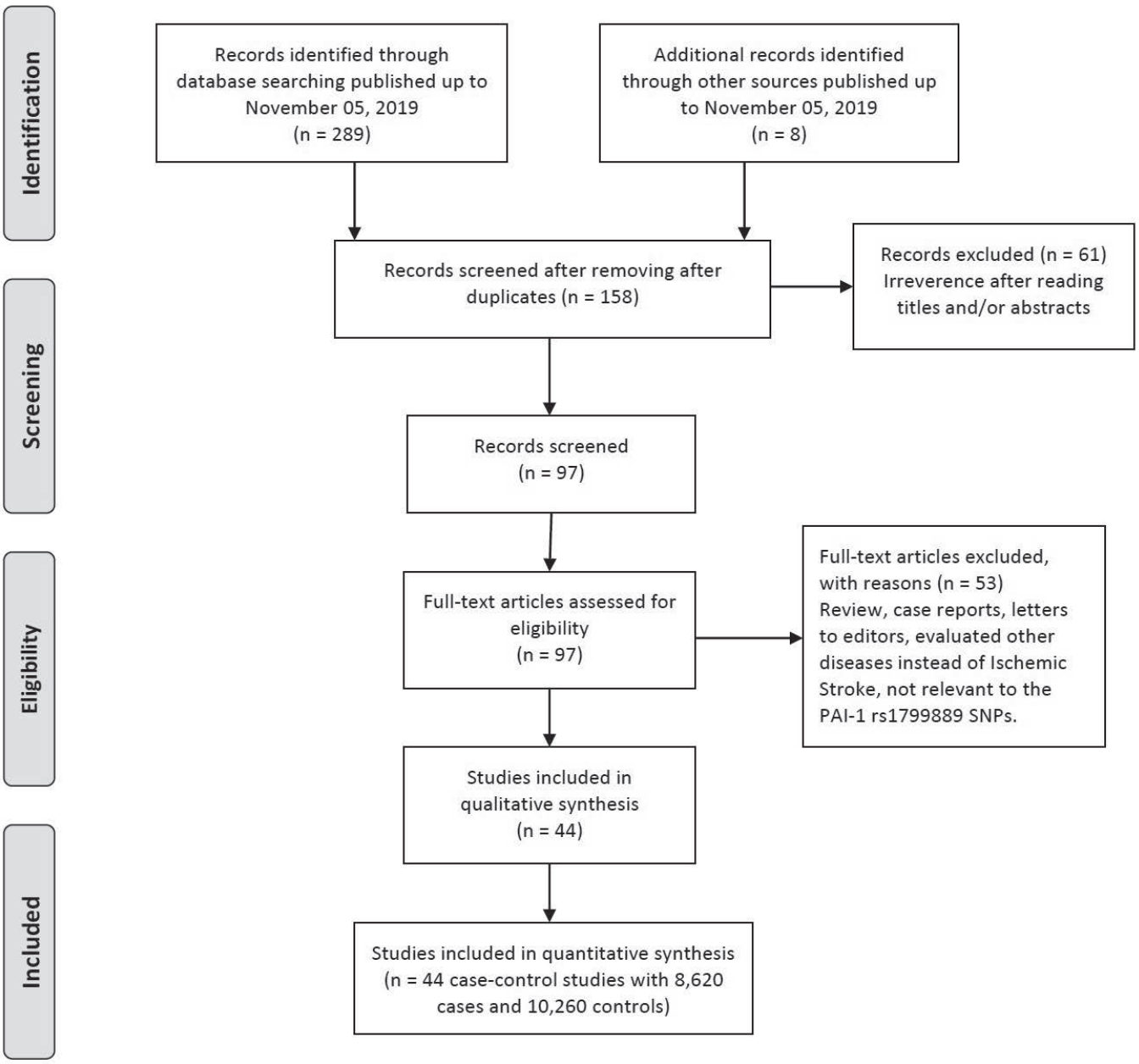

Fig. 1 The study selection and inclusion process.

majority of the votes. The collected data were: first author's name, publication year, country of origin, ethnicity (Caucasian, Asian, African, Mixed populations), total numbers of cases and controls, genotypes frequencies of cases and controls, minor allele frequencies (MAFs) and Hardy-Weinberg equilibrium test in control subjects.

STATISTICAL ANALYSIS

An ethical approval was not necessary as this study was a meta-analysis based on previous studies. The strength of the associations PAI-1 rs1799889 (4G/5G) polymorphism and susceptibility to ischemic stroke was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The statistical significance of the pooled OR was determined using the Z-test. Pooled estimates of the OR were obtained by calculating a weighted average of OR from each study. The pooled ORs was calculated under all five genetic models, i.e., allele (G vs. A), homozygote (GG vs. AA), heterozygote (GA vs. AA), dominant (GG+GA vs. AA) and recessive (GG vs. GA+AA). Between-studies heterogeneity was assessed by a Chi-squared Q-test and I^2 statistics ($P < 0.05$). The heterogeneity between studies was estimated by Cochran's $\chi 2$ based Q-statistic test, in which it was considered to be statistically significant at $P \leq 0.01$. In addition, I^2 test was used to quantify the effect of heterogeneity, with the range of 0 to 100%, and 0–40% meant no risk of heterogeneity, 30–60% meant a low risk of heterogeneity, 50–90% meant substantial heterogeneity and 75–100% meant considerable heterogeneity. Accordingly, when between-study heterogeneity existed a random-effects model weighted (the DerSimonian-Laird method) was applied to give a more conservative result; otherwise, a fixed-effects model weighted (the Mantel-Haenszel method) method was selected. Hardy-Weinberg equilibrium (HWE) of the genotype distribution in controls was conducted by Pearson's $\chi 2$ test, in which it was considered to be statistically significant at $P \leq 0.05$. A subgroup analysis by

Tab. 1 Characteristics of studies included in this meta-analysis.

First Author	Country (Ethnicity)	Case/ Control	Cases					Controls					MAFs	HWE
			Genotypes			Allele		Genotypes			Allele			
			AA	AG	GG	A	G	AA	AG	GG	A	G		
Catto 1997	UK(Caucasian)	558/172	150	274	134	574	542	56	80	36	192	152	0.442	0.454
Liu 1998	China(Asian)	107/95	44	43	20	131	83	25	48	22	98	92	0.484	0.910
Jeppesen 1998	Denmark(Caucasian)	177/93	48	92	37	188	166	26	49	18	101	85	0.457	0.552
Endler 2000	Austria(Caucasian)	136/115	42	63	31	147	125	48	48	19	144	86	0.373	0.287
Elbaz 2001	Netherlands(Caucasian)	461/461	125	223	113	473	449	129	245	87	503	419	0.454	0.123
Gottl 2001	Germany(Caucasian)	198/951	65	91	42	221	175	275	473	203	1023	879	0.462	0.988
Bang 2001	Korea(Asian)	60/100	25	25	10	75	45	21	53	26	95	105	0.525	0.530
Sun 2001	China(Asian)	50/60	21	20	9	62	38	15	30	15	60	60	0.500	1.000
Zhang 2001a	China(Asian)	95/60	50	31	14	131	59	15	30	15	60	60	0.500	1.000
Zhang 2001b	China(Asian)	65/60	28	25	12	81	49	16	35	9	67	53	0.441	0.157
Kain 2002	UK(Caucasian)	101/102	22	58	21	102	100	36	54	12	126	78	0.382	0.075
Hindorff 2002	USA(Caucasian)	41/385	7	24	10	38	44	115	187	83	417	353	0.458	0.668
Crainich 2003	USA(Caucasian)	265/753	81	143	41	305	225	200	387	166	787	719	0.477	0.410
Zhang 2003	China(Asian)	113/121	48	47	18	143	83	23	70	28	116	126	0.521	0.080
Chen 2003	Taiwan(Asian)	100/150	40	46	14	126	74	58	68	24	184	116	0.386	0.588
Zhan 2003	China(Asian)	54/83	11	30	13	52	56	25	30	6	80	42	0.344	0.485
Guan 2004	China(Asian)	222/215	75	105	42	255	189	46	121	48	213	217	0.504	0.065
Yeh 2004	China(Asian)	213/200	79	103	31	261	165	71	102	27	244	156	0.390	0.309
Yi 2004	China(Asian)	52/57	20	22	10	62	42	28	27	2	83	31	0.271	0.138
Tang 2005	China(Asian)	122/50	66	35	21	167	77	13	26	11	52	48	0.48	0.768
Jood 2005	Sweden(Caucasian)	600/600	162	307	131	631	569	186	280	134	652	548	0.456	0.144
Van Goor 2005	Netherlands(Caucasian)	123/123	33	61	29	127	119	36	58	29	130	116	0.472	0.550
Wiklund 2005a	Sweden(Caucasian)	89/218	42	33	14	117	61	67	109	42	243	193	0.442	0.844
Wiklund 2005b	Sweden(Caucasian)	222/542	94	85	43	273	171	174	261	107	609	475	0.438	0.609
Xu 2006	China(Asian)	72/77	15	29	28	59	85	5	35	37	45	109	0.707	0.386
Komitopoulou 2006	Greece(Caucasian)	87/101	23	50	14	96	78	23	55	23	101	101	0.500	0.370
Attia 2007	Australia(Caucasian)	171/182	63	71	37	197	145	62	89	31	213	151	0.415	0.922
Saidi 2007	Tunisia(African)	135/118	23	74	38	120	150	33	58	27	124	112	0.475	0.875
Liu 2008	China(Asian)	220/140	48	114	58	210	230	43	70	27	156	124	0.497	0.876
Tang 2008	China(Asian)	90/30	40	36	16	116	68	6	19	5	31	29	0.483	0.142
Adamski 2009	Poland(Caucasian)	390/291	120	189	81	429	351	89	136	66	314	268	0.377	0.018
Sabino 2011	Brazil(Mixed)	127/201	33	52	42	118	136	93	65	43	251	151	0.376	≤0.001
Balcerzyk 2011	Poland(Caucasian)	70/133	23	35	12	81	59	47	60	26	154	112	0.421	0.389
Pruissen 2011	Netherlands(Caucasian)	841/310	261	111	29	633	169	71	157	82	299	321	0.518	0.802
Maguire 2011	Australia(Caucasian)	612/600	198	279	135	675	549	169	302	129	640	560	0.467	0.784
Assawamakin 2012	Taiwan(Asian)	179/229	51	97	31	199	159	67	110	52	244	214	0.467	0.594
Babu 2012	India(Asian)	516/513	236	238	42	710	322	258	223	32	739	287	0.284	0.028
Huang 2014	China(Asian)	285/919	115	156	14	386	184	310	520	89	1140	698	0.380	≤0.001
Natesirinilkul 2014	Thailand(Asian)	29/40	2	20	7	24	34	1	32	7	34	46	0.575	≤0.001
Supanc 2014	Croatia(Caucasian)	155/150	44	51	60	139	171	28	46	76	102	198	0.660	≤0.001
García 2015	Mexico(Mixed)	204/204	23	94	87	140	268	16	87	101	119	289	0.708	0.646
Ranellou 2015	Greece(Caucasian)	40/65	2	36	2	40	40	4	44	17	52	78	0.600	≤0.001
Akhter 2017	India(Asian)	100/100	34	56	10	124	76	24	54	22	102	98	0.490	0.421
Coen Herak 2017	Croatia(Caucasian)	73/100	19	37	17	75	71	27	53	20	107	93	0.465	0.514

A

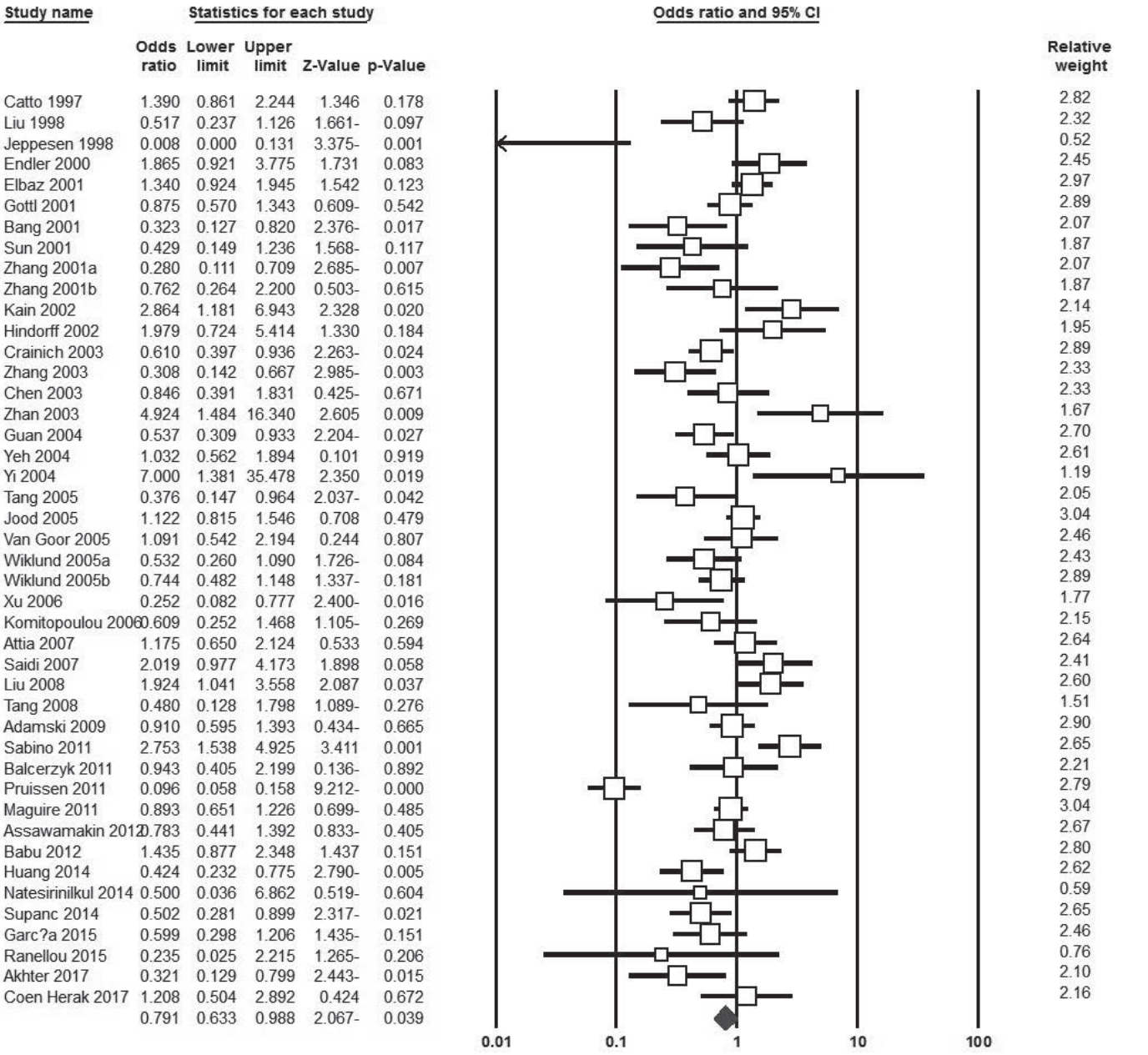

Fig. 2A Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: overall population (homozygote model: GG vs. AA).

ethnicity, country of origin, and source of controls was performed to explore potential sources of between-study heterogeneity (18, 19). To check the stability of the pooled data, a sensitivity analysis was performed by omitting each individual study in turn from the all selected studies and reanalyzing the remainder. Moreover, sensitivity analysis was performed by excluding HWE-violating studies. The potential publication bias was explored visually by Egger's linear regression test and Begg's quantitative test (20). The asymmetric plot of Egger's test and the P-value of Begg's test less than 0.05 were considered a significant publication bias. All statistical analyses were performed using Comprehensive Meta-Analysis (CMA) Software version 2.0 (Biostat, Englewood, NJ). All tests were two-sided, and the P values of < 0.05 were considered statistically significant.

RESULTS

CHARACTERISTICS OF INCLUDED STUDIES

By electronic and manual searches concerning the association of PAI-1 rs1799889 polymorphism and ischemic stroke risk, 297 relevant studies up to November 05, 2019 were identified. After reading titles and abstracts, 139 irrelevant and duplicate articles were excluded. Another 95 articles were subsequently excluded because not reporting useful data for meta-analysis, review, case only study, and not being case-control studies. Finally, a total of 44 case-control studies (5, 14–16, 21–49) with 8,620 ischemic stroke cases and 10,260 controls were included in the meta-analysis. Characteristics of included studies are presented in Table 1. All eligible studies were published in English and Chinese between April 1997 and November 2017. Among

B

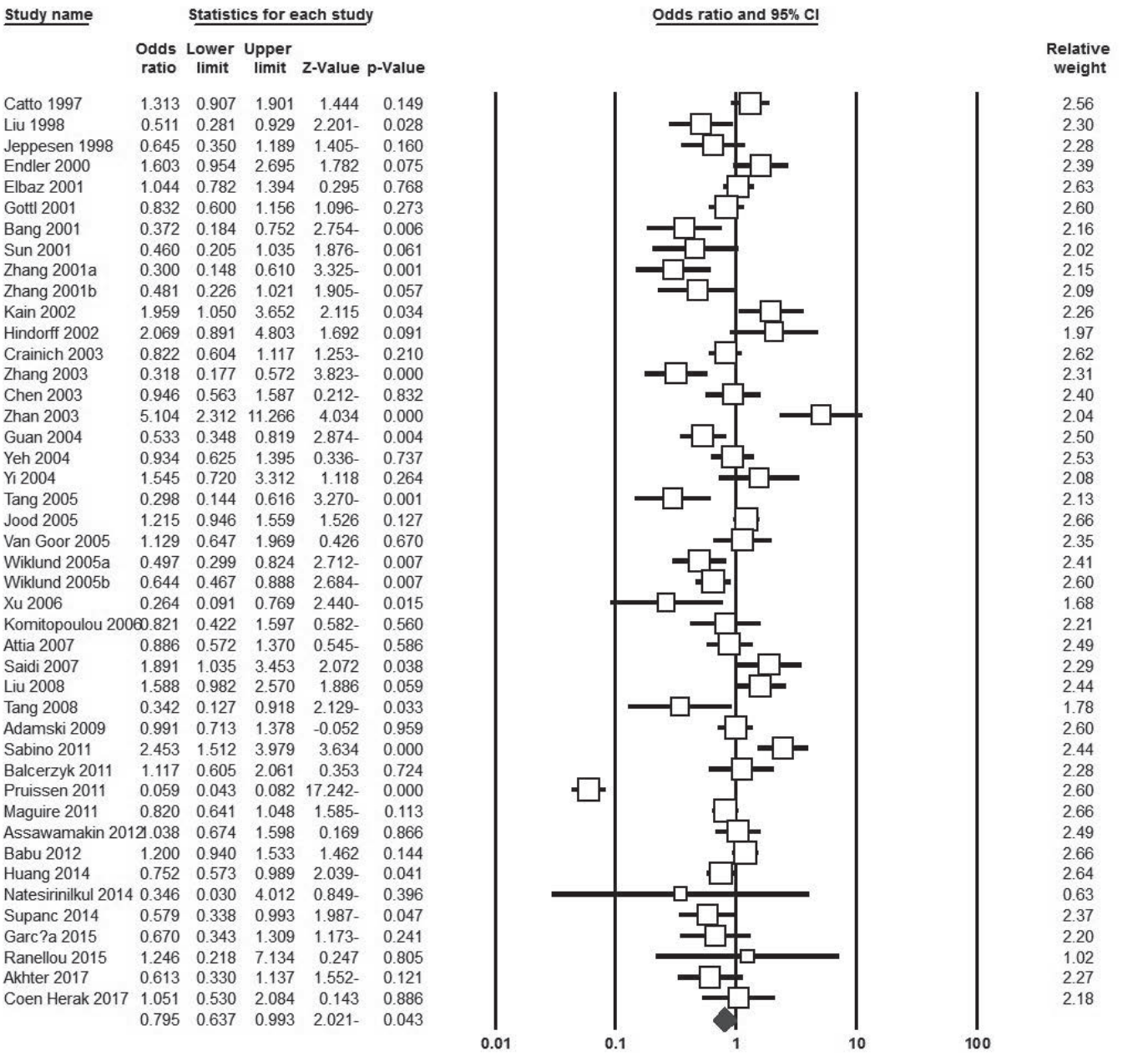

Fig. 2B Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: overall population (dominant model: GG + GA vs. AA).

them, 21 studies were based on Caucasian populations (5,410 cases and 6,438 controls), 20 studies based on Asian populations (3,137 cases and 3,700 controls), two studies based on mixed populations (331 cases and 405 controls), and one study was based on African populations (135 cases and 118 controls). The selected studies were conducted in UK, USA, Sweden, Greece, Australia, Austria, Poland, Denmark, Netherlands, Germany, Croatia, China, Taiwan, Thailand, Korea, India, Brazil, Mexico and Tunisia. The allele, genotype and minor allele frequency (MAF) distributions in the cases and controls are shown in Table 1. Moreover, the distribution of genotypes in the controls was in agreement with Hardy-Weinberg equilibrium (HWE) for all selected studies, except for seven studies (Table 1).

QUANTITATIVE DATA SYNTHESIS

The summary of the meta-analysis of the association of between PAI-1 rs1799889 polymorphism and ischemic stroke are shown in Table 2. Pooled data revealed that there was a significant association between PAI-1 rs1799889 polymorphism and an increased risk of ischemic stroke in the overall population under three genetic models, i.e., homozygote (GG vs. AA: OR = 0.791, 95% CI 0.633–0.988, p = 0.039, Fig 2A), heterozygote (GA vs. AA: OR = 0.807, 95% CI 0.683–0.953, p = 0.012) and dominant (GG+GA vs. AA: OR = 0.795, 95% CI 0.637–0.993, p = 0.043, Fig 2B). Moreover, we have performed subgroup analyses by ethnicity and country of origin. Subgroup analysis by ethnicity showed that there was a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk in

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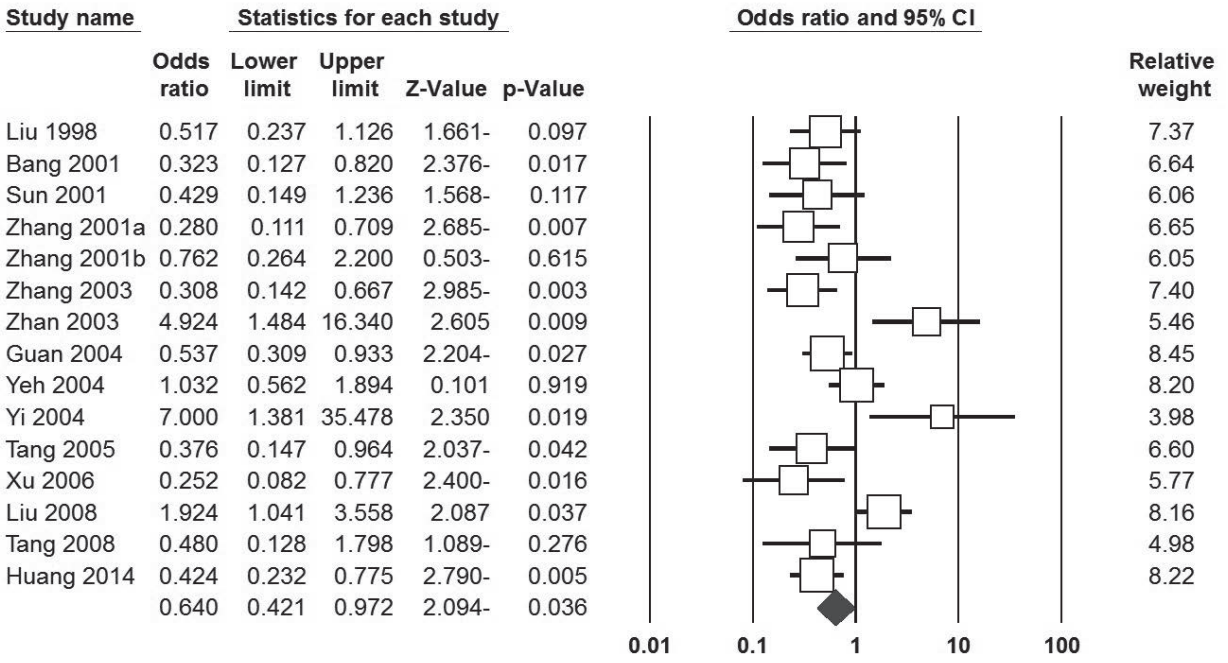

Fig. 2C Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: Chinese population (homozygote model: GG vs. AA).

Asians (G vs. A: OR = 0.829, 95% CI 0.697–0.987, p = 0.035; GA vs. AA: OR = 0.663, 95% CI 0.518–0.848, p = 0.001; and GG+GA vs. AA: OR = 0.683, 95% CI 0.521–0.897, p = 0.006) and Mixed population (G vs. A: OR = 3.255, 95% CI 1.041–10.181, p = 0.043), but not in Caucasians. When stratified analysis by country of origin performed a significant association was found among Chinese population (G vs. A: OR = 0.798, 95% CI 0.637–0.999, p = 0.049; GG vs. AA: OR = 0.640, 95% CI 0.421–0.972, p = 0.036, Fig 2C; GA vs. AA: OR = 0.577, 95% CI 0.427–0.778, p ≤ 0.001; and GG+GA vs. AA: OR = 0.620, 95% CI 0.438–0.876, p = 0.007), but not in Dutch (Netherlands) and Swedish.

BETWEEN-STUDY HETEROGENEITY TEST

As shown in Table 2, there was statistically moderate to high between-study heterogeneity in the overall population under all five genetic models, i.e., allele (I^2 = 91.95, $P_H \leq 0.001$), homozygote (I^2 = 79.67, $P_H \leq 0.001$), heterozygote (I^2 = 77.98, $P_H \leq 0.001$), dominant (I^2 = 89.86, $P_H \leq 0.001$), and recessive (I^2 = 78.03, $P_H \leq 0.001$). To explore the potential sources of heterogeneity, subgroup analyses by ethnicity, country of origin and HWE was performed. The results suggested that the above mentioned factors did not contribute to between-study heterogeneity in the current meta-analysis.

SENSITIVITY ANALYSIS

A sensitivity analysis was used to test the effects of each study on pooled ORs. There were no significant differences observed upon removal of any of the studies, suggesting that our findings were statistically robust and reliable. Moreover, we performed sensitivity analysis by excluding the HWE-violating study (Figure 3). When this study was excluded, the results were not changed in overall population and also by subgroup analyses, indicating that our meta-analysis was statistically robust and reliable.

PUBLICATION BIAS

Begg's funnel plot and Egger's test were inspected to evaluate the possible publication bias in this meta-analysis. Results of publication bias were shown in Table 2 and Figure 4. The shape of the funnel did not show any obvious asymmetry in all of the genetic models. Moreover, Egger's test was statistically revealed that there was no a significant bias under all five genetic models in the overall populations all five genetic models, i.e., allele (P_{Beggs} = 0.112; P_{Eggers} = 0.859), homozygote (P_{Beggs} = 0.198; P_{Eggers} = 0.307), heterozygote (P_{Beggs} = 0.107; P_{Eggers} = 0.267), dominant (P_{Beggs} = 0.172; P_{Eggers} = 0.841), and recessive (P_{Beggs} = 0.723; P_{Eggers} = 0.876).

DISCUSSION

The PAI-1 rs1799889 polymorphism association to ischemic stroke was first described by Catto et al. in 1997 (44). Since several epidemiological studies have been evaluated association between PAI-1 rs1799889 polymorphism and risk of ischemic stroke (17, 45). However, the results of these studies remain contradictory. It is clear that a single study may fail to demonstrate a complicated genetic relationship completely because of small sample size, which has low statistical power. Larger studies could overcome these disadvantages. Therefore, we performed a comprehensive meta-analysis of all eligible studies evaluated the association of PAI-1 rs1799889 polymorphism with risk ischemic stroke.

Tab. 2 Summary risk estimates for association of PAI-1 rs1799889 polymorphism with risk of ischemic stroke.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
Overall	G vs. A	Random	91.95	≤0.001	0.854	0.727-1.003	-1.928	0.054	0.112	0.859
	GG vs. AA	Random	79.67	≤0.001	0.791	0.633-0.988	-2.067	0.039	0.198	0.307
	GA vs. AA	Random	77.98	≤0.001	0.807	0.683-0.953	-2.526	0.012	0.107	0.267
	GG+GA vs. AA	Random	89.86	≤0.001	0.795	0.637-0.993	-2.021	0.043	0.172	0.841
	GG vs. GA+AA	Random	78.03	≤0.001	0.868	0.726-1.038	-1.555	0.120	0.723	0.876
Ethnicity										
Caucasian	G vs. A	Random	87.76	≤0.001	1.076	0.884-1.311	0.730	0.465	0.620	0.561
	GG vs. AA	Random	56.91	0.003	1.002	0.807-1.243	0.018	0.986	0.921	0.907
	GA vs. AA	Random	55.09	0.005	0.978	0.822-1.163	-0.255	0.798	0.373	0.588
	GG+GA vs. AA	Random	61.67	0.001	0.983	0.825-1.172	-0.189	0.850	0.428	0.611
	GG vs. GA+AA	Random	48.82	0.017	0.994	0.839-1.178	-0.072	0.942	0.766	0.681
Asian										
	G vs. A	Random	77.84	≤0.001	0.829	0.697-0.987	-2.113	0.035	0.820	0.389
	GG vs. AA	Random	94.75	≤0.001	0.988	0.446-2.189	-0.031	0.975	0.581	0.497
	GA vs. AA	Random	71.16	≤0.001	0.663	0.518-0.848	-3.276	0.001	0.144	0.014
	GG+GA vs. AA	Random	79.02	≤0.001	0.683	0.521-0.897	-2.749	0.006	0.284	0.079
	GG vs. GA+AA	Random	49.29	0.007	0.881	0.704-1.102	-1.111	0.267	0.314	0.410
Mixed										
	G vs. A	Random	96.28	≤0.001	3.255	1.041-10.181	2.029	0.043	NA	NA
	GG vs. AA	Random	90.73	0.001	1.301	0.292-5.795	0.345	0.730	NA	NA
	GA vs. AA	Random	83.11	0.015	1.333	0.455-3.908	0.524	0.600	NA	NA
	GG+GA vs. AA	Random	89.45	0.002	1.310	0.367-4.670	0.416	0.678	NA	NA
	GG vs. GA+AA	Random	86.25	0.007	1.156	0.492-2.719	0.333	0.739	NA	NA
Country										
China	G vs. A	Random	80.33	≤0.001	0.798	0.637-0.999	-1.967	0.049	0.766	0.871
	GG vs. AA	Random	71.92	≤0.001	0.640	0.421-0.972	-2.094	0.036	0.373	0.836
	GA vs. AA	Random	70.45	≤0.001	0.577	0.427-0.778	-3.599	≤0.001	0.373	0.104
	GG+GA vs. AA	Random	80.61	≤0.001	0.620	0.438-0.876	-2.706	0.007	0.766	0.383
	GG vs. GA+AA	Random	52.16	0.010	0.895	0.680-1.178	-0.793	0.428	0.373	0.243
Netherlands	G vs. A	Random	99.29	≤0.001	0.498	0.095-2.260	-0.822	0.411	1.000	0.959
	GG vs. AA	Random	96.06	≤0.001	0.586	0.184-1.862	-0.907	0.364	1.000	0.920
	GA vs. AA	Random	97.24	≤0.001	0.519	0.089-3.014	-0.731	0.465	1.000	0.825
	GG+GA vs. AA	Random	98.94	≤0.001	0.410	0.052-3.208	-0.849	0.396	1.000	0.899
	GG vs. GA+AA	Random	97.82	≤0.001	0.518	0.093-2.885	-0.751	0.453	1.000	0.730
Sweden	G vs. A	Random	75.65	0.016	0.855	0.647-1.129	-1.105	0.269	0.296	0.210
	GG vs. AA	Fixed	56.85	0.099	0.907	0.711-1.155	-0.794	0.427	0.296	0.219
	GA vs. AA	Random	87.54	≤0.001	0.737	0.401-1.352	-0.986	0.324	1.000	0.341
	GG+GA vs. AA	Random	86.60	0.001	0.751	0.437-1.292	-1.034	0.301	0.296	0.328
	GG vs. GA+AA	Fixed	0.00	0.829	0.951	0.769-1.177	-0.459	0.646	0.296	0.321
HWE	G vs. A	Random	92.62	≤0.001	0.843	0.700-1.015	-1.799	0.072	0.161	0.964
	GG vs. AA	Random	80.14	≤0.001	0.778	0.609-0.994	-2.010	0.044	0.277	0.418
	GA vs. AA	Random	79.04	≤0.001	0.765	0.633-0.926	-2.754	0.006	0.107	0.346
	GG+GA vs. AA	Random	90.70	≤0.001	0.763	0.590-0.988	-2.051	0.040	0.266	0.932
	GG vs. GA+AA	Random	79.09	≤0.001	0.871	0.714-1.062	-1.365	0.172	0.743	0.938

NA: Not Applicable.

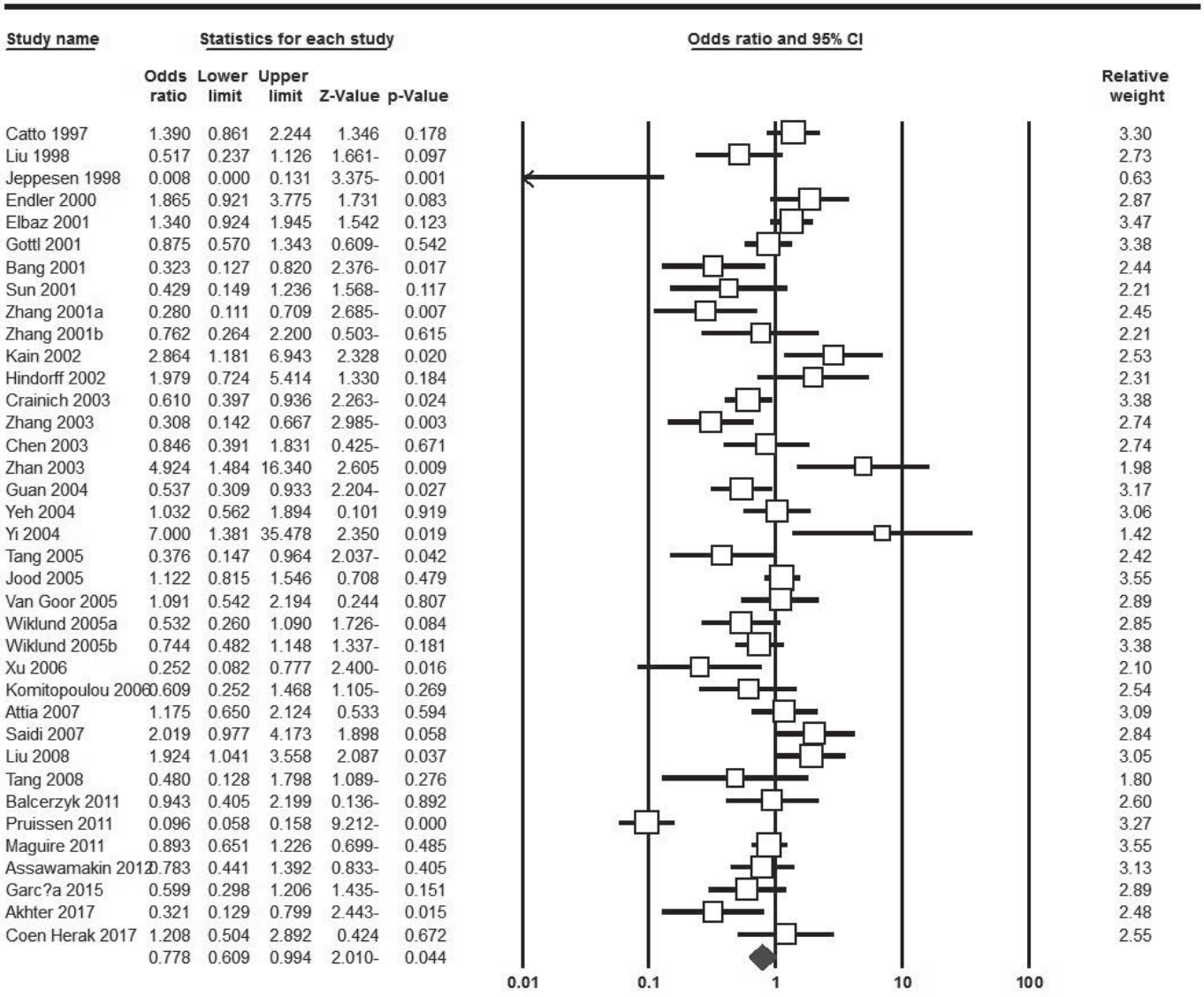

Fig. 3 Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke after excluding Hardy-Weinberg equilibrium (HWE) violating studies under the homozygote genetic model (GG vs. AA).

In the current meta-analysis, we have selected a total of 44 eligible case-control studies with 8,620 ischemic stroke cases and 10,260 controls to evaluate the association of PAI-1 rs1799889 polymorphism with ischemic stroke risk. Our pooled data showed that PAI-1 rs1799889 polymorphism was significantly associated with an increased risk of ischemic stroke in the overall population. Moreover, subgroup analyses revealed that PAI-1 rs1799889 polymorphism was associated with significantly increased risk of ischemic stroke in Asian and mixed populations, but not in Caucasians. When stratified analysis by country of origin performed a significant association was found among Chinese population, but not in Dutch (Netherlands) and Swedish. This finding indicated that the carriers with the 4G allele of the PAI-1 rs1799889 polymorphism in Asians and mixed populations might be predisposed to ischemic stroke, but not in Caucasian populations. Moreover, this finding suggested a possible influence among environmental exposures and different genetic backgrounds in development of ischemic stroke in different populations. Therefore, more studies are warranted to further validate genetic background difference in the effect of PAI-1 rs1799889 polymorphism in susceptibility to ischemic stroke, especially in Caucasians. Cao et al., in a meta-analysis of eleven case-control studies with 1,358 cases and 1,134 controls evaluated the association of PAI-1 rs1799889 polymorphism with susceptibility to ischemic stroke in the Chinese population. Their results showed a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk. However, their meta-analysis results reliability and the number of studies are considerably smaller than that needed to receive the robust conclusions (45). Here, we have extended the meta-analysis with a more relevant recently published studies and subgroup analysis by ethnicity. Moreover, Hu et al., in meta-analysis of 39 studies with 8,336 cases and 14,403 controls evaluated PAI-1 polymorphisms with risk of stroke. Their results revealed a significant association between PAI-1 rs1799889 polymorphism and an increased risk of ischemic stroke in adult, but not pediatric. Their stratified analysis showed a significant association in Asians, but not Caucasians. Moreover, they found that PAI-1–844 G>A, but not 11,053 T>G polymorphism was associated with an increased risk of ischemic stroke and a tendency

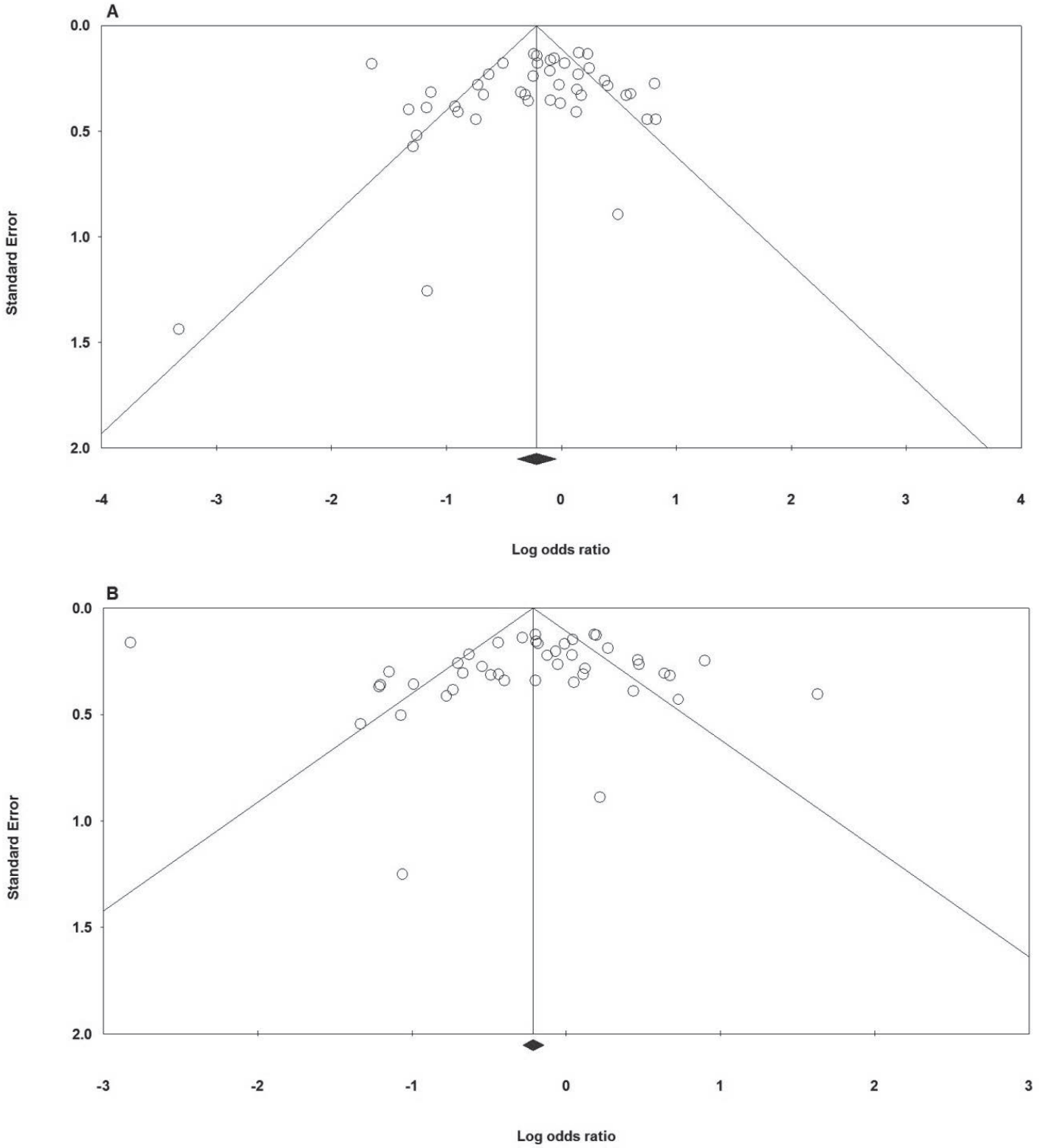

Fig. 4 Begg's funnel plots of between PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke. A: heterozygote model (GA vs. AA); B: dominant model (GG+GA vs. AA). Each point represents a separate study for the indicated association.

of PAI-1 rs1799889 polymorphism towards a decreased risk of hemorrhagic stroke (50).

Between-study heterogeneity is a common issue in a meta-analysis on genetic association (51–53). It could be attributable to differences in several factors such as environmental factors, including criteria or methodological factors in design and conduct of the studies (54, 55). Thus, identifying the potential sources of heterogeneity is one of the most important goals of meta-analysis. When all the eligible studies were pooled in this meta-analysis, there was significant between-study heterogeneity under all genetic models. However, after subgroup analyses by ethnicity the heterogeneity not effectively disappeared or decreased, which indicated that ethnicity did not play a crucial role in the existence of between-study heterogeneity in the current meta-analysis.

The current meta-analysis had some advantages. First, this was the most comprehensive and accurate me-

ta-analysis to evaluate association of PAI-1 rs1799889 polymorphism with ischemic stroke, which involved Asian, Caucasian, mixed populations. Second, the current meta-analysis search not restricted to studies published in indexed journals. Third, we have evaluated the association under all five genetic models. Forth, there was no evidence of publication bias by Begg's funnel plot and Egger's test in this meta-analysis. Finally, sensitivity analysis confers the reliability and stability of our pooled data. However, some limitations of this meta-analysis should be mentioned. First, the sample size of the included studies was not large enough by ethnicity among African and Mixed populations. Therefore, there was a lack of statistical power to better calculate association of PAI-1 rs1799889 polymorphism with risk of stroke among African and Mixed populations. Second, all included studies were published in English or Chinese which may be brought some bias. Third, in this meta-analysis between-study heterogeneity was detected under all five genetic models in the overall population and by subgroup analyses, which may be distorting the pooled data. Finally, our results were based on single-factor estimations without adjustment for other risk factors such as age, gender, and environmental factors.

In summary, this meta-analysis result revealed that PAI-1 rs1799889 polymorphism was significantly associated with an increased risk of ischemic stroke, especially in Asian populations. Moreover, there was a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk. Future studies with large sample sizes and well designs in the Mixed and African populations and gene-gene and gene-environment interaction studies are warranted to confirm these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Interdisciplinary Management of Visceral Artery Aneurysms and Visceral Artery Pseudoaneurysms

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ABSTRACT
The paper presents the results of treating 14 patients, namely eight patients with visceral artery aneurysms and six patients with visceral artery pseudoaneurysms. In 64.3% of the patients, the initial diagnosis was made based on the ultrasound examination. All the patients (100%) underwent CT angiography, while angiography was performed in 71.4% of the cases. Five (35.7%) patients with visceral artery pseudoaneurysms were emergently hospitalized; among them, the signs of bleeding were observed in 2 patients. In 9 patients, pathology was detected during tests for other conditions. Five (35.7%) patients underwent endovascular treatment, while 9 (64.3%) patients received surgical treatment. Endovascular interventions and open surgery demonstrated a nil mortality rate. After endovascular treatment, stent thrombosis was found in 1 patient. In the case of surgical treatment, visceral artery aneurysm was observed in 1 patient who underwent the resection of superior mesenteric artery pseudoaneurysm.
Conclusions. The choice of the method of treating visceral artery aneurysms and visceral artery pseudoaneurysms depends on the location, size, anatomic features of the visceral arteries and the clinical course of the disease. Both endovascular and surgical treatment demonstrate good postoperative outcomes. Visceral ischemia is one of the most serious complications in the postoperative period, which can complicate both the diagnosis and the choice of treatment tactics.

KEYWORDS
visceral artery pseudoaneurysm; visceral artery aneurysms; rupture risk; hemorrhage; visceral ischemia

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INTRODUCTION

Visceral artery aneurysms and pseudoaneurysms are rare yet serious vascular lesions that are quite difficult to diagnose. According to literature, visceral aneurysms account for 2–3% of all the cases of vascular aneurysms (1, 2). Risk factors for visceral aneurysm development include atherosclerosis, inflammatory conditions within the abdominal cavity and the retroperitoneal space, portal hypertension, connective tissue diseases, whereas visceral artery pseudoaneurysms are caused by destructive pancreatitis, iatrogenic injury to the visceral arteries, abdominal trauma (3, 4). The most common visceral artery aneurysm localization is as follows: the splenic artery – 60%, the common hepatic artery – 20%, the gastroduodenal artery – 6%, the superior mesenteric artery – 5.5%, the celiac artery – 4.5%, other arteries – 4%. The risk of visceral aneurysm rupture ranges from 5% for aneurysms that are 15–20 mm in diameter to 50–70% for aneurysms with diameters greater than 30 mm (5, 6). According to the international recommendations, endovascular or surgical treatment is indicated for the patients with visceral aneurysm larger than 20 mm, whereas, due to a high rupture risk, surgery is indicated for every patient with pseudoaneurysm (1, 7, 8). With the development of interventional radiology, endovascular methods of treating visceral artery aneurysms and pseudoaneurysms are implemented into practice (9, 10); however, traditional surgical treatment remains relevant, especially in the cases when it is impossible to perform the endovascular treatment (2, 11, 12). Considering a rarity of this pathology, specifics of its diagnosis and treatment, we have decided to share our own experience of treating visceral artery aneurysms and pseudoaneurysms.

MATERIAL

The paper presents the results of treating 14 patients during 2008–2018. Among them, there were 8 (57.1%) females and 6 (42.9%) males. The youngest patient was 47 years old; the oldest patient was 78 years old. According to the patients' past medical history, five of them underwent abdominal surgery (gastrectomy – 1 patient; pancreatoduodenal resection – 1 patient; pancreatic cyst drainage – 2 patients; surgical revision of the abdominal cavity in multi-trauma, splenectomy – 1 patient). All the patients underwent ultrasound examination of the abdominal cavity and the visceral arteries, enhanced CT angiography. Angiography of the aorta and the visceral arteries was applied in 10 cases. By the clinical course, five patients with complaints of severe epigastric pain and general weakness were emergently hospitalized, while nine patients complained of dull epigastric pain and epigastric heaviness.

RESULTS

DIAGNOSIS OF VISCERAL ARTERY ANEURYSMS AND PSEUDOANEURYSMS

In 9 (64.3%) out of 14 patients, the initial diagnosis was made based on ultrasound examination. Using angiography and CT angiography, a differential diagnosis between visceral artery aneurysm and visceral artery pseudoaneurysm was made, and the size of pathological formation was determined. The localization of visceral artery aneurysms and pseudoaneurysms is presented in Table 1.

Tab. 1 Visceral artery aneurysm localization.

Visceral artery	Visceral artery aneurysm	Visceral artery pseudoaneurysm
Splenic artery	3	3
Superior mesenteric artery	1	2
Celiac artery	1	1
Common hepatic artery	1	–
Pancreaticoduodenal artery	1	–
Left gastric artery	1	–

The smallest visceral artery aneurysm diameter was 23 × 27 mm, while the greatest one was 65 × 72 mm (Fig. 1).

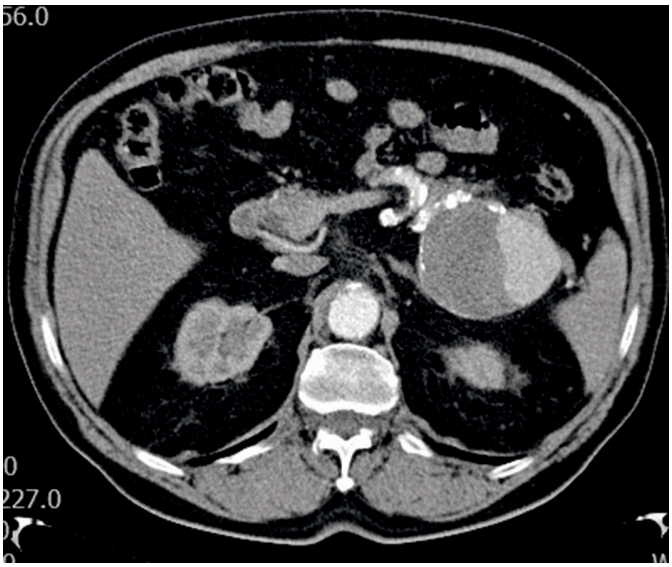

Fig. 1 CT angiography scan of splenic artery aneurysm with a diameter of 72 × 65 mm.

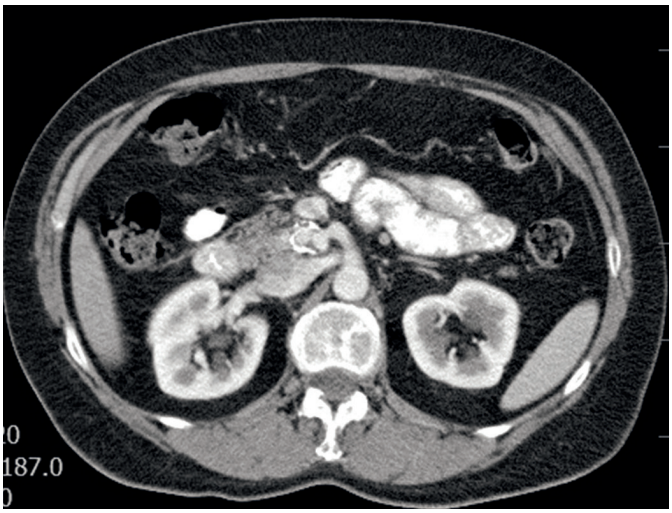

Fig. 2 CT angiography scan of superior mesenteric artery pseudoaneurysm with a diameter of 25 × 20 mm.

The smallest diameter of visceral artery pseudoaneurysm was 25 × 20 mm, while the greatest one was 52 × 25 mm (Fig. 2).

Multiple aneurysms in the splenic artery were detected in 1 patient. In 1 patient, in addition to splenic artery aneurysm, infrarenal aortic artery aneurysm with a diameter of 62 × 58 mm was found. In 1 case, celiac artery aneurysm extended to the hepatic artery (Fig. 3).

TREATMENT OF VISCERAL ARTERY ANEURYSMS AND PSEUDOANEURYSMS

Five (35.7%) patients received endovascular treatment. Endovascular treatment tactics were determined by both vascular surgeon and interventional radiologist. Embolization was used in 2 patients with pseudoaneurysm of the distal part of the splenic artery.

One patient with an aneurysm of the proximal part of the splenic artery underwent coil occlusion. Endovascular aneurysm repair was performed in 1 patient with celiac artery pseudoaneurysm and one patient with common hepatic artery aneurysm.

In other cases (64.3%), surgical treatment was preferred: ligature of the splenic artery – 1 patient; resection of splenic artery aneurysm with direct end-to-end anastomosis – 2 patients; resection of celiac artery aneurysm and celiac artery reconstruction with a polytetrafluoroethylene (PTFE) graft – 1 patient (Fig. 4); resection of pancreaticoduodenal artery aneurysm and direct suture of the pancreaticoduodenal artery – 1 patient; resection of left gastric artery aneurysm with left gastric artery plasty – 1 patient; resection of superior mesenteric artery aneurysm with vein plasty – 1 patient; resection of superior mesenteric artery pseudoaneurysm with direct sutures – 2 patients.

Endovascular interventions and open surgery demonstrated a nil mortality rate. After endovascular treatment, stent thrombosis was found in 1 patient. After surgical treatment in the early postoperative period, serious complication (visceral artery aneurysm) was observed in 1 patient who underwent the resection of superior mesenteric artery pseudoaneurysm.

Considering a particular interest of this case report, we propose its more detailed presentation.

A 57-year-old female patient with complaints of general weakness, mild epigastric pain, and diarrhea were hospitalized to the Department of Surgery No 1. According to past medical history, the patient had undergone pancreaticoduodenal resection for pancreatic head tumor three weeks prior hospitalization. According to the surgical treatment protocol, pancreatic tumor infiltrated the adventitia of the anterior surface of the superior mesenteric artery that required the preparation of malignant pancreatic formation at the subadventitial layer of the superior mesenteric artery. The pancreatic tumor was removed ad block without any signs of intraoperative bleeding.

At the hospitalization stage, abdominal ultrasound was performed that revealed the collection of hypoechoic fluid 5 × 2.5 × 1.5 cm in size in the epigastric region. To specify the diagnosis, there was prescribed CT angiography of the abdominal cavity, that, during the arterial phase of contrast administration, revealed a rounded area of contrast medium accumulation 50 × 42 mm in size to the left of the aorta and approximately 1.5–2 cm below the origin of the superior mesenteric artery.

The results obtained confirmed superior mesenteric artery pseudoaneurysm (Fig. 5).

The interventional radiologist did not recommend endovascular treatment of pseudoaneurysm as, according to CT angiography, significant anatomical and topographical changes in the superior mesenteric artery were observed.

Taking into account the presence of superior mesenteric artery pseudoaneurysm confirmed by CT angiography, the vascular surgeon recommended surgical resection of superior mesenteric artery pseudoaneurysm. The patient underwent elected laparotomy. During surgical revision,

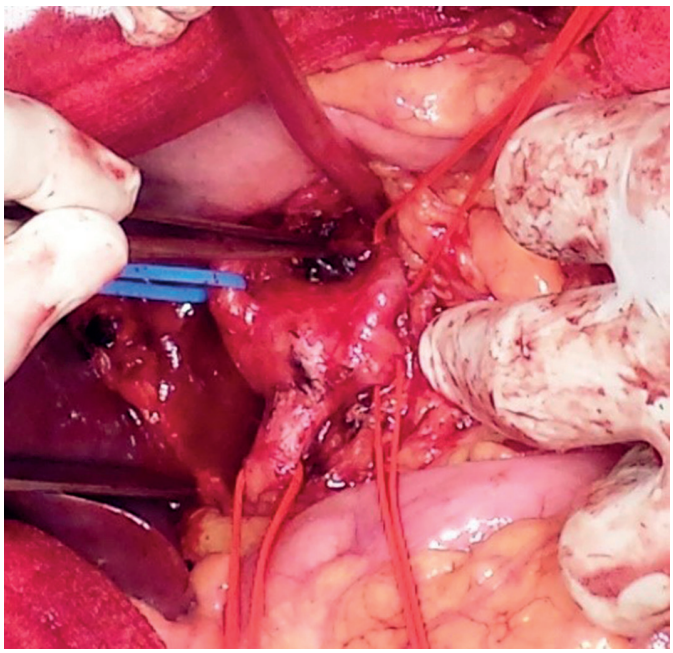

Fig. 3 Celiac artery aneurysm (Intraoperative image).

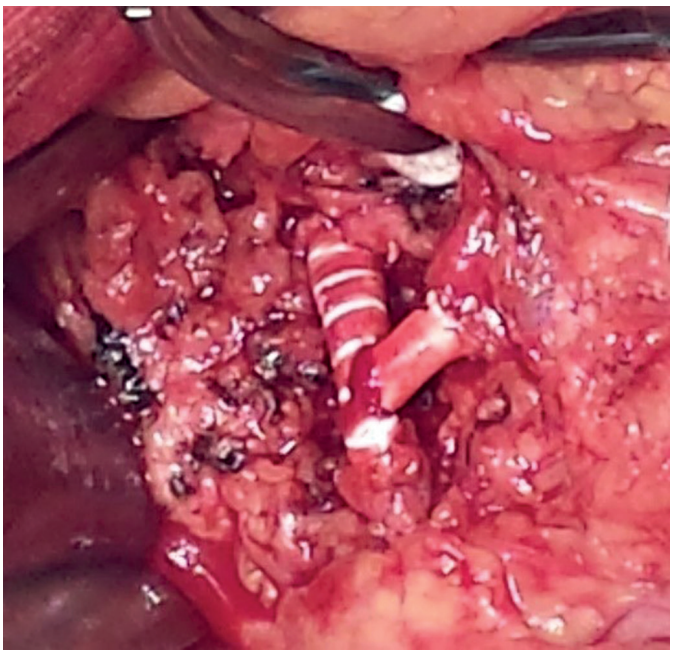

Fig. 4 Arterial reconstruction of the celiac artery with a PTFE Graft.

in the projection of the superior mesenteric artery, a pulsating mass 5 × 4 cm in diameter was detected. The superior mesenteric artery was prepared proximally and distally to the pseudoaneurysm. After injection of 5,000 units of heparin and clamping (compression) of the superior mesenteric artery, pseudoaneurysm was mobilized on the anterior surface of the superior mesenteric artery, where a 2–3-mm opening was found. Considering a pronounced subacute process, we have decided to perform minimally invasive surgery. Namely three single transverse sutures were applied to the superior mesenteric artery. After blood flow restoration, excellent pulsation of the superior mesenteric artery distal to sutures was detected.

However, the patient's clinical condition in the postoperative period was complicated; diffuse abdominal pain and weakly positive signs of peritoneal irritation were observed on the second day after surgery. CT angiography of the abdomen was emergently performed that revealed superior mesenteric artery occlusion. Relaparotomy and surgical revision were urgently performed, taking into account acute intestinal ischemia. Superior mesenteric artery occlusion at the site of pseudoaneurysm resection was found. Arterial reconstruction has been decided to be performed: iliac-mesenteric bypass with the great saphenous vein (Fig. 6).

As it was impossible to assess the viability of the small intestine, the abdomen was left open for eight hours for planned second-look reoperation; then, the small intestine was surgically revised. There was found the necrotic segment of the jejunum. There was performed segmental resection of the jejunum (70–80 cm). The postoperative course was uncomplicated. There were prescribed anti-inflammatory, detoxification, and antiplatelet therapies.

DISCUSSION

With the implementation of diagnostic methods such as angiography and CT angiography into clinical practice, visceral artery aneurysms and pseudoaneurysms can be detected before their complications develop that, certainly, increases the quality of treatment thereby reducing the risk of postoperative complications (8, 13).

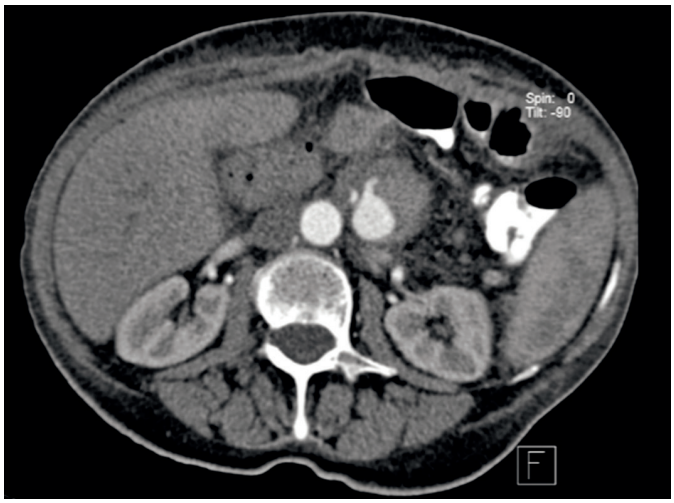

Fig. 5. CT scan of superior mesenteric artery pseudoaneurysm

Digital angiography is currently the gold standard for diagnosing pseudoaneurysms as it allows real-time assessment of the site of extravasation. Digital angiography has the highest sensitivity (100%), followed by CT (67%) and ultrasound (50%) (14). In our study, the initial diagnosis of visceral artery aneurysm based on ultrasound examination was made in 64.3% of the patients; all the patients (100%) underwent CT angiography, while angiography was performed in 71.4% of the cases.

The ratio of visceral artery aneurysms to visceral artery pseudoaneurysms depends on visceral artery location. The most common visceral artery aneurysm localization is the splenic artery while pseudoaneurysms of the gastroduodenal and superior mesenteric arteries are more common as compared to their aneurysms (89% vs. 11% and 67% vs. 33% respectively) (2, 9).

More than 60% of visceral artery pseudoaneurysms occur secondary to pancreatitis, and almost 10–17% of pseudocysts in patients with chronic pancreatitis are complicated by the development of visceral artery pseudoaneurysms (15).

Blunt or penetrating abdominal trauma and iatrogenic injury after hepatobiliary or vascular surgery, or pancreatic head biopsy may result in visceral artery pseudoaneurysm as well (7, 13).

Approximately 80% of visceral artery aneurysms are asymptomatic being detected during tests for other conditions. Almost 20% of visceral artery aneurysms have severe clinical manifestations; in 9% of the cases, they result in death (2). Clinical manifestations of visceral artery aneurysms are non-specific. The patients complain of abdominal discomfort and abdominal pain that is not related to food intake. At the same time, in most patients with visceral artery pseudoaneurysms, the symptomatic clinical course is found; they complain of abdominal and epigastric pain; hematemesis and melena may be observed (3). Among 14 patients, 5 (35.7%) individuals with visceral artery pseudoaneurysms were emergently hospitalized; among them, the signs of bleeding were observed in 2 patients. In 9 patients, pathology was detected during tests for other conditions; among them, three patients with recurrent symptoms of chronic pancreatitis were hospitalized in the surgical department.

The choice of the method for treatment of visceral artery aneurysms and pseudoaneurysms remains controversial, and the prognostic indicators of the clinical course depend on many factors, namely aneurysm localization and size, clinical manifestations, work experience of the surgical team and technical capabilities of a healthcare institution (7).

The method of choice should be endovascular treatment (selective embolization, coils, stent, gelatin foam,

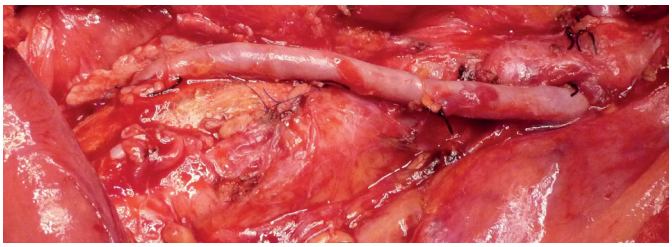

Fig. 6 Iliac-mesenteric bypass with the great saphenous vein

polyvinyl alcohol), which is performed under local anesthesia (9, 10). According to literature, endovascular treatment of visceral artery aneurysms was effective in 95–98% of cases. Reintervention was required in 3–5% of cases. Aneurysm-related thirty-day mortality rate was 3–4%, and the peri-procedural mortality rate was about 6% (3, 9).

In our study, only 5 (35.7%) patients underwent endovascular treatment. Two (14.3%) patients underwent attempted endovascular treatment that was not effective due to tortuosity of the affected visceral artery.

Contraindications to endovascular treatment may include vascular tortuosity and the length of the affected artery, especially in case of stent implantation when there is a need to fix the proximal and distal ends (8). Complications of endovascular surgery may include thrombosis resulting in visceral ischemia, stent or coil migration, stent occlusion, reperfusion, rebleeding, nephropathy, access-related complications (femoral pseudoaneurysm, hematoma, thrombosis or embolism, infection) (13).

When it is impossible to perform endovascular treatment, surgical treatment, that involves the exclusion of aneurysmal sac, arterial bypass, vessel ligature, is recommended. Among 14 patients, surgical treatment was performed in 64.3% of the cases. In some cases, organ resection (splenectomy, colon resection) is needed (11, 16). Due to the constant collateral circulation between the visceral arteries, most visceral artery aneurysms can be treated by ligation or embolization. However, they cannot be applied in superior mesenteric artery aneurysms when endovascular or surgical revascularization is always mandatory (4).

The choice of treatment tactics (surgical or endovascular) for hemodynamically unstable patients is controversial. We prefer surgical treatment, although there were a few articles on successful endovascular treatment of visceral artery aneurysms in hemodynamically unstable patients (10, 17).

Both in the case of endovascular surgery and traditional surgery, the most serious postoperative complication is visceral ischemia that not always is acute, thereby complicating both timely diagnosis and adequate treatment (18).

In the study group, visceral ischemia as a postoperative complication after surgical treatment of superior mesenteric artery pseudoaneurysm was observed in 1 patient.

The clinical picture was changed by a specific sign of the postoperative course (changes in the trajectory of the gastrointestinal contents passage after pancreaticoduodenal resection, adhesions, the significant extent of surgery). This case confirmed that in case of severe postoperative course, even if surgery was uneventful, the presence of iatrogenic injury to the visceral arteries should be taken into account. Ultrasonography was found to play an important role in the postoperative period. If there is any fluid collection, a differential diagnosis with the detection or exclusion of an active venous or arterial blood flow is required. If there are any abnormal abdominal masses, an objective diagnostic method is CT with intravenous bolus contrast medium injection.

The choice of surgery extent (resection of superior mesenteric artery pseudoaneurysm and application of direct transverse single sutures) was substantiated by the fact that, intraoperatively, the superior mesenteric artery was sufficiently wide that allowed us to apply sutures without stenosis formation. Moreover, our goal was to minimize the extent of surgery, as, in the case of a subacute process, anastomosis or plasty may result in the development of other postoperative complications.

Superior mesenteric artery occlusion was diagnosed on the second day after surgery, however. This was most likely due to pronounced infiltrative changes in the pancreaticoduodenal region. An unfavorable prognostic criterion in the postoperative period is intestinal ischemia. Therefore, rapid recognition of the patient's clinical condition is the key to treatment success (19). If the diagnosis of mesenteric ischemia is confirmed, emergency surgery is needed (20).

We performed emergency surgery and arterial reconstruction, namely iliac-mesenteric bypass. Next day, segmental resection of the jejunum (70–80 cm) was performed. As it is impossible to assess the viability of the small intestine, many surgeons use minimally invasive surgical interventions (resection of the necrotic segment) and delayed, second-look surgical revision of the intestine (21). On the other hand, practically no alternative approach to surgical treatment of the patients with mesenteric ischemia exists (22). The factor of acute intestinal ischemia duration is of extreme importance in the prediction of surgical treatment success for the patients with mesenteric ischemia (23). Therefore, careful attention should be paid to clinical signs of acute intestinal ischemia, especially in the patients who underwent visceral artery reconstruction.

CONCLUSIONS

The choice of the method for treatment of visceral artery aneurysms and visceral artery pseudoaneurysms depends on the location, size, anatomic features of the visceral arteries and the clinical course of the disease. Both endovascular and surgical treatment demonstrate good postoperative outcomes.

In the case of visceral artery reconstructions, the potential risk of both the development of acute mesenteric ischemia and visceral artery occlusion should be taken into account. Visceral ischemia is one of the most serious complications in the postoperative period, which can complicate both the diagnosis and the choice of treatment tactics. In acute intestinal ischemia, emergency surgery, that involves the revascularization of the intestine, the assessment of intestinal viability and segmental resection of the necrotic intestinal segment, is required.

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An Alternative Treatment for Vaginal Cuff Wart: a Case Report

Victoria Psomiadou, Christos Iavazzo*, Athanasios Douligeris, Alexandros Fotiou, Anastasia Prodromidou, Nikolaos Blontzos, Evgenia Karavioti, George Vorgias

ABSTRACT

Human papillomavirus (HPV) has been directly related to acuminate warts and cervical cancer, the second most common neoplasia among women. Given the lack of treatment against the virus itself, many medications have been utilised, mainly aiming in modifying the host's immunological response. We present the case of a 54 years old postmenopausal patient with a history of vaginal cuff wart and HPV persistence that we managed in our clinic for 6 months with a mix of curcumin, aloe vera, amla and other natural ingredients. As the patient was found to be intolerant to imiquimod (one of the most common conservative methods of treatment) we attempted the use of curcumin, which was applied to the area of the wart three times per week for 6 months. Both clinical and colposcopical improvement was noted in regular clinic visits with regression of the lesion. The outcome of this case encourages our view that curcumin should be considered as a significant treatment modality against HPV infection and acuminate warts.

KEYWORDS

HPV; vaginal cuff wart; curcumin; Indian Grapefruit (AMLA); aloe vera; Docosanol; lactic acid; CM-β glucans; SiloffGyn

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INTRODUCTION

Cervical cancer is the 4th most common cancer among women worldwide, with an estimated 527,624 new cases and 265,672 deaths in 2012 (1). HPV is recognized as a well-established causative factor, mainly through the work of Harald zur Hausen, who also later identified the two most oncogenic types of the virus, 16 and 18 (2). Nowadays there is upcoming conclusive evidence with respect to carcinogenicity of HPV in other anogenital cancers (anus, vulva, vagina and penis) as well as head and neck cancers (3).

Primary HPV infection usually occurs early in life and it is asymptomatic in most cases. Genital warts are the visible manifestation of the infection, typically caused by HPV types 6 and 11, which rarely are associated with invasive squamous cell carcinoma (4). Patients with condylomata (genital warts) can present with burning, itching, bleeding, and pain as well as psychological anxiety and embarrassment (5). The disease is estimated to lead to high morbidity and significant healthcare costs, since the lesions typically recur even after different ablative (electrocautery, liquid nitrogen, and laser therapy) techniques or surgical excision. Specifically, the recurrence rate with each technique reach 20–40% for cryotherapy, 15% for imiquimod, 5–50% for laser treatment, 5–30% for podofilox and 20-65% for podophyllin resin (6).

Currently many natural plant origin compounds have been identified as promising sources of drugs for treatment and prevention against recurrence of genital warts, with podofilox and imiquimod being the most recommended (7). Another non-invasive treatment agent is curcumin, a topical immune response modifier, isolated from the root of Curcuma longa. Curcumin, introduced as a safe and effective treatment for HPV-associated genital warts, has not been found to achieve its optimum therapeutic outcome, mainly because of its low solubility and poor bioavailability. Lately, it has been developed as a therapeutic drug through alterations in formulation properties and improvement of delivery systems (8).

We present a case of a vaginal cuff wart diagnosed and managed in our clinic with a mix of curcumin, aloe vera, amla and other natural ingredients. Our aim is to discuss an alternative option in the treatment of acute warts that persist or recur after other methods of therapy.

CASE REPORT

A 54-year-old woman, with a HGSIL Pap smear was referred to our clinic. The patient has a THBO medical history due to uterus fibroids ten years ago. Clinical examination and colposcopy revealed a vaginal cuff wart and histopathology confirmed the HPV infection. The patient was treated with ALDARA 5% cream, a regimen of imiquimod produced by Meda AB in Sweden, which was applied three times weekly. However, her clinical situation worsened after two cycles of therapy as she presented with symptoms of intense intolerance of the medicament, including burning and itchiness of the affected area. Administration of imiquimod was discontinued for the following two months. The patient was then reevaluated and an alternative treatment with a mixture of curcumin, Indian Grapefruit (AMLA), aloe vera, Docosanol, lactic acid and CM-β glucans was applied. The vaginal cream SiloffGyn, produced by Heremco Pharmaceuticals in Athens, was locally applied at the wart, daily for one week and then three times a week. Three months later the patient's clinical and colposcopy findings improved; both Pap smear result and colposcopy were negative. Two years later the patient has no clinical or laboratory suspicion of recurrence.

DISCUSSION

HPVs belong to the family of papilloma viruses, usually infecting exclusively skin and mucosal surfaces of the mouth, the anal, the female genitalia, and the epithelium of the endometrium resulting in cell proliferation and proliferative, precancerous, but also cancerous lesions. To date, 189 types of papillomaviruses have been identified, 120 of which infect humans (9).

HPV infection is the most common sexually transmitted disease in the U.S, affecting almost 1% of the sexually active population (10). Warts or condylomata are etiologically associated with some HPV types, mainly low risk types 6 or 11, but not exclusively. In contrast with the cervical intraepithelial neoplasias, which are silent, warts are usually noticed by the patient herself/himself and they vary from flat papules to large, cauliflower-like lesions. Diagnosis is clinical, but atypical lesions should be confirmed by histology. Therapy ranges between surgical (excision, electrosurgery, cryotherapy) and conservative. The latter depends on the topical application of various medicaments such as imiquimod, podofilox, podophyllin, bichloroacetic acid, and trichloroacetic acid. The cure rates are of each method are estimated to reach 30–50%, 45–80%, 30–80% and 50–80% respectively (6).

Derived from the perennial herb *Curcuma longa* (turmeric) curcumin is a polyphenol of plant origin known since many years as traditional Indian medicine. Turmeric

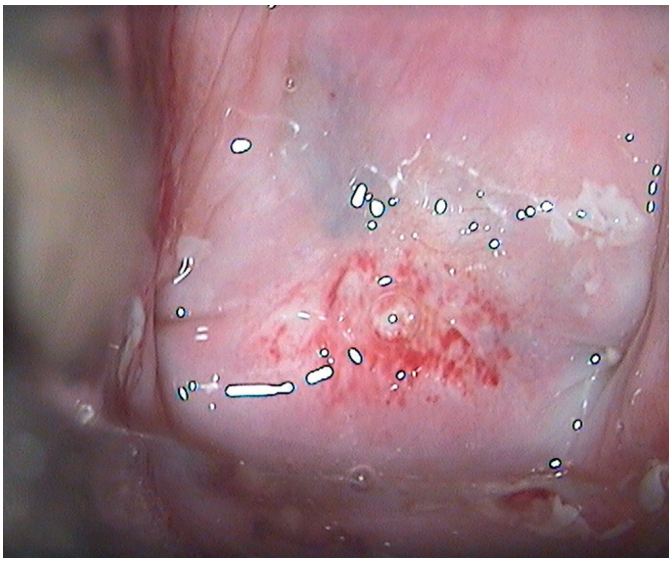

Fig. 1 Vaginal cuff wart before treatment.

was introduced into Europe in the 13th century by Marco Polo and surprisingly, since 1937, when an early study was published in The Lancet by Oppenheimer, it is in the last 15 years that it has gained increasing popularity and it has become the subject of many studies (8).

In the last decades, curcumin has been found to mediate in various cell signaling molecules and this way to downregulate inflammation mediators, cytokines, interleukins and enzymes, gaining anti-inflammatory effects and therapeutic potential against a wide range of pathologic conditions, such as many types of cancer, inflammatory bowel disease, osteoarthritis, H. Pylori infection, psoriasis, acute coronary syndrome, atherosclerosis, type 2 diabetes, renal transplantation and β-Thalassemia (11–13).

In the context of the aforementioned multiple properties, curcumin is considered cytotoxic against cervical cancer cells and has been found to downregulate the expression of HPV oncoproteins. Importantly, with regards to its safety, turmeric has been established to be safe and well-tolerated by human trials and is Generally Recognized As Safe (GRAS) by the US FDA (14). Interestingly, Debata et al, recently developed a curcumin-based vaginal cream that eradicates HPV positive cancer cells without affecting the healthy tissues (15).

CONCLUSIONS

As far as we know, most of the curcumin-related studies have highlighted its potential to clear HPV infection, and consequently the intraepithelial precancerous lesions it induces. Literature review about condylomata treatment with curcumin was poor but encouraging. The patient applied vaginally curcumin 2 times a week without further anti HPV treatment for 6 months and for 18 months now she has remained recurrent-free. Repeat cytology confirmed the recession of the wart. To the best of our knowledge, this is the first report in which curcumin has demonstrated an objective response in conventional treatment of a vaginal cuff condyloma.

LIST OF ABBREVIATIONS

THBO – Total Hysterectomy with Bilateral Oophorectomy

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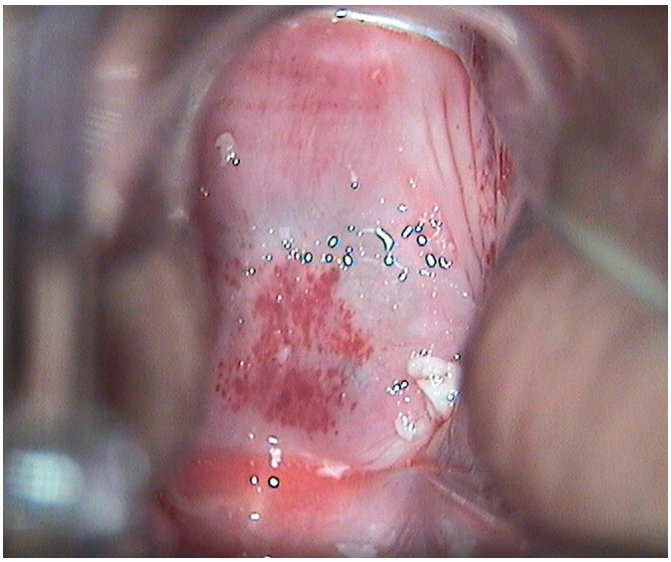

Fig. 2 Vaginal cuff wart after treatment with imiquimode.

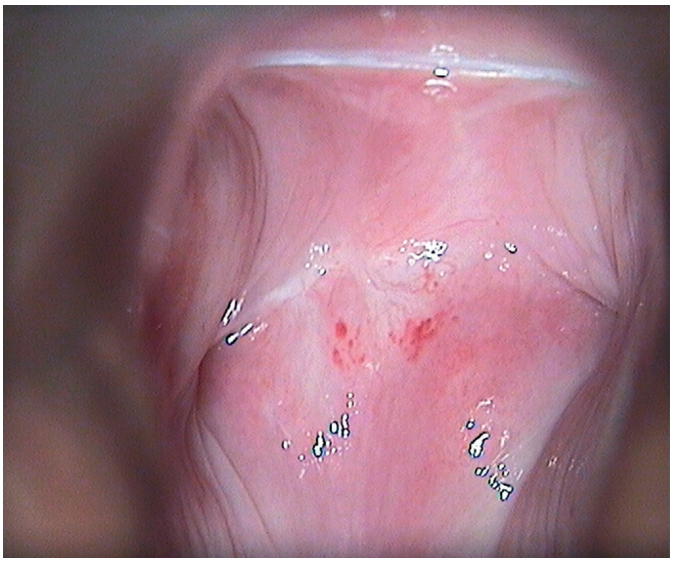

Fig. 3 Vaginal cuff wart after treatment with SiloffGyn.

Penile Degloving and Dorsal Dartos Flap Rotation Surgery in the Management of Severe Isolated Penile Torsion in a 6-Year-Old Boy

Zlatan Zvizdic[1], Emir Milisic[1], Semir Vranic[2,*]

ABSTRACT
Penile torsion is a rare congenital anomaly that is usually characterized by a counterclockwise rotation of the penile shaft or glans. Although several surgical techniques for its correction have been proposed, the consensus of choosing the most efficient technique remains controversial. Herein, we report our operational approach that successfully corrected a severe (>90 degrees) isolated penile torsion in the form of penile degloving and dorsal dartos flap rotation surgery.

KEYWORDS
isolated penile torsion; children; surgery; penile degloving; dorsal dartos flap rotation

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INTRODUCTION

Penile torsion is a rare congenital rotational defect of the penile shaft and glans on the longitudinal penile axis usually in a counterclockwise direction (1). Penile torsion is commonly seen in association with hypospadias or chordee while isolated penile torsion is rarely seen (2). Although the precise etiology of this anomaly is unclear, it is thought that penile torsion occurs because of the abnormality of the skin and dartos fascia attachment or abnormal development of the dartos fascia that causes disorientation of the penile shaft and corporeal rotation around its longitudinal axis (1). Recently, Zhou et al. proposed that the asymmetric development of the corpora cavernosa represented a major etiological factor of this anomaly (3). Regarding the degree of rotation, isolated penile torsion is divided into mild (<45 degrees), moderate (45–90 degrees) and severe (>90 degrees) forms (4). The precise incidence of isolated penile torsion is unknown but is believed to be in the range of 2–27% (5–7). However, surgical correction is required in only ~4% of patients (6).

Many operative techniques have been described for the correction of penile torsion including penile degloving and realignment technique, suturing the tunica albuginea to the pubic periosteum, dorsal dartos flap rotation, correction by mobilization of urethral plate and urethra, resection of Buck's fascia, modified Nesbit procedure, and diagonal corporal plication (1, 3, 4, 6, 8–11). However, the consensus on the most efficient and appropriate technique is still missing.

Herein, we report our operational approach for correction of severe (>90 degrees) isolated penile torsion in the form of penile degloving and dorsal dartos flap rotation surgery.

CASE REPORT

A 6-year-old uncircumcised boy presented with isolated penile torsion. Physical examination showed >90 degrees penile torsion, directed in a counterclockwise fashion with spiral deviation of the penile median raphe (Figures 1A–D). The surgical correction of penile torsion was carried out under general anesthesia. A circumferential subcoronal incision was taken and the penile skin and dartos were degloved to the penile root with division of all adhesion tissues. To achieve an artificial erection, we used a normal saline solution through a butterfly needle into one corporal body. The dorsal dartos flap technique was composed of dissection of the dorsal penile skin and dorsal dartos flap creation, which was rotated around the side of the penile shaft opposite to the direction of penile rotation and attached to the ventral aspect of the penile shaft. The operative technique was completed by a simple rearrangement of the skin on the shaft of the penis (Figure 2A). This operative technique has led to a complete correction of penile torsion, which was demonstrated by the presence of slit of the urethral meatus in one line with scrotal raphe (Figure 2B). Urinary catheter was not used during and after the procedure and no complications were recorded. Postoperative course of the patient was uneventful.

One-year follow-up revealed a satisfactory correction of the abnormal rotation in our patient.

DISCUSSION

For a long time after initial description of penile torsion by Verneuil in 1857, there has not been a proper recommendation for its operative correction (12). Recently, several researchers recommended a penile degloving as an adequate surgical procedure for correcting mild penile torsion (<45 degrees) while in moderate and severe degrees of penile torsion, other operative techniques were suggested. These approaches may, however, be associated with significantly higher risk of postoperative complications (1, 3, 4, 6, 8–11).

The dorsal dartos flap, previously used to cover the suture line urethroplasty in hypospadia surgery, proved as an effective technique for moderate and severe penile torsion (4, 13, 14). This technique was initially present-

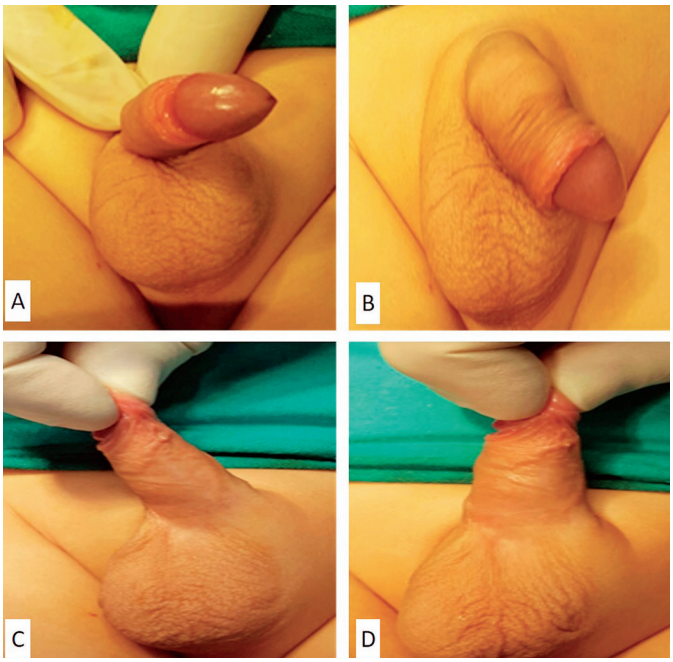

Fig. 1 (A–B): Isolated counterclockwise penile torsion (>90 degrees); (C–D): Median raphe pass in a spiral manner from the base of the penis ventrally and around the penile shaft.

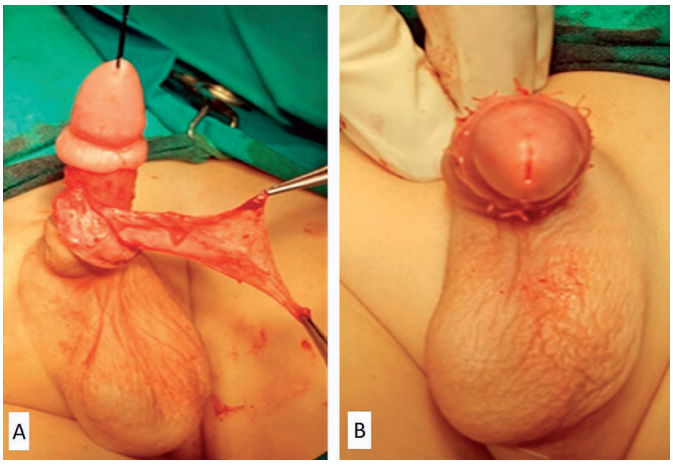

Fig. 2 (A): Intraoperative creation of dorsal dartos flap; (B): Postoperative view of complete correction of penile torsion.

ed by Fisher and Park in 2004 and implied performing a complete degloving of the penis, mobilization of a wide, well-vascularized dorsal dartos flap, its rotation around the right side of the penile shaft and fixation to the ventral aspect, causing clockwise penile rotation (4). This operative technique is completed by a simple rearrangement of the skin on the shaft of the penis (4).

The reported success rate of this technique in the complete correction of penile torsion was 100% in the Fisher and Park series (4), 97% in Marret et al. series (15), and only 64% in the Bauer and Kogan series (13). However, these authors found that 9/25 patients with incomplete penile torsion correction had a residual torsion of <10 degrees, which did not require an additional operative treatment (13). All these studies concluded that dorsal dartos flap rotation technique provides excellent short-term results.

CONCLUSIONS

Based on our experience and previous data, we confirm that the dorsal dartos flap rotation techniq isa suitable approach for the treatment of moderate and severe forms of penile torsion. It is a safe procedure that is free of major complications.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

CONSENT

The authors acknowledge the patient's family for consenting to the report of this illustrative case.

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