Oral Manifestations of Nutritional Deficiencies: Single Centre Analysis

Vladimíra Radochová[1,*], Radovan Slezák[1], Jakub Radocha[2]

ABSTRACT

Introduction: Oral manifestations of deficiency of iron, vitamin B12 and folic acid are thought to be common. Prevalence of these deficiencies among patients with compatible symptoms is not well known. The goal of this study was to summarize evidence from a dental practice of iron, vitamin B12 and folic acid deficiency in patients presenting with compatible oral manifestations.

Methods: 250 patients who presented with burning mouth syndrome, angular cheilitis, recurrent aphthous stomatitis, papillar atrophy of the tongue dorsum or mucosal erythema were identified. Patients underwent clinical examination, and the blood samples were taken.

Results: 250 patients (208 females; 42 males, mean age 44.1 years) with at least one corresponding symptom or sign were identified. The nutritional deficiency of one or more nutrients was found in 119 patients (47.6%). Seven times more females than males were noted to have one type of deficiency (104 females, 15 males). Iron deficiency as defined was diagnosed in 62 patients (24.8%), vitamin B12 or folic acid deficiency in 44 patients (17.6%) and both deficiencies (iron + vitamin B12/folic acid) in 13 patients (5.2%). The only predictive factor was gender and only for iron deficiency. The presence of more than one deficiency was noted in 10 patients (4.9%).

Conclusion: The most commonly observed deficiency in dental practice over the course of 11 years was an iron deficiency in the female population. Age, diet and reported co-morbidities did not show statistically significant predictable value in recognizing these deficiencies.

KEYWORDS

anemia; oral manifestations; iron deficiency; vitamin B12; folic acid

AUTHOR AFFILIATIONS

[1] Department of Dentistry, Faculty of Medicine in Hradec Králové, Charles University, and University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

[2] 4th Department of Internal Medicine – Hematology, Faculty of Medicine in Hradec Králové, Charles University, and University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

* Corresponding author: Department of Dentistry, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic, e-mail: vladimira.radochova@fnhk.cz

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INTRODUCTION

Vitamin and mineral nutritional deficiencies represent some of the most frequent disorders worldwide (1). Dental practitioners are often some of the first healthcare providers to observe oral manifestations of burning mouth, mucosal erythema or papillary atrophy of the tongue dorsum. The iron, folic acid and vitamin B12 deficiencies represent common challenging scenarios in routine dental practice. According to the World Health Organization (WHO), iron deficiency represents a common medical challenge with about 80% of anemias represented by iron deficiency, and 30% of the world population is at risk of developing iron deficiency (2). A vegetarian or vegan diet might influence the prevalence of nutrient deficiencies (3).

The clinical signs observed, and symptoms associated with oral cavity are often non-specific and may mimic or overlap with other types of deficiencies or disorders. The most frequently reported oral cavity symptoms of iron, vitamin B12 and folic acid deficiency are pale mucous membranes, erythema, glossitis, recurrent aphthous stomatitis, angular cheilitis and oral candidiasis (4, 5). Angular cheilitis and glossitis are mentioned as some of the common symptoms of iron deficiency (6). However, these two clinical signs may represent other pathology. The red beef tongue and burning mouth are more often reported to be related to vitamin B12 deficiency (7). The burning mouth, burning tongue, paresthesia and dysesthesia of the oral cavity often represent non-specific symptoms with nutritional deficiencies identified as some of the more common systemic factors (8).

The diagnosis of a nutritional or vitamin deficiency is based on patients' history, physical examination and laboratory findings. When oral signs and symptoms are identified, iron, vitamin B12 and folic acid deficiencies are commonly considered in differential diagnoses. The complete blood count, iron storage estimation and levels of vitamin B12 and folic acid represent necessary armamentarium for examination and development of differential diagnoses. These tests and exams are not commonly conducted by a general dentist. Furthermore, in large metropolitan or medical centre areas, the patient may easily be referred to an oral medicine specialist. This is not always possible in more rural areas or where there is limited access to an oral medicine specialist.

The goal of this retrospective study was to summarize clinical-based evidence from a dental practice of iron, vitamin B12 and folic acid deficiency in patients presenting with oral manifestations as an effort to improve health care delivery and provide information to help decision making and management of care with future patients.

PATIENTS AND METHODS

The patients who were referred to the consultation to the Department of Dentistry (tertiary dental clinic) University Hospital Hradec Kralove from January 2007 to December 2018 with one or more clinical symptoms or clinical signs were identified. The inclusion criteria for patients are summarized in Table 1. Patients with a known diagnosis of anemia were excluded from the study.

Tab. 1 Significant clinical signs and symptoms.

Objective signs	Subjective symptoms and history
glossitis with or without atrophic papillae	burning mouth syndrome
generalized or localized mucosal erythema	history of angular cheilitis
angular cheilitis	history of recurrent aphthous stomatitis
aphthous stomatitis	history of recurrent candidosis
oral candidosis	

A retrospective chart review was conducted of the identified patients. The research protocol was approved by the institutional review board and ethical committee.

The parameters of interest included: patient age, sex, special diets, the initial date of appointment, medical, dental, social, and family history; review of medications, allergies and medical co-morbidities. Initial intraoral and extraoral examination findings at the time of patient presentation, patient-reported symptoms and signs (Table 2), complete blood count with differential leukocyte count, vitamin B12 level, folic acid level, ferritin, serum iron and total iron-binding capacity, basic chemistry panel especially liver and kidney function tests, minerals, glucose) conducted as a routine practice to exclude potential frequent systemic disease.

Tab. 2 Frequency of specific deficiencies.

	N	(%)
Total No. Of patients with symptoms (in Table 1)	250	
Females	208	83.2
Males	42	16.8
Mean age (years)		
Females	46.4	
Males	51.7	
History of systemic disease		
Hypothyroidism	20	8.0
Autoimmune disease *	11	4.4
Arterial hypertension	7	2.8
Cancer (breast)	3	1.2
Diabetes mellitus	3	1.2
Other	4	1.6
Nutrient deficient patients		
Total number of deficiencies	119	47.6
Iron deficiency	62	24.8
Vitamin B12 and/or folic acid deficiency	44	17.6
Iron deficiency + B12 and/or folic acid	13	5.2
Total number without deficiency	131	52.4

* Includes rheumatoid arthritis, Sjögren syndrome, bronchial asthma.

The presence of iron deficiency was defined as serum ferritin level of less than 20 µg/l. Vitamin B12 deficiency was defined as serum levels less than 150 pmol/l and less than 9 nmol/l for folic acid deficiency. The latent deficiency was defined as presence of deficiency without anemia or other changes in the blood count. It was taken in account in the same manner as deficiency with anemia.

The continuous parameters of interest were summarized with means, medians, ranges. The categorical parameters were summarized with percentages and frequencies. Chi-square and Fisher's exact test was used to evaluate comparisons among parameters of interest. Data were analyzed using Microsoft Excel 2007 (Microsoft, USA) and MedCalc 9.5.2.0 (MedCalc, Belgium). P-values < .05 were considered statistically significant, and all tests were 2-sided.

RESULTS

PATIENT DEMOGRAPHICS

A total of 250 patients (208 females; 42 males, mean age 44.1 years) were identified. No patient was vegetarian or vegan. The nutritional deficiency of one or more nutrients was found in 119 patients (47.6%). Seven times more females than males were noted to have one type of deficiency (104 females, 15 males). Iron deficiency as defined was diagnosed in 62 patients (24.8%), vitamin B12 or folic acid deficiency in 44 patients (17.6%) and both deficiencies (iron + vitamin B12 and/or folic acid) in 13 patients (5.2%). Patients' characteristics are given in detail in Table 2. Females were more likely affected by iron deficiency than males (p = 0.020).

CLINICAL SIGNS AND SYMPTOMS

Distribution of each symptom and sign is shown in Table 3, and in specific groups of patients with corresponding deficiency is shown in Table 4. Comparison of a group of patients with and without deficiency related to symptom frequency is shown in Table 5. Erythema of the mucosa was present more frequently in patients with a deficiency compared to those without (29.4% versus 10.7%, p < 0.001). Angular cheilitis was significantly more associated with the presence of deficiency (36.1% with and 17.6% without deficiency, p = 0.001). The common clinical sign noted in patients with an identified deficiency was papillary atrophy regardless of deficiency type (31.9% with and 9.9% without deficiency, p < 0.001). Presence of burning mouth and recurrent aphthous stomatitis was not significantly different among patients with and without deficiency, see Table 6 for details.

Tab. 3 Frequency of symptoms and signs.

Symptom present	N	(%)
Burning mouth syndrome	129	51.6
Recurrent aphthous stomatitis	89	35.6
Angular cheilitis	66	26.4
Papillar atrophy of tongue dorsum	51	20.4
Erythema	49	19.6
Candidosis	9	3.6

Tab. 5 Differences in symptoms in patients with and without deficiency.

	N	%	p value
Erythema			
Deficiency	35/119	29.4	**< 0.001**
No deficiency	14/131	10.7	
Burning			
Deficiency	62/119	52.1	0.981
No deficiency	67/131	56.3	
Aphthae			
Deficiency	38/119	31.9	0.307
No deficiency	51/131	38.9	
Papillar atrophy			
Deficiency	38/119	31.9	**< 0.001**
No deficiency	13/131	9.9	
Angular cheilitis			
Deficiency	43/119	36.1	**0.001**
No deficiency	23/131	17.6	

Tab. 4 Symptoms according to deficiency.

	Burning	%	Recurrent aphthae	%	Angular cheilitis	%	Erythema	%	Papillar atrophy	%	Candidosis	%
Overall (N)	129		89		66		49		51		9	
Males	18	14.0	18	20.2	6	9.1	5	10.2	4	7.8	2	22.2
Females	111	86.0	71	79.8	60	90.9	44	89.8	47	92.2	7	77.8
Iron deficiency	34	26.4	25	28.1	29	43.9	19	38.8	28	54.9	3	33.3
B12 deficiency	34	26.4	14	15.7	18	27.3	21	42.9	16	31.4	4	44.4
Folic acid deficiency	6	4.7	5	5.6	3	4.5	5	10.2	3	5.9	2	22.2
None	67	51.9	51	57.3	23	34.8	14	28.6	13	25.5	1	11.1

Tab. 6 Comparison of symptoms in iron and vitamin B12/folic acid deficiency.

	N	%	p value
Burning			**0.0002**
Iron deficiency	34/62	54.8	
B12/folic acid deficiency	40/44	90.9	
Papillar atrophy of the tongue dorsum			0.997
Iron deficiency	28/62	45.2	
B12/folic acid deficiency	19/44	43.2	
Angular cheilitis			0.92
Iron deficiency	29/62	46.8	
B12/folic acid deficiency	21/44	47.7	
Aphthae			0.924
Iron deficiency	25/62	40.3	
B12/folic acid deficiency	19/44	43.2	
Erythema			**0.007**
Iron deficiency	19/62	30.6	
B12/folic acid deficiency	26/44	59.1	

IRON, VITAMIN B12, FOLIC ACID

The most common deficiency was iron deficiency representing 52.1% of all pathologies followed by vitamin B12 and/or folic acid deficiency (36.9%). Both deficiencies (iron + vitamin B12 and/or folic acid) represented 10.9% of all deficiencies. Anemia in the blood count was found in more than half of patients with iron, vitamin B12 or folic acid deficiency (n = 61/119, 51.3%).

CLINICAL MANAGEMENT AND FOLLOW UP

Local treatment was advised for patients with candidosis (antifungal oral rinses) and topical treatment with hydrocortisone, natamycin and neomycin ointment was advised for patients with angular cheilitis. The patients were referred to the general practitioner when the deficiency was found. Supplementation of missing nutrient, search for the underlying cause was recommended. Patient counselling of such deficiencies was completed together with a discussion of their impact on overall health. None of the patients whose symptoms were due to nutrient deficiency was in need of additional referrals for further follow up to our department. Results of supplementation by the general practitioner are not available.

DISCUSSION

Nutritional deficiencies represent a significant medical challenge even in the contemporary European population. Diet habits and socioeconomic status may contribute to the development of such problems (9). Women are generally at higher risk of iron deficiency because of their regular iron loss during the menstrual cycle and pregnancy (overall, 58 females with iron deficiency compared to only four males in the current study). One of the most significant studies by Viñas published in 2011 described the prevalence of micronutrient deficiencies in the European population. 21–30% of adult males from Finland and adult females from Ireland (both countries with high social and economic standards) had insufficient vitamin B12 intake. The prevalence of vitamin B12 deficiency increases with age as well. It reached 19% in a Swiss population of seniors above 80 years of age (10). Another study from Ireland described only 2.7% of vitamin B12 deficiency in the adult population (11). Results of our group of folic acid and vitamin B12 did not vary in age compared to the group without deficiency. Older patients might especially suffer from folic acid deficiency (12). Iron deficiency seems to affect a more extensive age range. Children as young as 12 to 36 months of age could have up to 11.8% of iron deficiency (13). Adolescent children between 12 and 17 years of age have iron deficiency present in 17.6% across Europe (14). Finally, the prevalence of iron deficiency anemia can reach up to 19.9% in the adult European population (15). The age of our iron-deficient patients (mean age 41.9 years) was significantly lower than in those without deficiency.

Since the prevalence of nutritional deficiencies is high, their presence is expected to be high as well in the general population. The first signs and symptoms of these deficiencies do not necessarily need to be anemia and often manifest themselves in other organ symptoms. Graells published a small pivotal study of 4 patients with Hunter glossitis and normal blood count. The organ symptoms in all 4 patients preceded the development of anemia (16). In the presented study, 48.7% of patients (N = 58) had a deficiency without any change in the blood count. These findings are consistent with the study of Asian population where a total of 149 patients with vitamin B12 and iron deficiency revealed normal blood counts in 36.2% of patients matching almost entirely results from our Czech sample (17).

Burning mouth is one of the most frequent signs that bring patients to dental medicine clinics. It represents a wide variety of diseases ranging from benign to severe disorders. The exact etiology of burning mouth syndrome cannot be frequently identified (18). Nutrition deficiencies and anemia represent the frequent and often reversible cause of burning mouth. Lin et al. described a decrease of hemoglobin in a cohort of 399 Asian patients with the burning mouth in 22.3%. 20.3% of these 399 patients had iron deficiency, 2.5% vitamin B12 deficiency and 1.5% folic acid deficiency (19). Very similar findings are shown by the current study, where 24.8% of patients had an iron deficiency in a slightly smaller European population.

Similarly, our current data showed a more significant portion of patients with vitamin B12 and the folic acid deficiency (17.6%). An Italian study showed 11% of vitamin B12 and 12.5% of folic acid deficiency in patients with burning mouth syndrome (20). There are apparent geographic differences in the prevalence of each deficiency. Even subtle changes in mean cell erythrocyte volume can be a sign of deficiency. All patients (100%) with increases mean cell volume had burning mouth in the study by

Chang published in 2015. 66.7% of these patients had concomitants atrophic glossitis (21).

The oral symptoms are not similar for various deficiencies. While the most frequent symptom (burning mouth) is similarly distributed among patients with and without underlying deficiency, objective signs differ in groups of patients with and without deficiency in our cohort of patients. Likewise, the most frequent sign described is angular cheilitis (58%) and papillar atrophy of the tongue dorsum (42%) in a smaller cohort of patients with oral symptoms from 2004 as reported by Lu et al. (4). The patients with iron deficiency represented 40% of symptomatic patients and vitamin B12 20% (4). Analysis of our group revealed one fourth (24%) of patients with iron deficiency and 11.7% of patients with vitamin B12 deficiency. Very similar findings were observed in a study by Wu et al. (22) The authors described the cohort of iron-deficient patients showing papillar atrophy present in 26.7% of patients with iron deficiency compared to 54.9% in our group.

According to Sun et al., patients with papillar atrophy more frequently have iron deficiency (26.7%) than vitamin B12 deficiency (7.4%) (23). Andrès et al. described atrophic glossitis in 62% of hospitalized patients who were found to have vitamin B12 deficiency which is more compared to our study (43.2%) (24). Recurrent aphthous stomatitis is also a frequent finding. Iron deficiency can be identified in around 20% of patients with aphthous stomatitis which is a bit lower than 28.1% in our group (25). Kozlaka et al. found that patients with recurrent aphthous stomatitis have a lower daily intake of vitamin B12 compared to healthy population (26). We observed a very low prevalence of oral candidiasis in our group. Recently published data by Lu showed the prevalence of oral pseudomembranous and erythematous candidiasis around 40% in patients in a similar cohort of patients (27). The atrophic glossitis can have the very similar clinical picture, and the deficiency can be overlooked (28).

Reversal of symptoms by supplementation of missing nutrients has been shown to be effective in a cohort of 399 patients (18). The burning mouth disappeared within 5–10 months after supplementation of missing nutrient in virtually all affected patients (29). None of our patients with revealed deficiency came back with the same symptoms after the recommended treatment.

CONCLUSION

Nutritional deficiencies and their symptoms are very frequent and commonly present without corresponding anemia. The most common deficiency identified in our cohort was iron deficiency with an expected female predominance. Other deficiencies were evenly distributed among males and females. The diagnosis of the deficiency might also lead to the identification of potentially life-threatening underlying diseases and maybe the first guide to the correct diagnosis of systemic disease and treatment. The dentists should be familiar with the above to identify the signs and symptoms, forcing the proper evaluation adequately.

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CONFLICTS OF INTEREST

None declared.

REFERENCES:

1. GlobalHealthRisks_report_full.pdf [Internet]. Available at: http://www.who.int/healthinfo/global_burden_disease/ GlobalHealthRisks_report_full.pdf
2. WHO | Worldwide prevalence of anaemia 1993-2005 [Internet]. WHO. Available at: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596657/en/
3. Barr SI. Vegetarian diets. World Rev Nutr Diet 2015; 111: 53–7.
4. Lu S-Y, Wu H-C. Initial diagnosis of anemia from sore mouth and improved classification of anemias by MCV and RDW in 30 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 679–85.
5. Adeyemo TA, Adeyemo WL, Adediran A, Akinbami AJA, Akanmu AS. Orofacial manifestations of hematological disorders: anemia and hemostatic disorders. Indian J Dent Res 2011; 22: 454–61.
6. Long RG, Hlousek L, Doyle JL. Oral manifestations of systemic diseases. Mt Sinai J Med N Y 1998; 65: 309–15.
7. Greenberg MS. Clinical and histologic changes of the oral mucosa in pernicious anemia. Oral Surg Oral Med Oral Pathol 1981; 52: 38–42.
8. Coculescu EC, Tovaru S, Coculescu BI. Epidemiological and etiological aspects of burning mouth syndrome. J Med Life 2014; 7: 305–9.
9. Huffman FG, Vaccaro JA, Zarini GG, Biller D, Dixon Z. Inadequacy of micronutrients, fat, and fiber consumption in the diets of Haitian-, African- and Cuban-Americans with and without type 2 diabetes. Int J Vitam Nutr Res 2012; 82: 275–87.
10. Risch M, Meier DW, Sakem B, et al. Vitamin B12 and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. BMC Geriatr 2015; 15: 82.
11. McNamee T, Hyland T, Harrington J, et al. Haematinic deficiency and macrocytosis in middle-aged and older adults. PloS One 2013; 8(11): e77743.
12. Roman Viñas B, Ribas Barba L, Ngo J, et al. Projected prevalence of inadequate nutrient intakes in Europe. Ann Nutr Metab 2011; 59: 84–95.
13. Akkermans MD, van der Horst-Graat JM, Eussen SRBM, van Goudoever JB, Brus F. Iron and Vitamin D Deficiency in Healthy Young Children in Western Europe Despite Current Nutritional Recommendations. J Pediatr Gastroenterol Nutr 2016; 62: 635–42.
14. Ferrari M, Mistura L, Patterson E, et al. Evaluation of iron status in European adolescents through biochemical iron indicators: the HELENA Study. Eur J Clin Nutr 2011; 65: 340–9.
15. Fonseca C, Marques F, Robalo Nunes A, Belo A, Brilhante D, Cortez J. Prevalence of anaemia and iron deficiency in Portugal: the EMPIRE study. Intern Med J 2016; 46: 470–8.
16. Graells J, Ojeda RM, Muniesa C, Gonzalez J, Saavedra J. Glossitis with linear lesions: An early sign of vitamin B12 deficiency. J Am Acad Dermatol 2009; 60: 498–500.
17. Chang JY-F, Wang Y-P, Wu Y-C, Cheng S-J, Chen H-M, Sun A. Blood profile of oral mucosal disease patients with both vitamin B12 and iron deficiencies. J Formos Med Assoc 2015; 114: 532–8.
18. Sun A, Wu K-M, Wang Y-P, Lin H-P, Chen H-M, Chiang C-P. Burning mouth syndrome: a review and update. J Oral Pathol Med 2013; 42: 649–55.
19. Lin H-P, Wang Y-P, Chen H-M, Kuo Y-S, Lang M-J, Sun A. Significant association of hematinic deficiencies and high blood homocysteine levels with burning mouth syndrome. J Formos Med Assoc 2013; 112: 319–25.
20. Sardella A, Lodi G, Demarosi F, Uglietti D, Carrassi A. Causative or precipitating aspects of burning mouth syndrome: a case–control study. J Oral Pathol Med 2006; 35: 466–71.
21. Chang JY-F, Wang Y-P, Wu Y-C, Cheng S-J, Chen H-M, Sun A. Hematinic deficiencies and pernicious anemia in oral mucosal dis-

ease patients with macrocytosis. J Formos Med Assoc 2015; 114: 736–41.
22. Wu Y-C, Wang Y-P, Chang JY-F, Cheng S-J, Chen H-M, Sun A. Oral manifestations and blood profile in patients with iron deficiency anemia. J Formos Med Assoc 2014; 113: 83–7.
23. Sun A, Lin H-P, Wang Y-P, Chiang C-P. Significant association of deficiency of hemoglobin, iron and vitamin B12, high homocysteine level, and gastric parietal cell antibody positivity with atrophic glossitis. J Oral Pathol Med 2012; 41: 500–4.
24. Andrès E, Nachit M, Guillet-Thibault J. Oral manifestations of vitamin B12 and B9 deficiencies: a prospective study. J Oral Pathol Med 2016; 45: 154–154.
25. Sun A, Chen H-M, Cheng S-J, et al. Significant association of deficiencies of hemoglobin, iron, vitamin B12, and folic acid and high homocysteine level with recurrent aphthous stomatitis. J Oral Pathol Med 2015; 44: 300–5.
26. Kozlak ST, Walsh SJ, Lalla RV. Reduced dietary intake of vitamin B12 and folate in patients with recurrent aphthous stomatitis. J Oral Pathol Med 2010; 39: 420–3.
27. Lu S-Y. Perception of iron deficiency from oral mucosa alterations that show a high prevalence of Candida infection. J Formos Med Assoc 2016; 115: 619–27.
28. Terai H, Shimahara M. Atrophic tongue associated with Candida. J Oral Pathol Med 2005; 34: 397–400.
29. Sun A, Lin H-P, Wang Y-P, Chen H-M, Cheng S-J, Chiang C-P. Significant reduction of serum homocysteine level and oral symptoms after different vitamin-supplement treatments in patients with burning mouth syndrome. J Oral Pathol Med 2013; 42: 4.

Association of XPC Polymorphisms with Cutaneous Malignant Melanoma Risk: Evidence from a Meta-Analysis

Fatemeh Asadian[1], Seyed Mohammadreza Niktabar[2,*], Yaser Ghelmani[3], Shadi Kargar[2], Elahe Akbarian[4], Seyed Alireza Emarati[4], Jalal Sadeghizadeh-Yazdi[5], Hossein Neamatzadeh[6, 7]

ABSTRACT

Background: A number of studies have reported that the xeroderma pigmentosum complementation group C (XPC) polymorphisms are associated with cutaneous malignant melanoma (CMM) susceptibility. But the results of those studies were inconsistent. Here, we performed a study to obtain a more conclusive result on the association of XPC polymorphisms with risk of CMM.
Methods: The XPC Lys939Gln and Ala499Val polymorphisms were genotyped in 150 CMM cases and 150 controls by PCR-RFLP assay. Subsequently, all published relevant studies were identified through a comprehensive literature search in PubMed, Web of Science, and CNKI databases. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of correlation.
Results: There was no significant association between XPC Lys939Gln and Ala499Val polymorphisms and CMM risk in our population. A total of 15 case-control studies including ten studies with 5,990 cases and 7,697 controls on XPC Lys939Gln and five studies with 3,139 cases and 3,721 controls on XPC Ala499Val polymorphism were selected. Pooled data revealed that XPC Lys939Gln (C vs. A: OR = 1.108, 95% CI 1.008–1.217; P = 0.033) and Ala499Val (C vs. A: OR = 0.918, 95% CI 0.850–0.992; p = 0.031; CC+CA vs. AA: OR = 0.904, 95% CI 0.819–0.997; p = 0.043) polymorphisms were significantly associated with an increased risk of CMM. Moreover, stratified analyses by ethnicity revealed that the XPC Ala499Val and Lys939Gln polymorphisms were significantly associated with risk of CMM in Caucasians and mixed populations, respectively.
Conclusions: This meta-analysis result suggested that XPC Lys939Gln and Ala499Val polymorphisms were significantly associated with risk of CMM.

KEYWORDS

cutaneous melanoma; malignant melanoma; XPC gene; polymorphism; meta-analysis

AUTHOR AFFILIATIONS

[1] Department of Medical Laboratory Sciences, School of Paramedical Science, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Department of Surgery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Clinical Research Development Center of Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5] Department of Food Science and Technology, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[6] Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[7] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
* Corresponding author: Department of Surgery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; e-mail: niktabarsmn@gmail.com

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INTRODUCTION

Cutaneous malignant melanoma (CMM) is an aggressive tumor of melanocytes in skin with rapidly increasing incidence causing a major public health problem (1). CMM responsible for less than 5% of all skin cancers but over 75% of skin cancer related deaths (2–4). Its incidence varies dramatically between different ethnicities (3, 4). Analysis of the data from 29 countries suggested that Australia and New Zealand has by far the greatest incidence, illustrating the connection between white populations near the equator and CMM (4). There are numerous risk factors such as age, fair skin type, family history of CMM and presence of many or large nevi identified for developing CMM (5). It is firmly established that around 8–12% of CMM cases have a family history of CMM (6). UV-light is the most important cause, and the incidence of CMM in individuals with a susceptible skin type increases with proximity to the equator (7). Despite several decades of research on CMM, both etiology and pathogenesis of this disease is still unknown.

The etiology of CMM is likely to be multifactorial, involving UV exposure and genetic predisposition (6–8). The nucleotide excision repair (NER) is a versatile system that repairs a wide variety of DNA damage, including UV photoproducts (9). Thus, genetic mutations of NER proteins may be the natural candidate for development of CMM in association studies (10). There are at least eight core NER proteins participating in the pathway, and mutations in their genes may alter NER functions (11). The xeroderma pigmentosum complementation group C (XPC) is one of the key members in the NER pathway (10, 12). The XPC protein can form a XPC-RAD23B complex with RAD23B, which involved in global genome repair and works as the earliest damage detector to initiate the NER pathway. In addition, XPC may also possess some functions in base excision repair (BER) via attenuation with thymine DNA glycosylate and the human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) (13, 14). Mutation in this gene can lead to Xeroderma pigmentosum (XP), a rare autosomal recessive disorder characterized by extreme UV-sensitivity (15).

The human XPC gene is located on chromosome 3p25.1, consists of 16 exons, and encodes a 940 amino acid protein (16). To date, at least 102 coding-region single nucleotide polymorphisms (SNPs) in the XPC gene have been identified, among which two common SNPs including Lys939Gln (rs2228001) in exon15 and Ala499Val (rs2228000) in exon 8 most frequently studied in CMM (16, 17). Moreover, it has been shown that XPC Lys939Gln and Ala499Val polymorphisms may be a risk factor in various cancers such as bladder cancer, prostate cancer, lung cancer, head and neck cancer and digestive system cancer (18–20). Over the last decade, several epidemiological studies evaluated association of XPC Lys939Gln and Ala499Val polymorphisms with risk of CMM. However, the associations remain controversial in susceptibility to CMM, partially because of a possible weak effect of the polymorphisms on CMM risk, ethnicity, sample size, study design, and also using different genotyping methods. Hence, we performed a meta-analysis to derive a relatively comprehensive assessment of the association between XPC Lys939Gln and Ala499Val polymorphisms and CMM risk.

MATERIALS AND METHODS

CASE-CONTROL STUDY

Study Population

The melanoma patient group consisted of 714 unselected participants, 451 women (mean age, 63 years) and 263 men (mean age, 65.5 years) from Poland.

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A total of 150 cases diagnosed with CMM consisted of 150 participants included 83 women (mean age, 61 years) and 67 men (mean age, 63 years) were enrolled from central cities of Iran. All cases had undergone surgical treatment for primary (54%) or metastatic melanoma (56%) between June 2015 and July 2017. In addition, 150 age and sex matched, unrelated healthy subjects without cancer family history (first- and second-degree relatives) were recruited after dermatological examination form same cities. All participants were Persian. The study was approved by the Medical Research Ethics Committee and the written informed consent was obtained from the study participants.

SNPs Genotyping

DNA samples were obtained from peripheral blood of CMM cases and healthy subjects using the Qiagen Blood DNA Mini Kit (QIAGEN, Venlo, Netherlands) according to the instructions of the manufacturer. DNA was diluted to 50 ng/μL concentration and was stored at –70 °C until genotyping. Genotype analyses of XPC Lys939Gln and Ala499Val polymorphisms were performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay as described previously. Primer sequences were: XPC Lys939Gln, 5′-ACCTGTCCAGAGTGAGGCAG-3′ (forward) and 5′-TCAAAGGGTGAGTGGGCTTT-3′ (reverse), and XPC Ala499 Val, 5′-TGGCCTCCAGGGTGTCTTAT-3′ (forward) and 5′-ACTGTCAATGCCCACCACAT-3′ (reverse). PCR amplification conditions included denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 67 °C for 30 seconds, polymerization at 72 °C for 40 seconds, and a final stage of polymerization at 72 °C for 7 minutes. The PCR products were then digested with restriction endonucleases. For XPC Lys939Gln, the PCR products were with one unit of PvuII and AciI restriction enzymes for XPC Lys939Gln and Ala499Val overnight at 37 °C, respectively. DNA fragments were resolved on 3% agarose gels and stained with ethidium bromide. A difference between cases and controls regarding alleles and genotypes of XPC Lys939Gln and Ala499Val polymorphisms was analyzed by Chi-square test. Goodness-of-fit $\chi 2$ test was performed to test whether the genotype frequency distribution of each polymorphism in controls was in Hardy-Weinberg equilibrium (HWE). All statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). A two-sided statistical significance level of 0.05 was chosen.

META-ANALYSIS

Search Strategy

This meta-analysis conformed to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) criteria. Eligible studies were identified through computer-aided literature searching in PubMed, EMBASE, Web of Science, Science Direct, Scopus, Cochrane Library database, Springer Link, Chinese Biomedical Database (CBD), China National Knowledge Infrastructure (CNKI), VIP, SID, Wanfang and Chinese Biomedical database up to September 25, 2019. The following terms and keywords were used for this search: ("Skin Cancer" OR "Melanoma" OR "Cutaneous Melanoma" OR "Malignant Melanoma" OR "Cutaneous Malignant Melanoma") AND ("Xeroderma pigmentosum" OR "Complementation Group C" OR "XPC") AND ("Lys939Gln" OR "rs2228001") OR ("Ala499Val OR "rs2228000") AND ("Gene" OR "Polymorphism" OR "SNPs" OR "Mutation" OR "Variation" OR "Allele"). We also included additional studies by a hands-on search of references of original studies. Of the studies with the same or overlapping data, the most recent ones with the most subjects were selected.

Selection Criteria

The inclusion criteria of studies in the meta-analysis were defined as follows: 1) original and published data; 2) studies with case-control or cohort design; 3) evaluates the associations of XPC Lys939Gln and Ala499Val polymorphisms with CMM risk; 4) provides sufficient data for calculation of odds ratio (OR) with 95% confidence interval (CI). In addition, the following exclusion criteria were used: 1) none-case control studies; 2) no usable data reported; 3) case only studies (without controls); 4) linkage studies, twins, sibling and other family based studies; 5) animal studies; 6) abstracts, case reports, posters, editorials, reviews, conference articles and previous meta-analyses; and 7) duplicated publications and repeated literatures.

Data Extraction

Two authors independently evaluated the articles for compliance with our inclusion criteria and data was carefully extracted from all eligible studies. Any potential disagreements were resolved by discussion until consensus was reached. The following data were extracted from each study: first author name, publication year, country of origin, ethnicity, source of controls, genotyping methods, genotype distribution of XPC Lys939Gln and Ala499Val polymorphisms in CMM cases and controls, minor allele frequencies (MAFs) in control groups, and result of HWE test in control subjects. Diverse ethnicity descents were categorized as Asian, Caucasian, and African. In the case of multiple studies by the same authors with overlapping data, the most recent published study with the largest number of participants was included in the current meta-analysis.

STATISTICAL ANALYSIS

The strength of the association between XPC Lys939Gln and Ala499Val polymorphisms and risk of CMM was measured by odd ratios (ORs) with 95% confidence intervals (CIs). Z-test was carried out to evaluate the statistical significance of pooled ORs. The pooled ORs were performed under the following five genetic models: allele model (B vs. A), homozygote model (BB vs. AA), heterozygote model (BA vs. AA), dominant model (BA+BB vs. AA) and recessive model (BB vs. BA+AA). The heterogeneity between studies was assessed with the chi-squared based Q-test and I^2 statistics. A significant p value (< 0.10) was used to indicate heterogeneity among studies. Moreover, a high value of I^2 indicated a higher probability of the existence of heterogeneity ($I^2 = 0\%$ to 25%, no heterogeneity; $I^2 = 25\%$ to 50%, moderate heterogeneity; $I^2 = 50\%$ to 75%, large heterogeneity; and $I^2 = 75\%$ to 100%, extreme heterogeneity). When between-study heterogeneity was found a random-effects model was performed; otherwise, a fixed-effects model (Mantel-Haenszel method) was accepted. Stratification and meta-regression analyses were used to detect the potential heterogeneity among studies. HWE of genotype distribution in the controls of included studies was conducted using an online program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl), and $P < 0.05$ was considered significantly deviating from HWE. To validate the reliability and stability of the results, sensitivity analysis was performed with a single study in the meta-analysis being deleted each time to reflect the influence of the individual data set on the pooled OR, as well as limiting this meta-analysis to studies which were conformed to HWE. Publication bias was assessed by Begg's test and Egger's test. The funnel plot was employed to examine the publication bias. Egger's regression analysis was used for reevaluation of publication bias. The significance of the intercept was determined by the t test suggested by Egger, with $p < 0.10$ considered representative of statistically significant publication bias. Funnel plots and Egger's linear regression tests were used to provide a diagnosis of the potential publication bias. In the presence of a bias, we utilized the Duval and Tweedie non-parametric "trim and fill" methods to adjust results. All of the statistical calculations were performed using Comprehensive Meta-Analysis (CMA) software version 2.0 (Biostat, USA). Two-sided P-values < 0.05 were considered statistically significant.

RESULTS

CASE-CONTROL STUDY

In this case-control study, a total of 300 samples including 150 patients diagnosed with CMM and 150 controls were recruited. Age and gender did not show a statistically different distribution between cases and controls. All observed genotype frequencies of the XPC Lys939Gln and Ala499Val polymorphisms in the control group were in accordance with the HWE ($p = 0.492$ and $p = 0.698$, respectively). Distribution of XPC Lys939Gln and Ala499Val polymorphisms in melanoma cases and controls are shown in Table 1. The frequencies of XPC Lys939Gln polymorphism AA, AC, and CC genotypes in patients were 28.7%,

Tab. 1 Distribution of XPC gene polymorphisms in CMM cases and controls.

Polymorphism	Cases (n = 150)	Control (n = 150)	OR (95% CI)	p-value
XPC Lys939Gln				
Genotypes				
AA	55 (28.7%)	49(28.0%)	Ref.	
AC	69 (50.0%)	77(53.3%)	0.808 (0.513–1.271)	0.356
CC	26 (21.3%)	24 (18.7%)	1.101 (0.600–2.021)	0.757
Allele				
A	179 (53.7%)	175 (54.7%)	Ref.	
C	121 (46.3%)	125 (45.3%)	0.946 (0.683–1.310)	0.740
XPC Ala499Val				
Genotypes				
GG	80 (27.3%)	79 (31.3%)	Ref.	
GA	57 (48.0%)	61 (46.0%)	0.894 (0.563–1.422)	0.636
AA	13 (24.7%)	10 (22.7%)	1.328 (0.564–3.131)	0.516
Allele				
G	217 (51.3%)	219 (54.3%)	Ref.	
A	83 (48.7%)	81 (46.7%)	1.034 (0.722–1.481)	0.855

OR: Odds Ratio; CI: Confidence Interval

50.0%, and 21.3%, respectively, which were similar to those in the control (28.1%, 53.3% and 18.6%, respectively). For XPC Ala499Val polymorphism GG, GA and AA genotypes were found in 27.3%, 48.0% and 24.7% cases, respectively. In control group, GG, GA and AA genotypes were found in 31.3%, 46.0% and 22.7%, respectively. The chi-square test results showed that there was no significant difference between the genotypic frequencies of XPC Lys939Gln and Ala499Val polymorphisms between CMM cases and controls (Table 1).

META-ANALYSIS

Figure 1 shows the flowchart of literature search and selection process. Based on the search criteria, 117 individual literatures were found. After screening the titles and abstracts, 45 publications were excluded. Therefore, 83 full text publications were preliminarily identified for further detailed evaluation. Subsequently, 68 studies were excluded: were not relevant to the XPC Lys939Gln and Ala499Val polymorphisms on CMM risk, not presenting sufficient data of genotype for calculating OR and 95% CI, reviews, previous meta-analyses, and case reports. Finally, a total of 15 case-control studies including ten case-control studies with 5,990 cases and 7,697 controls on XPC Lys939Gln polymorphism and five case-control studies with 3,139 cases and 3,721 controls on XPC Ala499Val polymorphism were selected (10, 21–28). The baseline characteristics of the included studies are shown in Table 2. The main characteristics of the studies were presented in Table 2. All included studies were published between 2005 and 2013. The studies have been carried out in Germany, USA, Brazil, Spain, Poland, and Iran. As for ethnicity, eleven studies were conducted among Caucasians, two studies among Asians, two studies among Africans. Four different genotyping approaches were applied by the selected studies including: PCR-RFLP, TaqMan, Illumina GoldenGate Assay, and Sequenom. The genotype and minor allele frequency (MAF) distributions in the studies considered in the present meta-analysis are shown in Table 2. Moreover, the distribution of genotypes in the controls was in agreement with HWE for all selected studies, except for one study (23) on XPC Lys939Gln polymorphism (Table 2).

QUANTITATIVE DATA SYNTHESIS

XPC Lys939Gln Polymorphism

Table 3 listed the main results of the meta-analysis of XPC Lys939Gln polymorphism and CMM risk. Overall, after the ten case-control studies were pooled into meta-analysis, there was a significant association between XPC Lys939Gln polymorphism and risk of CMM under the recessive model (CC vs. CA+AA: OR = 1.108, 95% CI 1.008–1.217; P = 0.033, Fig. 2A). The studies were further stratified by ethnicity and genotyping methods. Subgroup analysis by ethnicity showed that there was a significant association between XPC Lys939Gln polymorphism and CMM risk in mixed populations under all five genetic model, i.e., allele

(C vs. A: OR = 1.543, 95% CI 1.237–1.926, P ≤ 0.001), homozygote (CC vs. AA: OR = 2.778, 95% CI 1.691–4.563, P ≤ 0.001), heterozygote (CA vs. AA: OR = 1.389, 95% CI 1.005–1.920, P = 0.046), dominant (CC+CA vs. AA: OR = 1.574, 95% CI 0.158–2.140, P = 0.004), and recessive (CC vs. CA+AA: OR = 2.246, 95% CI 1.413–3.572, P = 0.001), but not in Caucasians. Moreover, in the PCR-RFLP group, significantly increased association between XPC Lys939Gln polymorphism and CMM risk was found under the recessive model (TT vs. TC+CC: OR = 1.297, 95% CI 1.056–1.594, P = 0.013). However, no significant association was found in the TaqMan group (Table 3).

XPC Ala499Val Polymorphism

Table 4 listed the main results of the meta-analysis of XPC Ala499Val polymorphism and CMM risk. When all the eligible studies were pooled into the meta-analysis of XPC Ala499Val polymorphism was significantly increased risk of CMM was found under two genetic models i.e., allele (T vs. C: OR = 0.918, 95% CI 0.850-0.992; P = 0.031, Fig 2B) and dominant (TT+TC vs. CC: OR = 0.904, 95% CI 0.819-0.997; P = 0.043). When stratified by ethnicity and genotyping method, no significant association was found in Caucasians and subgroup analysis by genotyping technique in PCR-RFLP and TaqMan subgroups (Table 4).

Tab. 2 Characteristics of studies included in the meta-analysis.

First Author/ Year	Country (Ethnicity)	SOC	Genotyping Technique	Case/ Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
XPC Lys939Gln					**AA**	**AC**	**CC**	**A**	**C**	**AA**	**AC**	**CC**	**A**	**C**		
Blankenburg 2005	Germany (Caucasian)	PB	PCR-RFLP	294/373	113	128	53	354	234	138	185	50	461	285	0.382	0.330
Li 2006	USA (Caucasian)	PB	PCR-RFLP	602/603	223	281	98	727	477	195	311	97	701	505	0.418	0.144
Millikan 2006	USA (Caucasian)	PB	TaqMan	1209/2439	409	580	220	1398	1020	785	1252	402	2822	2056	0.421	≤ 0.001
Figl 2010	Germany (Caucasian)	PB	TaqMan	1185/1273	420	568	197	1408	962	460	597	216	1517	1029	0.404	0.348
Goncalves 2011	Brazil (Mixed)	HB	PCR-RFLP	192/205	61	93	38	215	169	102	85	21	289	127	0.305	0.597
Ibarrola-Villava 2011	Spain (Caucasian)	PB	TaqMan	684/406	281	289	114	851	517	154	198	54	506	306	0.376	0.439
Paszkowska-Szczur 2013	Poland (Caucasian)	PB	Sequenom	635/1336	227	314	94	768	502	480	647	209	1607	1065	0.398	0.711
Oliveira 2013	Brazil (Mixed)	HB	PCR-RFLP	146/146	59	65	22	183	109	64	72	10	200	92	0.315	0.084
Torres 2013	USA (Caucasian)	PB	IGGA	893/766	304	451	138	1059	727	273	382	111	928	604	0.394	0.222
Present study	Iran (Asian)	PB	PCR-RFLP	150/150	55	69	26	179	121	49	77	24	175	125	0.416	0.492
XPC Ala499Val					**CC**	**CT**	**TT**	**C**	**T**	**CC**	**CT**	**TT**	**C**	**T**		
Li 2006	USA (Caucasian)	PB	PCR-RFLP	602/603	338	214	50	890	314	318	248	37	881	322	0.267	0.212
Figl 2010	Germany (Caucasian)	PB	TaqMan	1184/1274	626	477	81	1729	639	670	516	88	1856	692	0.271	0.397
Ibarrola-Villava 2011	Spain (Caucasian)	PB	TaqMan	684/406	408	227	49	1043	325	225	158	23	608	204	0.251	0.488
Paszkowska-Szczur 2013	Poland (Caucasian)	PB	Sequenom	519/1288	245	240	34	730	308	548	563	177	1659	917	0.356	0.093
Present study	Iran (Asian)	PB	PCR-RFLP	150/150	80	57	13	217	83	79	61	10	219	81	0.270	0.698

SOC: source of control; PB: Population based; HB: hospital based; IGGA: Illumina GoldenGate Assay; PCR-RFLP: restriction fragment length polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg Equilibrium.

NEITY AND SENSITIVITY ANALYSIS

As shown in Table 3, significant between-study heterogeneity appeared under four genetic models except under recessive model for overall analysis, thus, we have utilized a random-effect model to calculate the pooled estimates. Moderate between-study heterogeneity were observed in the overall analysis evaluating the association between XPC Lys939Gln polymorphism and CMM under four genetic models, i.e., allele (I^2 = 50.18% and P_H = 0.034), homozygote (I^2 = 48.81% and P_H = 0.040), heterozygote (I^2 = 64.42% and P_H = 0.003), and dominant (I^2 = 48.78% and P_H = 0.040). To the XPC Ala499Val polymorphism, a significant between-study heterogeneity were observed among overall studies in the two genetic models, i.e., the homozygote model (I^2 = 47.33% and P_H = 0.002) and recessive model (I^2 = 80.15% and $P_H \leq 0.001$). In addition, we have performed leave-one-out sensitivity analysis validated the stability of results that no single study changed the pooled ORs qualitatively (data not shown). However, the pooled ORs of XPC Lys939Gln and Ala499Val polymorphisms were not influenced by sequentially removing individual studies, suggesting that the included studies to this meta-analysis were statistically accurate.

PUBLICATION BIAS

We have used both Begg's funnel plot and Egger's test to assess the publication bias of literatures. The results of Egger's regression test and relative asymmetry of funnel plot provided sufficient evidence for publication bias for

Tab. 3 Summary of meta-analysis for the association of XPC Lys939Gln polymorphism with risk of CMM.

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	C vs. A	Random	50.18	0.034	1.040	0.963–1.123	1.010	0.312	0.152	0.124
	CC vs. AA	Random	48.81	0.040	1.127	0.961–1.322	1.472	0.141	0.107	0.046
	CA vs. AA	Random	64.42	0.003	0.934	0.812–1.074	–0.955	0.339	0.591	0.650
	CC+CA vs. AA	Random	48.78	0.040	0.998	0.895–1.113	–0.039	0.969	0.720	0.417
	CC vs. CA+AA	Fixed	7.78	0.107	1.108	1.008–1.217	2.128	0.033	0.049	0.024
Ethnicity										
Caucasians	C vs. A	Fixed	0.00	0.897	1.002	0.951–1.055	0.069	0.945	0.763	0.923
	CC vs. AA	Fixed	0.00	0.839	1.035	0.929–1.153	0.630	0.529	0.367	0.450
	CA vs. AA	Fixed	18.60	0.288	0.939	0.867–1.016	–1.563	0.118	0.548	0.359
	CC+CA vs. AA	Fixed	0.00	0.581	0.962	0.893–1.037	–1.006	0.315	0.763	0.497
	CC vs. CA+AA	Fixed	0.00	0.531	1.074	0.974–1.984	1.433	0.152	0.229	0.321
Genotyping										
Mixed	C vs. A	Fixed	42.50	0.187	1.543	1.237–1.926	3.840	≤ 0.001	NA	NA
	CC vs. AA	Fixed	0.00	0.653	2.778	1.691–4.563	4.036	≤ 0.001	NA	NA
	CA vs. AA	Fixed	71.69	0.060	1.389	1.005–1.920	1.991	0.046	NA	NA
	CC+CA vs. AA	Fixed	67.64	0.079	1.574	0.158–2.140	2.895	0.004	NA	NA
	CC vs. CA+AA	Fixed	0.00	0.825	2.246	1.413–3.572	3.420	0.001	NA	NA
PCR–RFLP	C vs. A	Random	75.85	0.002	1.143	0.906–1.442	1.130	0.259	0.462	0.255
	CC vs. AA	Random	72.84	0.005	1.441	0.901–2.305	1.524	0.127	0.462	0.185
	CA vs. AA	Random	79.46	0.001	0.876	0.595–1.291	–0.667	0.504	1.000	0.995
	CC+CA vs. AA	Random	73.47	0.005	1.065	0.777–1.461	0.339	0.694	0.806	0.346
	CC vs. CA+AA	Fixed	52.18	0.079	1.297	1.056–1.594	2.482	0.013	0.462	0.125
TaqMan	C vs. A	Fixed	0.00	0.996	1.000	0.941–1.064	0.013	0.990	1.000	0.796
	CC vs. AA	Fixed	0.00	0.861	1.026	0.903–1.166	0.398	0.691	1.000	0.727
	CA vs. AA	Fixed	22.90	0.273	0.947	0.861–1.041	–1.130	0.258	0.734	0.732
	CC+CA vs. AA	Fixed	0.00	0.654	0.967	0.884–1.057	–0.745	0.456	0.734	0.743
	CC vs. CA+AA	Fixed	6.609	0.360	1.059	0.944–1.189	0.979	0.328	1.000	0.800

NA: Not Applicable

XPC Lys939Gln in the homozygote model ($P_{Begg's}$ = 0.107, P_{Eggers} = 0.046, Fig. 3A) and the recessive model ($P_{Begg's}$ = 0.049, P_{Eggers} = 0.024, Fig. 3B), suggesting that there was obvious publication bias in the genetic contrast. Therefore, we have performed the Duval and Tweedie nonparametric "trim and fill" method to adjust for publication bias. The "trim and fill" method did not change conclusion, indicating the results were statistically robust. Moreover, there was no publication bias of literatures for XPC Lys939Gln polymorphism by subgroup analyses (Table 3). To the XPC Ala499Val polymorphism, the shapes of the funnel plots and Egger's test revealed no obvious asymmetry for association in the overall and by subgroup analyses (Table 3).

MINOR ALLELE FREQUENCIES (MAFS)

The minor allele frequencies (MAFs) of the XPC Lys939Gln and Ala499Val polymorphisms by ethnicity are presented in Tables 2. The XPC 939Gln allele frequencies in the overall, Caucasian and mixed populations were 36.30% (30.50–42.10%), 39.85% (37.60–42.10%), and 31.0% (30.50–31.50%), respectively. The XPC 499Val allele frequency in the overall population was 30.35% (25.10–35.60%). Therefore, the frequencies of the XPC 939Gln allele in Caucasians were greater than overall and mixed populations.

DISCUSSION

Our case-control study showed that there was no a significant association between XPC Lys939Gln and Ala499Val polymorphisms and an increased risk of CMM in our population. One limitation was the relatively small sample size to establish an association between polymorphisms at XPC gene and risk of CMM. Similarly, three studies by Ibarrola-Villava et al., Li et al., and Blankenburg et al., with large sample size did not find an association between the XPC Val499Ala polymorphism on melanoma risk (21, 22, 26). However, Paszkowska-Szczur et al., in case-control study with 714 unselected melanoma patients and 1,841

Tab. 4 Summary of meta–analysis for the association of XPC Ala499Val polymorphism with risk of CMM.

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	T vs. C	Fixed	47.33	0.108	0.918	0.850–0.992	–2.157	0.031	1.000	0.865
	TT vs. CC	Random	77.08	0.002	0.926	0.598–1.434	–0.346	0.729	1.000	0.646
	TT vs. CC	Fixed	0.00	0.535	0.911	0.822–1.009	–1.786	0.074	0.220	0.426
	TT+TC vs. CC	Fixed	0.00	0.652	0.904	0.819–0.997	–2.028	0.043	1.000	0.657
	TT vs. TC+CC	Random	80.15	≤ 0.001	0.973	0.616–1.536	–0.117	0.906	0.806	0.590
Ethnicity										
Caucasians	T vs. C	Fixed	58.07	0.067	0.913	0.844–0.988	–2.249	0.024	0.734	0.816
	TT vs. CC	Random	82.03	0.001	0.882	0.541–1.439	–0.502	0.616	0.734	0.800
	TT vs. CC	Fixed	4.27	0.371	0.910	0.820–1.011	–1.756	0.079	0.089	0.077
	TT+TC vs. CC	Fixed	0.00	0.503	0.900	0.814–0.995	–2.051	0.040	0.734	0.135
	TT vs. TC+CC	Random	84.49	≤ 0.001	0.927	0.555–1.547	–0.289	0.772	0.734	0.704
Genotyping										
PCR–RFLP	T vs. C	Fixed	0.00	0.750	0.980	0.835–1.154	–0.226	0.821	NA	NA
	TT vs. CC	Fixed	0.00	0.985	1.274	0.852–1.904	1.180	0.238	NA	NA
	TT vs. CC	Fixed	0.00	0.638	0.833	0.673–1.031	–1.680	0.093	NA	NA
	TT+TC vs. CC	Fixed	0.00	0.669	0.891	0.727–1.091	–1.114	0.265	NA	NA
	TT vs. TC+CC	Fixed	0.00	0.932	1.373	0.928–2.033	1.586	0.113	NA	NA
TaqMan	T vs. C	Fixed	0.00	0.591	0.973	0.875–1.083	–0.497	0.619	NA	NA
	TT vs. CC	Fixed	0.00	0.573	1.034	0.787–1.359	0.240	0.811	NA	NA
	TT vs. CC	Fixed	49.84	0.158	0.928	0.807–1.067	–1.047	0.295	NA	NA
	TT+TC vs. CC	Fixed	13.93	0.281	0.943	0.826–1.078	–0.854	0.393	NA	NA
	TT vs. TC+CC	Fixed	0.00	0.393	1.603	0.814–1.388	0.446	0.656	NA	NA

NA: Not Applicable.

controls demonstrated that XPC Val499Ala polymorphism at XPC gene is associated with melanoma susceptibility in the polish population, but not XPC Lys939Gln polymorphism (10).

In the present meta-analysis, a total of ten studies with 5,990 cases and 7,697 controls were retrieved on XPC Lys939Gln polymorphism. Our pooled analysis showed that the XPC Lys939Gln polymorphism was significantly associated with increased risk of CMM. Our results were inconsistent with the previous meta-analyses showed that the XPC Lys939Gln polymorphism might be associated with risk of CMM. In 2013, Zhou et al., in a meta-analysis of seven studies (with 3,971 cases and 5,873 controls) have reported that the XPC Lys939Gln polymorphism was not significantly associated with CMM risk under all five genetic models (29). Jiang et al., in a more recently published meta-analysis with eight studies of 4,631 cases and 5,111 controls have found also negative results (30). The current meta-analysis has a number of strengths when compared to the previous meta-analyses. We have evaluated the association of XPC Lys939Gln polymorphism with CMM with the largest sample size to date. We expanded sample size by adding two more studies and our case-control study data and improved statistical power to derive a more precise risk estimate for the associations. Remarkably, the both previous meta-analyses did not report the significant association between XPC Lys939Gln and risk of CMM. Therefore, the positive results of the current meta-analysis might have been caused by large sample size.

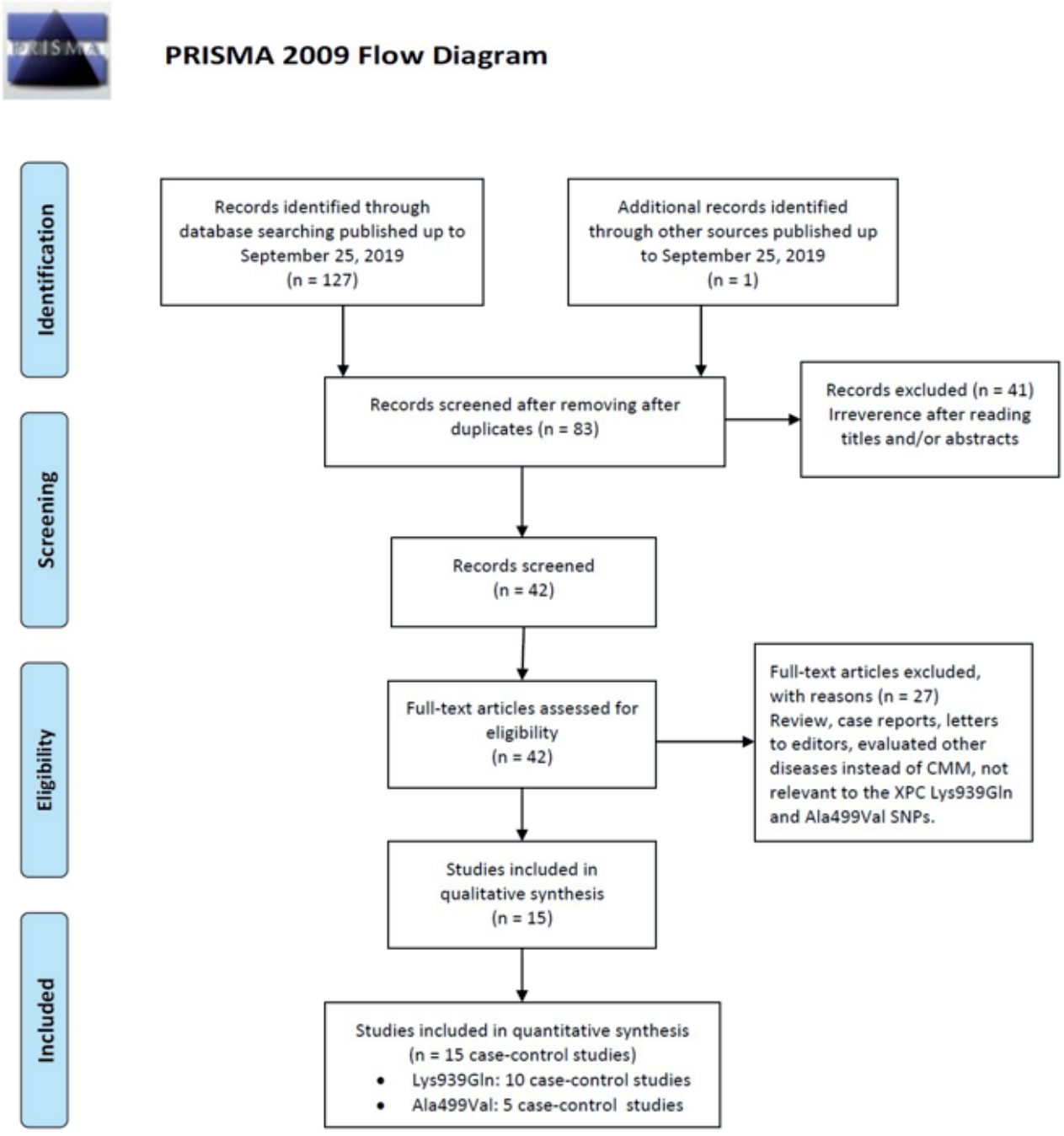

Fig. 1 Flowchart of literature search and selection process.

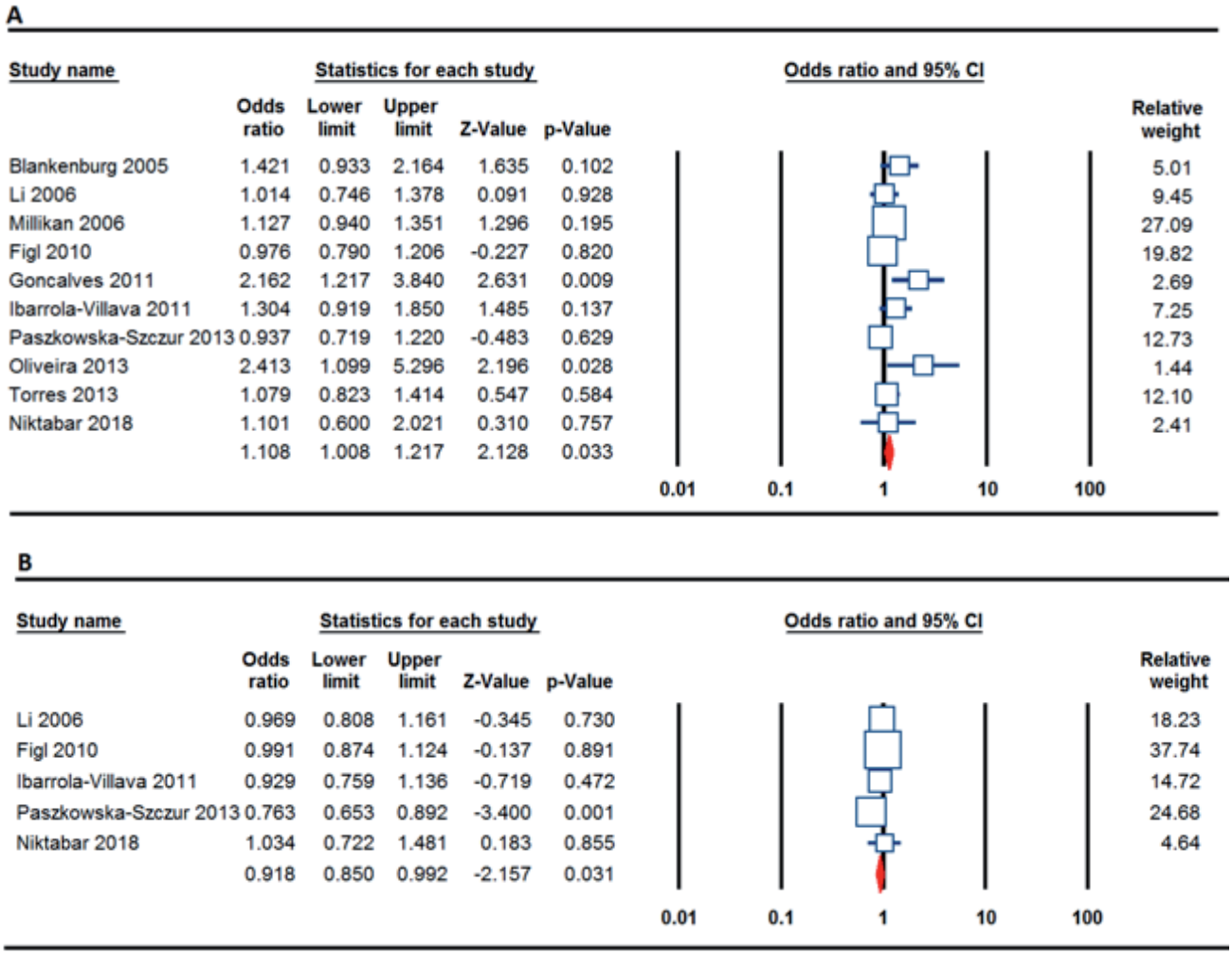

Fig. 2 Forest plots for association between XPC Lys939Gln and Ala499Val polymorphisms and CM risk. **A:** XPC Lys939Gln (recessive model: CC vs. CA+AA); **B:** XPC Ala499Val (allele model: T vs. C).

The XPC Ala499Val polymorphism is one of the more widely studied SNPs in the XPC gene involving a substitution of alanine for valine (31). Mutations of the XPC genes may increase malignancy susceptibility by causing a severe depression of NER and consequently altering DNA repair activity (32). However, the exact consequences of the Ala499Val substitution on protein function or structure not well established (33). In the current meta-analysis based on five case-control studies with 3,139 CMM cases and 3,721 controls we evaluated the association of XPC Ala499Val polymorphism with CMM. Overall, we found that there was a significant association between XPC Ala499Val and increased risk of CMM under two genetic models i.e., allele (T vs. C: OR = 0.918, 95% CI 0.850–0.992; P = 0.031) and dominant (TT+TC vs. CC: OR = 0.904, 95% CI 0.819–0.997; P = 0.043). In recent years, several meta-analyses have investigated the role XPC Ala499Val in susceptibility to different human malignancies. Interestingly, those meta-analyses results showed that the XPC Ala499Val polymorphism was not associated with increased risk of most tumors including breast, colorectal, gastric, and lung. However, their results demonstrated that the XPC Ala499Val polymorphism may contribute to susceptibility to bladder cancer, especially among Caucasians (33, 34). Moreover, our pooled results showed that this polymorphism is significantly associated with risk of CMM.

Between-study heterogeneity is a potential problem in genetic association meta-analysis studies that may affect the interpretation of the pooled results (35–37). It may be due to various factors, such as diversity in the population characteristics (ethnicity, age, and sun exposure), differences in the number of cases and controls, diverse genotype distribution of XPC gene polymorphisms in different ethnicities, using different genotyping methods and study design (38–41). In the present meta-analysis we found relatively high heterogeneity in overall analysis. However, after subgroup analyses by ethnicity, source of controls and genotyping method, the between studies heterogeneity was removed or significantly decreased. Thus, subgroup analyses showed that the origin of the heterogeneity among the studies was ethnicity and genotyping methods.

The current meta-analysis has some advantages. First, to the best of our knowledge, this was the first meta-analysis to evaluate the association between XPC Ala499Val polymorphism and CMM risk. Second, in the current meta-analysis, more studies were included than previous meta-analyses on XPC Lys939Gln polymorphism association with CMM. Third, we have performed sensitive analysis by excluding studies deviating from HWE, due that

deviations from HWE in healthy subjects may be a sign of selection bias. However, the pooled ORs did not significantly influenced, suggesting that the included studies to this meta-analysis were statistically accurate. Fourth, compared with the previous meta-analysis, subgroup analysis by ethnicity and genotyping methods were also carried out.

Despite of the advantages mentioned above, there were still several limitations that should be noted in the meta-analysis. First, although we were able to discern a significant association of XPC Lys939Gln and Ala499Val polymorphisms with CMM in the overall population, the sample size was still relatively small. Second, the included studies involved in the meta-analysis were mainly were performed among Caucasian populations, so it is uncertain whether these results are generalizable to other ethnicities. Thus, to strengthening the statistical power will require more data from different ethnicities. Third, we have included only English published studies in this meta-analysis, which might have led to literature biases. Fourth, although we have performed a comprehensive search to identify all eligible case-control studies and included our unpublished original data, some relevant studies with negative results might be still missed, which might have led to literature biases. Fifth, there was significant between-study heterogeneity for XPC Lys939Gln polymorphism under four genetic models and for XPC Ala499Val under two genetic models. Even though the random-effects model was used to calculate pool ORs, the precision of outcome might be affected. Sixth, the lack of available data prevented an adjustment for subgroup factors such as age, gender, exposure to environmental risk

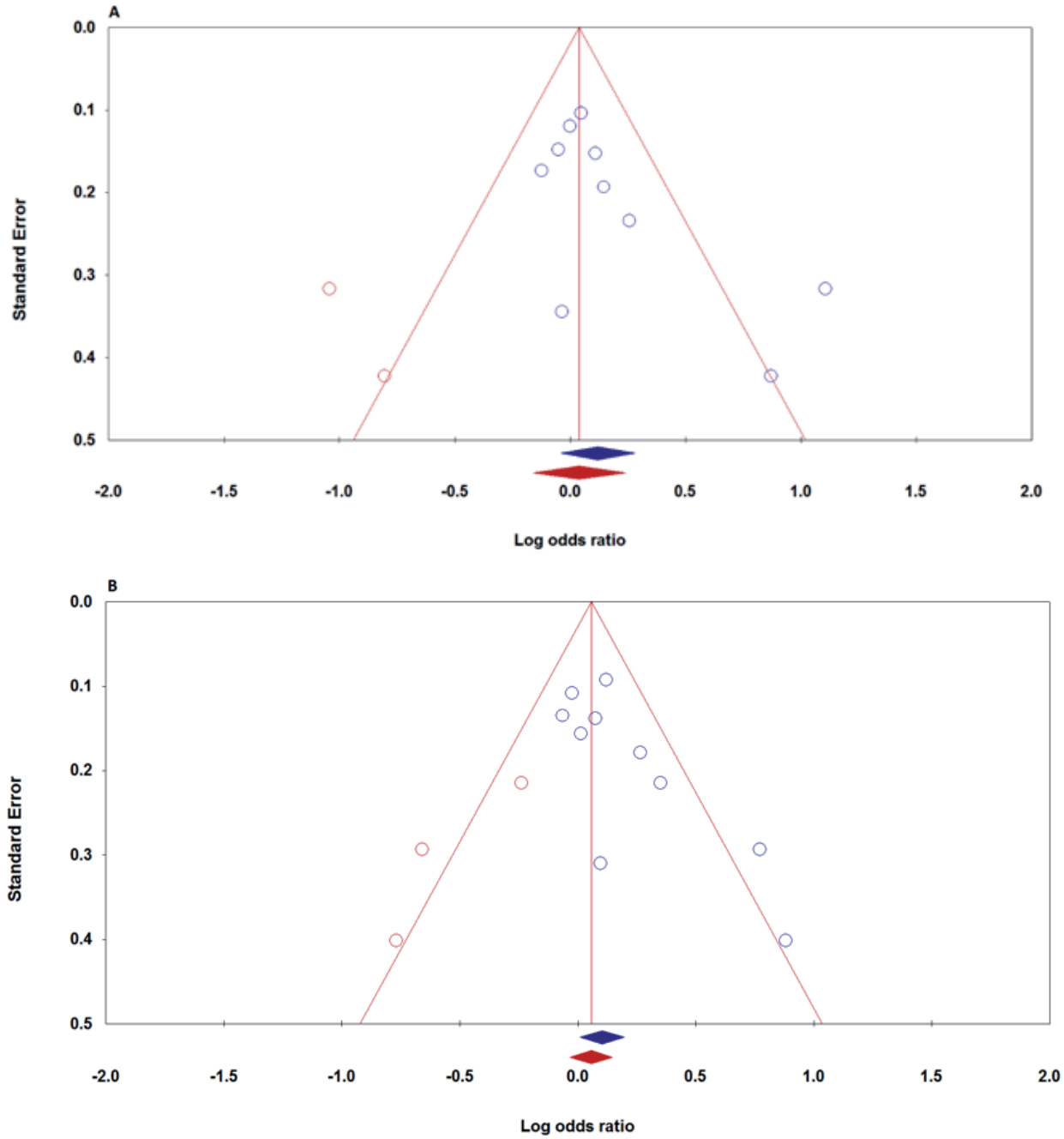

Fig. 3 Begg's funnel plot of publication bias test for association between XPC Lys939Gln polymorphism and CM risk before (blue) and after (red) trim-and-fill method. **A:** homozygote model (CC vs. AA); **B:** recessive model (CC vs. CA+AA).

factors, lifestyle and other confounding factors that can interact with genetic factors to influence the association of XPC Lys939Gln and Ala499Val polymorphisms with susceptibility to CMM. Finally, lack of original data from included studies limited our results because the interactions between gene- gene, gene -environment, and also XPC Lys939Gln/Ala499Val may be modulated the association of XPC Lys939Gln and Ala499Val polymorphisms on development of CMM.

In summary, our results demonstrated that the Lys939Gln and Ala499Val polymorphisms at XPC gene were significantly associated with an increased risk of CMM in the global population. Moreover, stratified analysis by ethnicity revealed that XPC Ala499Val and Lys939Gln polymorphisms were significantly associated with risk of CMM in Caucasians and mixed populations, respectively. Thus, these polymorphisms may serve as genetic biomarker for development of CMM. However, considering the limitations mentioned above, more well-designed studies with larger sample sizes are needed in future.

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COMPETING INTERESTS

The authors declare that there are no competing interests associated with the manuscript.

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ABBREVIATIONS

XPC:	Xeroderma Pigmentosum Complementation Group C
CMM:	Cutaneous Malignant Melanoma
ORs:	Odds Ratios
CIs:	Confidence Intervals
NER:	Nucleotide Excision Repair
BER:	Base Excision Repair
hOGG1:	human 8-oxoguanine DNA N-glycosylase 1
XP:	Xeroderma Pigmentosum
HWE:	Hardy-Weinberg Equilibrium
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-analyses
MAFs:	Minor Allele Frequencies
CMA:	Comprehensive Meta-Analysis
SOC:	Source of Control
PB:	Population Based
HB:	hospital Based
IGGA:	Illumina GoldenGate Assay
RFLP:	Restriction Fragment Length Polymorphism

REFERENCES

1. Situm M, Buljan M, Kolić M, Vučić M. Melanoma-clinical, dermatoscopical, and histopathological morphological characteristics. Acta Dermatovenerologica Croatica: ADC 2014; 22: 1–12.
2. Erdei E, Torres SM. A new understanding in the epidemiology of melanoma. Expert Review of Anticancer Therapy 2010; 10: 1811–23.
3. Wu S, Han J, Song F, et al. Caffeine Intake, Coffee Consumption, and Risk of Cutaneous Malignant Melanoma. Epidemiology 2015; 26: 898–908.
4. Ossio R, Roldán-Marín R, Martínez-Said H, Adams DJ, Robles-Espinoza CD. Melanoma: a global perspective. Nature Reviews Cancer 2017; 17: 393–4.
5. Noto G. On the clinical significance of cutaneous melanoma's precursors. Indian Dermatology Online Journal 2012; 3: 83–8.
6. Casula M, Colombino M, Satta MP, et al. Factors predicting the occurrence of germline mutations in candidate genes among patients with cutaneous malignant melanoma from South Italy. European Journal of Cancer 2007; 43: 137–43.
7. Godar DE, Subramanian M, Merrill SJ. Cutaneous malignant melanoma incidences analyzed worldwide by sex, age, and skin type over personal Ultraviolet-B dose shows no role for sunburn but implies one for Vitamin D3. Dermato-endocrinology 2017; 9: e1267077.
8. Niktabar SM, Latifi SM, Moghimi M, et al. Association of vitamin D receptor gene polymorphisms with risk of cutaneous melanoma. A meta-analysis based on 40 case-control studies. Dermatology Review/Przegląd Dermatologiczny 2019; 106: 268–79.
9. Leibeling D, Laspe P, Emmert S. Nucleotide excision repair and cancer. Journal of Molecular Histology 2006; 37: 225–38.
10. Paszkowska-Szczur K, Scott RJ, Serrano-Fernandez P, et al. Xeroderma pigmentosum genes and melanoma risk. International Journal of Cancer 2013; 133: 1094–100.
11. Li C, Hu Z, Liu Z, et al. Polymorphisms in the DNA repair genes XPC, XPD, and XPG and risk of cutaneous melanoma: a case-control analysis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2006; 15: 2526–32.
12. Sobhan MR, Yazdi MF, Mazaheri M, Shehneh MZ, Neamatzadeh H. Association between the DNA repair gene XRCC3 rs861539 polymorphism and risk of osteosarcoma: A systematic review and meta-analysis. Asian Pacific Journal of Cancer Prevention 2017; 18.
13. Shimizu Y, Iwai S, Hanaoka F, and Sugasawa K. Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. The EMBO Journal 2003; 22: 164–73.
14. Nakamura T, Murakami K, Tada H, et al. Thymine DNA glycosylase modulates DNA damage response and gene expression by base excision repair-dependent and independent mechanisms. Genes to Cells 2017; 22: 392–405.
15. DiGiovanna JJ, Kraemer KH. Shining a Light on Xeroderma Pigmentosum. Journal of Investigative Dermatology 2012; 132: 785–96.
16. Hua R-X, Zhu J, Jiang D-H, et al. Association of XPC Gene Polymorphisms with Colorectal Cancer Risk in a Southern Chinese Population: A Case-Control Study and Meta-Analysis. Genes 2016; 7: 73.
17. He J, Shi T-Y, Zhu M-L, Wang M-Y, Li Q-X,Wei Q-Y. Associations of Lys939Gln and Ala499Val polymorphisms of the *XPC* gene with cancer susceptibility: A meta-analysis. International Journal of Cancer 2013; 133: 1765–75.
18. Wu H, Lv Z, Wang X, Zhang L, Mo N. Lack of association between XPC Lys939Gln polymorphism and prostate cancer risk: an updated meta-analysis based on 3039 cases and 3253 controls. International journal of clinical and experimental medicine 2015; 8: 17959–67.
19. Yu G, Wang J, Dong J, Liu J. XPC Ala499Val and XPG Asp1104His polymorphisms and digestive system cancer risk: a meta-analysis based on model-free approach. International Journal of Clinical and Experimental Medicine 2015; 8: 6621–30.
20. Zhang Y, Li Z, Zhong Q, et al. Polymorphisms of the XPC gene may contribute to the risk of head and neck cancer: a meta-analysis. Tumour Biology: the Journal of the International Society for Oncodevelopmental Biology and Medicine 2014; 35: 3917–31.
21. Blankenburg S, König IR, Moessner R, et al. Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case–control study. Carcinogenesis 2005; 26: 1085–90.
22. Li C, Hu Z, Liu Z, et al. Polymorphisms in the DNA Repair Genes XPC, XPD, and XPG and Risk of Cutaneous Melanoma: a Case-Control

Analysis. Cancer Epidemiology Biomarkers & Prevention 2006; 15: 2526–32.
23. Millikan RC, Hummer A, Begg C, et al. Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study. Carcinogenesis 2006; 27: 610–8.
24. Figl A, Scherer D, Nagore E, et al. Single-nucleotide polymorphisms in DNA-repair genes and cutaneous melanoma. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 2010; 702: 8–16.
25. Gonçalves FT, Francisco G, de Souza SP, et al. European ancestry and polymorphisms in DNA repair genes modify the risk of melanoma: A case–control study in a high UV index region in Brazil. Journal of Dermatological Science 2011; 64: 59–66.
26. Ibarrola-Villava M, Peña-Chilet M, Fernandez LP, et al. Genetic polymorphisms in DNA repair and oxidative stress pathways associated with malignant melanoma susceptibility. European Journal of Cancer 2011; 47: 2618–25.
27. Oliveira C, Rinck-Junior JA, Lourenço GJ, Moraes AM, Lima CSP. Assessment of the XPC (A2920C), XPF (T30028C), TP53 (Arg72Pro) and GSTP1 (Ile105Val) polymorphisms in the risk of cutaneous melanoma. Journal of Cancer Research and Clinical Oncology 2013; 139: 1199–206.
28. Torres SM, Luo L, Lilyquist J, et al. DNA repair variants, indoor tanning, and risk of melanoma. Pigment Cell & Melanoma Research 2013; 26: 677–84.
29. Zhou L, Lu Y, Yang G, Wu J. Quantitative assessment of the association between XPC Lys939Gln polymorphism and cutaneous melanoma risk. Tumor Biology 2014; 35: 1427–32.
30. Jiang W, Zhang H, Chen QW, Xie S. A meta-analysis of XPC Lys939Gln polymorphism and melanoma susceptibility. Journal of the European Academy of Dermatology and Venereology 2016; 30: 1327–31.
31. Yang X, Liu D, Wu H, et al. Association of XPC polymorphisms with susceptibility and clinical outcome to chemotherapy in breast cancer patients. Cancer Science 2012; 103: 1207–14.
32. Peng Q, Chen Z, Lu Y, et al. Current evidences on XPC polymorphisms and gastric cancer susceptibility: a meta-analysis. Diagnostic Pathology 2014; 9: 96.
33. Wang Y, Li Z, Liu N, and Zhang G. Association between CCND1 and XPC polymorphisms and bladder cancer risk: a meta-analysis based on 15 case–control studies. Tumor Biology 2014; 35: 3155–65.
34. Sankhwar M, Sankhwar SN, Bansal SK, Gupta G, Rajender S. Polymorphisms in the XPC gene affect urinary bladder cancer risk: a case-control study, meta-analyses and trial sequential analyses. Scientific Reports 2016; 6: 27018.
35. Aghili K, Sobhan MR, Mehdinezhad-Yazdi M, et al. Association of GDF-5 rs143383 polymorphism with radiographic defined knee osteoarthritis: A systematic review and meta-analysis. Journal of Orthopaedics 2018; 15: 945–51.
36. Moghimi M, Kargar S, Jafari MA, et al. Angiotensin Converting Enzyme Insertion/Deletion Polymorphism is Associated with Breast Cancer Risk: A Meta-Analysis. Asian Pac J Cancer Prev 2018; 19: 3225–31.
37. Namazi A, Abedinzadeh M, Nourbaksh P, Neamatzadeh H. Association between the XRCC3 Thr241Met polymorphism and risk of colorectal cancer: A meta analysis of 5,193 cases and 6,645 controls. Asian Pac J Cancer Prev 2015; 16: 2263–8.
38. Namazi A, Forat-Yazdi M, Jafari M, et al. Association of interleukin-10 -1082 A/G (rs1800896) polymorphism with susceptibility to gastric cancer: meta-analysis of 6,101 cases and 8,557 controls. Arq Gastroenterol 2018; 55: 33–40.
39. Jafari-Nedooshan J, Moghimi M, Zare M, et al. Association of Promoter Region Polymorphisms of IL-10 Gene with Susceptibility to Lung Cancer: Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev 2019; 20: 1951–7.
40. Moghimi M, Sobhan MR, Jarahzadeh MH, et al. Association of GSTM1, GSTT1, GSTM3, and GSTP1 Genes Polymorphisms with Susceptibility to Osteosarcoma: a Case-Control Study and Meta-Analysis. Asian Pac J Cancer Prev 2019; 20: 675–82.
41. Yazdi MF, Rafieian S, Gholi-Nataj M, Sheikhha MH, Nazari T, Neamatzadeh H. CYP2D6 Genotype and Risk of Recurrence in Tamoxifen Treated Breast Cancer Patients. Asian Asian Pac J Cancer Prev 2015; 16: 6783–7.

Uroflowmetry in Non-Monosymptomatic Nocturnal Enuresis in Children of Coastal Region of Croatia

Sandra Prgomet[1], Marjan Saraga[1], Sandra Benzon[2,*], Daniel Turudić[3], Dragan Ledina[4], Danko Milošević[3]

ABSTRACT

Purpose: The aim of the study was to describe clinical characteristics and bladder assessment in children with Non-Monosymptomatic Nocturnal Enuresis (NMNE) in coastal region of Croatia.

Materials and methods: Records on 85 patients with NMNE were retrospectively reviewed. Bladder assessments were performed in all children. In this research we: (i) compare clinical characteristics and features of bladder assessment: uroflowmetry, post void residuals (PVR) and bladder wall thickness between boys and girls with NMNE and we compare (ii) clinical characteristics and bladder assessment between children with primary and secondary NMNE.

Results: There were 46 girls and 39 boys. The total of 59 children had primary NMNE and 26 children had secondary NMNE. Uroflow pattern was abnormal in 42% of all children with NMNE. Abnormal uroflow pattern in children with NMNE was more often in girls than in boys ($P < 0.05$) and in children with secondary than in children with primary NMNE ($P < 0.05$). Ultrasound evidence of bladder wall thickness was more frequent in boys than in girls. Girls were more likely to have dysfunctional voiding and larger residual urinary volume than boys.

Conclusions: Abnormal uroflow pattern in children with NMNE was more often in girls than boys and in children with secondary than in children with primary NMNE.

KEYWORDS

nocturnal enuresis; non-monosymptomatic nocturnal enuresis; uroflowmetry

AUTHOR AFFILIATIONS

[1] Department of Pediatrics, Split University Hospital, University of Split, Split, Croatia
[2] Department of Obstetrics and Gynecology, Split University Hospital, University of Split, Split, Croatia
[3] Department of Pediatrics, Zagreb University Hospital, University of Zagreb, Zagreb, Croatia
[4] Department of Infectology, Split University Hospital, University of Split, Split, Croatia
* Corresponding author: Department of Gynecology and Obstetrics, Split University Hospital, Spinčićeva 1, 21000 Split; e-mail: sbenzon68@gmail.com

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INTRODUCTION

The International Children's Continence Society (ICCS) defines enuresis (or nocturnal enuresis, NE) as wetting in discrete portions while asleep in a child older than five (1). Primary nocturnal enuresis (PNE) is defined as nocturnal wetting in a child who has never been dry on consecutive nights for longer than 6 months. Secondary enuresis is the re-emergence of enuresis after continence has been established for at least 6 months (1, 2). It has been documented that 15–30% of enuretic children can experience daytime incontinence (3–6). Enuresis which occur without lower urinary tract symptoms or a history of bladder dysfunction is monosymptomatic. Enuresis with lower urinary tract symptoms such as change in voiding frequency, daytime wetting, dribbling, and holding manoeuvres is non-monosymptomatic (or polysymptomatic). Among those children with frequent NE (2 or more wet nights per week), 68.5% were classified as monosymptomatic and 31.5% as non-monosymptomatic (7). Nocturnal polyuria along with abnormal circadian release of antidiuretic hormone (ADH) or arginine vasopressin (AVP) is an important contributor to NE (8). The other etiological factors are an arousal disturbance during sleep and lack of inhibition of the micturition reflex. These developmental disturbances are genetically based and can be modulated by environmental factors (9). The etiological factors of lower urinary tract symptoms and disorders are heterogeneous, based on the symptoms present (urge, voiding postponement, dysfunctional voiding etc.). Diagnosis consists of detailed medical history, clinical examination, frequency-volume charts, and appropriate investigations. In general, urodynamic studies are not required (10, 11).

Imaging and urodynamic studies are reserved for children with significant daytime symptoms, history or diagnosis of urinary tract infections, features suggesting structural renal abnormalities, or refractory cases (12–14). In the evaluation of non-monosymptomatic nocturnal enuresis (NMNE) the approach is still controversial with little evidence-based medicine supporting diagnostic evaluation. In children with NMNE, two diagnosis need to be considered according to the ICCS: one for lower urinary tract disorder and another for nocturnal enuresis. Proposed algorithm of strategy in managing children with daytime incontinence include bladder ultrasound, post void residuals (PVR) together with uroflowmetry (15).

MATERIALS AND METHODS

We assessed total of 85 children with NMNE (median 7.6 years, 5–18 years) who referred to the Pediatric Nephrology Clinic of the Split University Hospital from January 2014 to December 2017. The outpatient clinic for voiding disorders is the only one in the coastal region of Croatia. Informed consent has been obtained. Study have been performed according to the Declaration of Helsinki, and the procedures have been approved by the local ethics committee. We compare clinical characteristics and features of bladder assessment: uroflowmetry, PVR and bladder wall thickness between boys and girls with NMNE and we compare clinical characteristics and bladder assessment between children with primary and children with secondary NMNE. We analysed medical records data retrospectively. Baseline parameters were obtained from a questionnaire for toilet training age, the history of urinary tract infection, presence of frequency, urgency, daytime incontinence and constipation; frequency volume chart for two consecutive days and first morning urine osmolality. After taking detailed individual history, a detailed physical examination and urine analysis was carried out and recorded by pediatrician. International Children's Continence Society criteria were used to define enuresis, its subtypes and lower urinary tract terminology (1). Children with neurologic deficit (myelodysplasia, spinal cord disorders and mentally retarded children) and urogenital malformations were excluded from the study. The age at which a child began to void on her or his own has been expressed in months. We have defined urinary tract infection as a history of symptomatic significant bacteriuria, with or without fever in the period prior to arriving at Pediatric Nephrology Clinic. The largest voiding volume of urine from a frequency- volume chart for two days is considered as maximum bladder capacity (MBC). We used Koff's formula to measure expected bladder capacity (EBC) according to the age: Volume (ml) = (age in years + 1) × 30. Bladder capacity (BC) was expressed as a percentage according to the formula: BC (%) = MBC/EBC × 100. Nocturnal polyuria was calculated from frequency volume chart and defined as nocturnal production of the urine > 130% of expected bladder capacity according to the age. The Roma IV criteria was used for diagnose constipation (16). We performed a retrospective chart review of all bladder assessments: uroflowmetry, post void residuals and bladder wall thickness. Uroflowmetry and pelvic floor electromyography (EMG) were performed in all children. For the uroflowmetry test, children were asked to wait until they felt a strong desire to void. The uroflowmetry test was performed for two consecutive times and only those curves generated after adequate voided volume (> 50% of the expected bladder capacity) were analysed. Pelvic floor EMG was done by spacing skin electrodes. We used ICCS suggested categories for normal and abnormal urine flow patterns: the normal urine flow curve is bell shaped, the abnormal urine flow patterns are tower, plateau, staccato and interrupted. Bladder ultrasound (US) was performed immediately (within 5 minutes) after voiding with 5 MHz suprapubic ultrasound. Post void residual was estimated by the equation of height × width × depth (of the bladder) × 0.52 ml (17). Bladder wall thickness > 4 mm in an empty bladder was considered as increased. Dysfunctional voiding was defined as contraction of the external sphincter during voiding with staccato pattern with or without an interrupted flow with post voiding residual urine above 10 ml. We compared all the clinical characteristics and features of bladder assessment between boys and girls. We also compared all the clinical characteristics and features of bladder assessment between children with primary and secondary NE and lower urinary tract symptoms.

Statistical analysis was performed using SPSS version 23, using basic descriptive statistics, the corresponding

parametric (t-test) and nonparametric (Chi-square) test. The level for statistical significance was set at $p < 0.05$.

RESULTS

Among a total of 85 children, 46 (54%) were girls and 39 (46%) boys (Table 1). The median age of children with NMNE was 7.6 years (Min–Max: 5–18 years). The total of 59 children (69%) had primary NMNE and 26 (31%) children had secondary NMNE. Patients with NMNE started to void on their own at median of 30 months (Min–Max: 14–54 months). Nocturnal urine production was increased in 27 children who had nocturnal polyuria (37%). The mean first morning urine osmolality was 827.5 mOsm/kgH_2O (± 257.7 mOsm/kgH_2O). Children with nocturnal polyuria had lower first morning urine osmolality (721:905 mOsm/kg, $p = 0.0089$). The lower urinary tract symptoms were: daytime incontinence in 71 (83%) cases, urgency in 66 (80%) cases and frequency in 40 (51%) cases.

Tab. 1 Descriptive statistics of the total sample ($N = 85$).

Characteristics of patients	Number of patients (%)
Sex	
Male	39 (45.88)
Female	46 (54.12)
Enuresis	
Primary	59 (69.41)
Secondary	26 (30.59)
Age (years)	7.6118 ± 2.75635
The age of sphincter control (months)	30.4138 ± 6.87746
UTI[1]	23 (28.40)
Constipation	29 (36.25)
Daytime incontinence	71 (83.53)
Urgency	66 (80.49)
Frequency	40 (50.63)
Nocturnal polyuria	27 (36.99)
First morning urine osmolality (mOsmol/kg)	827.5400 ± 257.76830
Bladder capacity %	70.8922 ± 29.07430
Abnormal urine flow patterns	35 (41.67)
Post void residual urine (ml)	14.2262 ± 13.39156
Dysfunctional voiding	20 (23.52)
Thickened bladder wall	15 (18.29)

Legend: Values are presented as number (%) or arithmetic mean ± standard deviation; [1] UTI – positive history of urinary tract infection.

23 (28%) children had urinary tract infection. The constipation was recorded in 29 (36%) children. According to frequency volume charts the average percentage of bladder capacity was 71% (±29). Uroflow pattern was abnormal in 35 (42%) cases. Average residual urine volume was 14.2 ml (±13.3 ml). Bladder wall thickness in an empty bladder was abnormal in 15 (18%) cases. Dysfunctional voiding was recorded in 20 (24%) of all children.

There is no statistically significant difference in the frequency of primary or secondary NMNE between boys and girls (Table 2). There was no difference according to the clinical features including the age, age of toilet training, nocturnal polyuria, bladder capacity, frequency and daytime incontinence between the boys and girls. Girls had statistically more often urinary tract infections, constipation and larger residual urine volume than boys. Abnormal uroflow and dysfunctional voiding were statistically more often in girls than boys. Urgency and ultrasound evidence of bladder wall thickness were statistically significantly more frequent in boys than girls. Abnormal uroflow pattern was statistically more often in children with secondary NMNE than in children with primary NMNE (Table 3). Uroflowmetry and pelvic floor electromyography (EMG) revealed abnormal features in 34.5% children with primary NMNE (group 1) and in 57.7% children with secondary NMNE (group 2). The following abnormal patterns in flow shapes were noted in group 1 patients: tower in 7 patients, staccato in 7 patients, interrupted in 5 patients, plateau in 1 patient. The group 2 had the following abnormal patterns: tower in 2 patients, staccato in 10 patients, interrupted in 1 patient, plateau in 2 patients. There was no statistically significant difference in other clinical characteristics between children with primary and secondary NMNE. Dysfunctional voiding was statistically more often in children with secondary NMNE than and in children with primary NMNE.

DISCUSSION

In our study, NMNE was slightly more common in females (57%) than in males (43%). According to the literature, the bedwetting is more common in boys whereas daytime incontinence is more common in girls (18). The one of the main etiological factors for NE is nocturnal polyuria. In our study nocturnal polyuria was equally represented in boys and girls and in both primary and secondary NMNE. Children with nocturnal polyuria, had lower first morning urine osmolality. Because of difficulty to measure the amount of urine output at night, we suggest first urine osmolality. The most important lower urinary tract symptom registered in our patients (83%) was daytime incontinence. In the evaluation of NE by an evidence-based strategy, careful assessment of NE-related symptoms by questionnaire, physical examination, urine analysis, and frequency volume chart are only essential in the initial evaluation (13). Furthermore, the recently published National Institute for Health and Clinical Excellence (NICE) guidelines do not recommend even urine analysis unless there is a suspicion of recent onset, daytime symptoms, urinary tract infection, or diabetes mellitus (19). In the evaluation of NMNE, specialist referral is almost mandatory, and the approach is still controversial with little evidence-based medicine to guide treatment (11). In children with NMNE, two diagnosis need to be considered according to the ICCS: one for lower urinary tract syndrome and another for nocturnal enuresis. Proposed algorithm of

Tab. 2 Descriptive statistics and significance of differences between boys (N = 39) and girls (N = 46).

Variable	Boys (%)	Girls (%)	p
Enuresis			
primary	28 (71.8)	31 (67.4)	.661[a]
secondary	11 (28.2)	15 (32.6)	
Age (years)	7.03 ± 1.885	8.11 ± 3.261	.060[b]
The age of sphincter control (months)	30.04 ± 6.523	30.77 ± 7.286	.690[b]
UTI	2 (5.6)	21 (46.7)	.000[a]
Constipation	7 (19.4)	22 (50.0)	.005[a]
Daytime incontinence	33 (84.6)	38 (82.6)	.804[a]
Urgency	34 (89.5)	32 (72.7)	.056[a]
Frequency	22 (59.5)	18 (42.9)	.141[a]
Nocturnal polyuria	13 (37.1)	14 (36.8)	.979[a]
First morning urine osmolality (mOsmol/kg)	871.28 ± 253.811	767.14 ± 256.899	.161[b]
Bladder capacity %	67.79 ±28.080	73.61±29.999	.385[b]
Abnormal urine flow patterns	11 (28.2)	24 (53.3)	.020[a]
Post void residual urine(ml)	10.64 ± 8.106	17.33 ± 16.132	.021[b]
Dysfunctional voiding	5 (12.8)	15 (32.6)	.003[a]
Thickened bladder wall	11 (28.2)	4 (9.3)	.027[a]

Legend: Values are presented as number (%) or arithmetic mean ± standard deviation; a – chi-square; b – t-test.

Tab. 3 Descriptive statistics and significance of differences between a group of people with primary (N = 59) and secondary enuresis (N = 26).

Characteristics of patients	Primary enuresis (%)	Secondary enuresis (%)	p
Age (years)	7.4068 ± 2.53335	8.0769 ± 3.21152	.304[b]
The age of sphincter control (months)	30.4722 ± 7.76250	30.3182 ± 5.28598	.935[b]
Urinary tract infection	14 (25.5)	9 (34.6)	.393[b]
Constipation	20 (36.4)	9 (36.0)	.975[a]
Daytime incontinence	49 (83.1)	22 (84.6)	.858[a]
Urgency	47 (83.9)	19 (73.1)	.249[a]
Frequency	28 (52.8)	12 (46.2)	.577[a]
Nocturnal polyuria	20 (40.8)	7 (29.2)	.333[a]
First morning urine osmolality (mOsmol/kg)	833.7222 ± 247.82858	811.6429 ± 291.04602	.789[b]
Bladder capacity %	72.4453 ± 28.81554	67.4625 ± 29.96836	.490[b]
Abnormal urine flow patterns	20 (34.5)	15 (57.7)	.046[a]
Post void residual urine (ml)	13.6949 ± 12.42391	15.4800 ± 15.64747	.580[b]
Dysfunctional voiding	10 (16.9)	10 (38.4)	.003[a]
Thickened bladder wall	10 (17.5)	5 (20.0)	.791[a]

Legend: Values are presented as number (%) or arithmetic mean ± standard deviation; a – chi-square; b – t-test.

strategy in managing children with daytime incontinence include bladder ultrasound, PVR together with uroflowmetry (15). Uroflowmetry is time-consuming as for proper evaluation of bladder emptying, at least two bladder filling cycles need to be done.

Association of enuresis and bladder dysfunction has been reported and small capacity bladder and detrusor overactivity have been the most important (common) urodynamic findings (20–23). In our study there was no difference according to the bladder capacity between the boys and girls and between children with primary and children with secondary NMNE. However, urodynamic studies have indicated that bladder wall thickness was significantly higher in patients with detrusor overactivity and detrusor overactivity was seen in children with primary NMNE as compared to primary MNE (24). According to our study bladder wall thickness was statistically more often in boys. Those findings are probably related to detrusor overactivity in boys. Small capacity bladder and detrusor overactivity cause symptoms of urinary bladder storage function while uroflowmetry examines bladder emptying.

According to the literature it is also crucial to differentiate primary NE from secondary NE or daytime incontinence with a nocturnal component (25, 26). In our study an abnormal bladder emptying detected using uroflowmetry was more likely to be associated with secondary NMNE. Maybe this is because in primary NMNE the more common problem is overactive bladder which cause storage bladder problem and in secondary NMNE and in girls the more common are emptying difficulties which are measured by uroflowmetry. Abrams et al. have already proposed that secondary enuresis was more likely to be associated with an organic cause (18).

According to our study dysfunctional voiding was more often in children with secondary NMNE.

Naseri and Hiradfar urodynamic studies had revealed abnormalities in 43% of MNE and 53% of NMNE, which shows that abnormal urodynamics is as common in MNE as in NMNE. The majority of their patients had small capacity and low compliance bladders which was indicative of an overactive bladder (27). Some authors found that the only significant difference between the patients with primary NE and those with secondary NE was in the prevalence of constipation which is significantly associated with primary NE (28). In our study the incidence of constipation was less than 30% of total number of children. Constipation was significantly more often in girls than in boys. Urinary tract infections (UTI) are already recognized as related to staccato flow pattern and dyssinergic voiding (29, 30). In our study both dysfunctional voiding and UTI were more often in girls than in boys.

Limitation of this study was in the number of the patients so our conclusions should be confirmed in future larger study. In children with NMNE, children with primary and secondary NMNE should be distinguished.

Despite the fact that some children with primary enuresis also had daily symptoms, uroflow pattern was mostly normal, bell shaped. It is probably because the emptying function of the bladder in those children is normal. In children with primary NMNE we propose primary assessment by bladder ultrasound with PVR, and uroflowmetry to be part of secondary assessment if primary assessment is suspected of obstruction or if therapy failed. Emptying difficulties are more often in girls than boys with NMNE. As for therapy, standard urotherapy, adequate water intake, and regular voiding was implemented in all children. Constipation was treated along with standard urotherapy. Some of children were treated with desmopressin, some with anticholinergic therapy with oxybutinine, and some with dual therapy with both desmopressin and oxybutinin. Animated biofeedback was used for dysfunctional voiding.

CONCLUSIONS

The UTI, constipation and urinary dysfunctions are statistically significant NMNE symptoms in our study. In our patients with NMNE an abnormal uroflow pattern was in almost half of the cases. It was more often in girls than boys and in children with secondary than in children with primary NMNE.

Based on this finding we propose that in children with secondary NMNE, uroflowmetry always be performed as a first-line examination. In contrast, in children with primary NMNE, it is not necessary to initially perform uroflowmetry, but only as a second-line examination.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Austin PF, Bauer S, Bower W, et al. The standardization of terminology of lower urinary tract function in children and adolescents: update report from the Standardization Committee of the International Children's Continence Society. Neurourol Urodyn 2016; 35: 471–81.
2. Graham KM, Levy JB. Enuresis. Pediatr Rev 2009; 30: 165–72.
3. Gumus B, Vurgun N, Lekili M, Iscan A, Muezzinoglu T, Buyuksu C. Prevalence of nocturnal enuresis and accompanying factors in children aged 7–11 years in Turkey. Acta Paediatr 1999; 88: 1369e72.
4. Jarvelin MR, Vikevainen-Tervonen L, Moilanen I, Huttunen NP. Enuresis in seven-year-old children. Acta Paediatr Scand 1988; 77: 148e53.
5. Forsythe WI, Redmond A. Enuresis and spontaneous cure rate. Study of 1129 enuretis. Arch Dis Child 1974; 49: 259e63.
6. Hellstrom A, Hanson E, Hansson S, Hjalmas K, Jodal U. Micturition habits and incontinence at age 17ereinvestigation of a cohort studied at age 7. Br J Urol 1995; 76: 231e4.2e5.
7. Butler R, Heron J. The Alspac Study T. Exploring the differences between mono- and polysymptomatic nocturnal enuresis. Scand J Urol Nephrol 2006; 40: 313e9.
8. Abdelfatah D, Shaker H, Ismail M, Ezzat M. Nocturnal polyuria and arginine vasopressin (AVP): A key factor in the pathophysiology of monosymptomatic nocturnal enuresis. Neurorol Urodyn 2009; 28: 506–9.
9. Av Gontard, Neve'us T. Management of disorders of bladder and bowel control in childhood. London: Mac Keith Press; 2006.
10. Franco I, von Gontard A, De Gennaro M; International Children's Continence Society. Evaluation and treatment of nonmonosymptomatic nocturnal enuresis: a standardization document from the International Children's Continence Society. J Pediatr Urol 2013; 9(2): 234–43.
11. Vande Walle J, Rittig S, Bauer S, Eggert P, Marschall-Kehrel D, Tekgul S. American Academy of Pediatrics; European Society for Paediatric Urology; European Society for Paediatric Nephrology; International

Children's Continence Society. Practical consensus guidelines for the management of enuresis. Eur J Pediatr 2012; 171: 971–83.
12. Fritz G, Rockney R, Bernet W, et al. Practice parameter for the assessment and treatment of children and adolescents with enuresis. J Am Acad Child Adolesc Psychiatry 2004; 43(12): 1540–50
13. Hjalmas K, Arnold T, Bower W, et al. Nocturnal enuresis: an international evidence-based management strategy. J Urol 2004; 171(6 pt 2): 2545–61.
14. Kalyanakrishnan Ramakrishnan. Evaluation and Treatment of Enuresis. Am Fam Physician 2008; 78(4): 489–96.
15. Chang SJ, van Laecke E, Bauer SB, et al. Treatment of daytime urinary incontinence: a standardization document from the International Children's Continence Society. Neurourol Urodyn 2017; 36(1): 43–50.
16. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Functional Disorders: Children and Adolescents. Gastroenterology 2016; 150(6): 1456–68.
17. Erasmie U, Lidefelt KJ. Accuracy of ultrasonic assessment of residual urine in children. Pediatr Radiol 1989; 19: 388–90.
18. Abrams P, Cardozo L, Khoury S, Wein A. Incontinence (editors). Paris. Health Publication 2002; 513–51.
19. Wootton J, Norfolk S. Nocturnal enuresis: assessing and treating children and young people. Community Pract 2010; 83: 37–9.
20. Yeung CK, Chin HN, Sit FK. Bladder dysfunction in children with refractory nocturnal mono-symptomatic primary nocturnal enuresis. J Urol 1999; 162: 1049–54.
21. Kajiwara M, Inouse K, Kato M, Usui A, Kurihara M, Usui T. Nocturnal enuresis and overactive bladder in children: an epidemiological study. Int J Urol 2006; 13: 36–41.
22. Frank HJS, Pannek BJ. Voiding dysfunction in children: role of urodynamic studies. Urol Int 2002; 69: 297–301.
23. Naseri M, Hiradfar M. Abnormal urodynamic findings in children with nocturnal enuresis. Indian Pediatr 2012; 49: 401–3.
24. Charalampous S, Printza N, Hashim H, et al. Bladder wall thickness and urodynamic correlation in children with primary nocturnal enuresis. J Pediatr Urol 2013; 9: 334–815.
25. Schaeffer AJ, Diamond DA. Pediatric urinary incontinence: Classification, evaluation, and management. Afr J Urol 2014; 20: 1–13.
26. Lettgen B. Differenial diagnosis for nocturnal enuresis. Scand J Urol Nephrol Suppl 1997; 183: 47–8.
27. Naseri M, Hiradfar M. Monosymptomatic and non-monosymptomatic nocturnal enuresis: a clinical evaluation. Arch Iran med 2012; 15(11): 702–6.
28. Robson WL, Leung AK, Van Howe R. Primary and secondary nocturnal enuresis: similarities in presentation. Pediatr 2005; 115(4): 956–9.
29. Chase J, Austin P, Hoebeke P, mcKenna P. The management of dysfunctional voiding in children: a report from the standardisation comitee of the International Children's continence society. J Urol 2010; 183: 1296–1302.
30. Hoebeke P, Bower W, Combs A, De Jong T, Yang S. Diagnostic evaluation of children with daytime incontinence. J Urol 2010; 183(2): 699–703.

Clinical Probe of Cyp2C8*2 Mutants in a Malaria Hyperendemic Zone: Evidence from North-Central, Nigeria

Olalere Shittu[1,*], Olufunke Adenike Opeyemi[1], Olumuyiwa Babagbemi Omotesho[2], Oluwatosin Fakayode[3], Nnaemeka Asogwa[4], Opeyemi Margaret Adeniyi[1], Ifeoluwa Margaret Fatoba[1], Kayode Muritala Salawu[5], Olusola Ajibaye[6], Olarewaju Abdulkareem Babamale[1], Oluyinka Ajibola Iyiola[8], Olusola Isaac Aremu[7]

ABSTRACT

Background: A tremendous level of success has been achieved since the introduction of chloroquine and the combination of amodiaquine and artemisinin for the treatment of both complicated and uncomplicated malaria infections in sub-Saharan Africa. However, the recent discovery of drug resistant strains of *Plasmodium falciparum (P. f.)* and the ability of the parasite to ingest CYP2C8 into its digestive vacuole is of great public health concern. This study probes the occurrence of CYP2C8*2 allelic mutant amongst malaria patients in North-Central Nigeria.

Methods: Three hundred and eighty five (385) unrelated study participants were screened for current malaria episodes using routine microscopy and/or rapid diagnostic test strips (RDTs). Chelex extraction method was used for single nucleotide polymorphisms (SNPs) and identification of CYP2C8*2 (805A > T) variant respectively. Wild-type (A) and the defective allele (T) were differentiated with the use of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The results obtained were further validated with Sanger sequencing of a few samples and thereafter, the genotype data were statistically processed. All alleles obtained were in Hardy Weinberg equilibrium.

Results: Out of the 385 participants (45.5% Male and 54.5% Female) genotyped for SNPs, 75 (19.5%) had the autosomal recessive mutant trait. Occurrence of mutant traits was gender and ethnic independent ($p > 0.05$). Yoruba ethnic group recorded a reduction in proportion of genotypic defective CYP2C8*2 allele (T) (1 in every 8 persons) with a carrier percentage of 13.3% compared with Hausa (26.62%); Igbo (25.37%) and other minority ethnic groups (17.6%).

Conclusions: A remarkable inter-ethnic differences in autosomal recessive CYP2C8*2 allele was observed. By implication, there is a gradual incursion of genetic drift for poor CQ and AQ-Artemisinin metabolizers among the inhabitants.

KEYWORDS

Plasmodium falciparum; Chloroquine; Amodiaquine-Artemisinin combination therapy; CYP2C8*2; Hausa, Igbo, Yoruba, Nigeria

AUTHOR AFFILIATIONS

[1] Parasitology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria
[2] Unilorin Clinic, University of Ilorin, Ilorin, Nigeria
[3] Children Specialist Hospital, Centre Igboro, Ilorin, Nigeria
[4] Department of Biochemistry, University of Ilorin, Ilorin, Nigeria
[5] Department of Pharmacognosy and Drug Development, University of Ilorin, Ilorin, Nigeria
[6] Biochemistry Division, Nigerian Institute of Medical Research, Lagos, Nigeria
[7] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria
[8] Cell Biology and Genetics Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria
* Corresponding author: Parasitology Unit, Department of Zoology, University of Ilorin, Ilorin, Nigeria; e-mail: eternity403@yahoo.com

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INTRODUCTION

Infections arising as a result of *Plasmodium falciparum* is the major cause of malaria-related deaths and it has been reported to be the most common of the four human malaria parasites across sub-Saharan Africa (1). Despite wide documented chloroquine (CQ) and Amodiaquine (AQ) resistance; majority of the populace still rely on therapeutic CQ and AQ medications. The increasing failure of these drugs against falciparum malaria constitutes a notable setback in the eradication efforts of malaria in many African countries (2–4). Previous studies have established that host genetic variations with respect to cytochrome P450 (CYP) 2C8 (CYP2C8) as drug metabolizers is responsible for the metabolism of about 20–50% clinical drugs and endogenous substances. The emerging mutations with respect to these metabolites is one of the main risk factors for the drug resistant strains of *P. falciparum* in Africa (5). Genotype-inferred low metabolizers were reported in 1–4% of African populations corresponding to millions of expected exposures to AQ (6). Recent studies further revealed that resistant strain are often characterized with the genetic defective variant (CYP2C8*2) identified as being responsible for the hepatic metabolism of CQ and AQ, consequently altering chloroquine flux or reduced drug binding to hematin inside the parasite digestive vacuole. This mechanism culminates into *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) point mutations (5, 7). Similarly, quinoline ring in 4Qs is resistant to degradation by cytochrome P450 enzymes (CYP) CYP2C8 and CYP3A4, with potentials to mediate 80% of the total metabolism of 4AQs (8). However, the common occurrence of genetic variant CYP2C8*2 in malaria infected host has been linked to the presence of drug-resistant parasites in the infected host (*pfcrt-76Y* and *pfmdr1-86Y P. falciparum* alleles). This anomaly is documented as a strong factor that is chronically hindering the efficacy of CQ and AQ in Africa. A recent longitudinal study in Africa reported that the prevalence of the defective allele "CYP2C8*2" is statistically insignificant among ethnic groups in Nigeria, although comparable with what was obtained in Senegal and Madagascar (9). However, it is pertinent to further explicate the presence of this allele in other African descents, because of the crucial role it plays in the epidemiology of falciparum infections. This study will investigate the occurrence and determine the allele frequencies of CYP2C8*2 amongst residents in a malaria high transmission zone of North-Central Nigeria. This is to guide intervention for better understanding of the metabolic mechanism of CQ and AQ artemisinin-based combination therapy (ACT) in the study area.

MATERIALS AND METHODS

STUDY AREA, DESIGN AND PROTOCOLS

The study was conducted within Ilorin metropolis, an urban area, in the North-Central zone and the capital of Kwara State, Nigeria. It is located on longitude 4°35′E and latitude 8°35′N. It covers an area of about 38 square miles, with an estimated population of 1.4 million people. The area is associated with intense rainfalls from April to October and daily temperature of between 23 °C and 37 °C. Inhabitants are mostly farmers, civil servants, traders and students. Out-patients from four randomly selected hospitals (Civil Service Hospital, Temitope Hospital, Children Specialist Hospital and University of Ilorin Health Centre) in Ilorin were used for the study. A simple structured questionnaire was administered to volunteers after written informed consent was sought and approved to obtain some basic information on ethnicity and Knowledge about usage of antimalarials like CQ, AQ and ACTs (viz; Artemether-lumefantrine, Artesunate-mefloquine and Dihydroartemisinin-piperaquine). Only volunteers with a record of past CQ and AQ medications were considered for the present study. Intravenous blood samples of subjects were spotted on Whatman number 3 filter papers, air dried and separately stored in sealed plastic containers. Routine malaria diagnosis was initially performed by microscopic examination of Giemsa-stained thick blood smears and/or a rapid diagnostic test (Malaria Antigen *P. f.*, Standard diagnostics, INC. Ingbert, Germany). Single nucleotide polymorphisms in CYP2C8*2 was screened for according to Marwa et al. (10). DNA extraction and subsequent identification of CYP2C8*2 (805A > T) variant was carried out using Chelex extraction method and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) respectively as described by Paganotti et al. (11). Briefly, 2µl of DNA template was amplified by PCR, at 107bp fragment for the CYP2C8 gene forward primer (i.e. at 5′-GAACACCAAGCATCACTGGA-3′) and reverse primer (i.e. at 5′-GAAATCAAAATACTGSTCTGTTGC-3′). The products from PCR analysis was incubated with Bcl 1 enzyme that cuts the wild type allele only (A); undigested products then represent the variant allele (T). In order to detect the size polymorphisms, both types were allowed to run on a metaphor 3% gel. Controls for human genotyping were then utilized after sequencing of the PCR product obtained from each different genotype. Genotyping errors were avoided by double checking for the heterozygous samples.

ETHICAL APPROVAL

This study was performed according to the Declaration of Helsinki and the procedure followed was part of a study approved by the University of Ilorin Ethical Consideration with protocol approval number: UERC/ASN/2012/221. Consent form was administered and collected from the volunteers before the commencement for the study.

STATISTICAL ANALYSIS

Data obtained were analyzed with SigmaPlot for Windows version 12.0 (Systat. Software, Inc.). The prevalence of recorded alleles (i.e. wild and mutant alleles) were subjected to Chi-square ($\chi 2$) analysis and statistical significance was set at $p < 0.05$. The results obtained were further validated with Sanger sequencing of a few samples and thereafter, the genotype data were statistically processed. Hardy-Weinberg equation ($p^2 + 2pq + q^2 = 1$) was used to estimate the frequency of the carrier state (2pq) for autosomal recessive trait among the study population (12).

Tab. 1 Characteristics of samples taken for CYP2C8*2 analysis.

Factor	No examined (%)	Allele frequency (%)		
		Mutants	Wild Type	p-value
Total no examined	385	75 (19.5)	310 (80.5)	
Gender				0.814
Male	175 (45.5)	35 (46.7)	140 (45.2)	
Female	210 (54.5)	40 (53.3)	170 (54.8)	
Age group				0.003
0–5	100 (26.0)	20 (26.7)	80 (25.8)	
6–15	90 (23.4)	10 (13.3)	80 (25.8)	
16–25	50 (13.0)	20 (26.7)	30 (9.7)	
26–35	35 (9.1)	5 (6.7)	30 (9.7)	
36–45	60 (15.6)	10 (13.3)	50 (16.1)	
> 45	50 (13.0)	10 (13.3)	40 (12.9)	
Ethnic group				0.102
Yoruba	195 (50.6)	45 (60.0)	150 (48.4)	
Hausa	40 (10.4)	10 (13.3)	30 (9.7%)	
Igbo	45 (11.7)	5 (6.7)	40 (12.9)	
Others*	105 (29.9)	15 (20.0)	90 (29.0)	

* Idoma (30, 7.8%), Fulani (10, 2.6%), Nupe (40, 10.4%), Igala (10, 2.6%) and Benue/Igede (15, 3.9%).

RESULTS

Three hundred and eighty five (385) individuals consisting of 175 (45.5%) male and 210 (54.5%) female with a past record of CQ and AQ use voluntarily participated in this study. Seventy five (75 (19.5%)) was analysed to have recessive mutant traits of CYP2C8*2 allele. Mutant population with respect to gender and ethnic group were not statistically significant ($p > 0.05$). However, the defective CYP2C8*2 allele in comparison with the wild dominant allele was significant with respect to distribution among the respective age groups sampled ($p = 0.003$) (Table 1).

The genotype and allele frequencies in the Nigerian major and minor ethnic groups domicilied in the study area was assessed with Hardy-Weinberg equilibrum calculator (12). The genotype frequencies obtained obeyed the assumptions layed down for the principle. For instance, the allele frequency for this generation was done by pooling together the alleles from each genotype of the same generation according to the expected contribution from the homozygote and heterozygote genotypes. Yoruba ethnic group recorded a reduction in proportion of CYP2C8*2 allele (T) frequency (1 in every 8 persons) with a carrier percentage of 13.3% despite the large sample size screened (N = 195) compared with others (viz; Hausa: 26.62%; Igbo: 25.37%; Others: 17.6%) (Table 2).

DISCUSSION

The recent discovery of molecular markers for drug resistance in genomic studies is gradually eliciting various dimension (13). Investigation on defective CYP2C8*2 is essential to evaluate emergence of antimalarial drug resistance markers (*P. falciparum*) population among the three major Nigerian ethnic groups. In this study, a non-negligible frequency (19.5%) of autosomal recessive CYP2C8*2 mutants was obtained among malaria patients. This outcome is similar to the report of Adehin et al. (14) in South-west Nigeria. However, this study reported a lower

Tab. 2 Genotypes for CYP2C8*2 and T allele frequency among the studied Ethnic groups.

Ethnic groups	CYP2C8*2 (rs11572103, A > T)					
	Genotype frequencies				Allele frequency	
	N	AA	AT	TT	T	Carrier (%)
Yoruba	195	0.862	0.133	0.005	1 in 8	26 (13.3)
Hausa	40	0.709	0.266	0.025	1 in 4	11 (26.62)
Igbo	45	0.724	0.254	0.022	1 in 4	11 (25.37)
Others*	105	0.814	0.176	0.010	1 in 6	18 (17.61)

* Idoma (30, 7.8%), Fulani (10, 2.6%), Nupe (40, 10.4%), Igala (10, 2.6%) and Benue/Igede (15, 3.9%). AA – homozygous wild-type; AT – heterozygous carrier; TT – homozygous mutant; T – Phenotype.

prevalence of CYP2C8*2 status when compared with several early studies reports in African populations (10, 11, 15, 16). In a similar vein, CYP2C8*2 allele was successfully genotyped in 75% (213/285) of children in Congo Brazzaville. The CYP2C8*2A allele had a frequency of 63%, whereas the CYP2C8*2T allele had a frequency of 37%. Genotypes CYP2C8*2AA (rapid metabolizer), CYP2C8*2AT (intermediate metabolizer), and CYP2C8*2TT (poor metabolizer) were reported in 44%, 38%, and 18% of the investigated participants, respectively (17). This suggests that mutations in specific *P. f.* genes may confer resistance to antimalarial drugs, climaxing into sustained drug pressure (18). Also, this finding may serve as an important tool at predicting the level of resistance to CQ and AQ + Artemisinin combinations drugs. It is suffice to mention that in sub-Saharan Africa, people carrying CYP2C8*2 C.805A > T (CYP2C8*2; rs11572103) allele suffer impaired amodiaquine metabolism, increased risk of amodiaquine-related adverse events, and may promote the selection of drug-resistant parasite strains (17). CYP2C8 accounts for the metabolism of > 20% of drugs used in the treatment of varying ailments with over 60 clinically important therapeutic agents of which malaria is one (19). However, CYP2C8*2T allele occurs mostly in people with a sub-Saharan Africa ancestry (19%) and it is less frequent (≤ 1%) in individuals of European, Asian, or American origin (20). The 4-Qs become resident in the acidic digestive vacuole, where they are believed to bind b-hematin and interfere with heme detoxification (21). In human, AQ is mainly metabolized in the liver, and CYP2C8 is the main hepatic isoform that catalyzes the formation of N-desethylaminodiaquine (DEAQ) (22). From the aforementioned, it is obvious that the defective allele give rise to a number of different point mutations affecting the heme and or substrate binding ability of CYP2C8 as it is the most abundant form expressed in the liver and other extrahepatic tissues (23). In our study, genotypic data obtained showed that the Yoruba ethnic group' chances of outcome with the defective allele was one in eight which appears to be the least because the carrier frequency was 13.3%, amongst the studied population. The observed variance may be due to the differences in population sampled or it may be ascribed to activity impacting nature of the SNPs playing less relevance in some descents (10, 14). CYP2C8 also metabolizes arachidonic acid and the anticancer drug paclitaxel, and CYP2C8 variants have been shown to be defective in the metabolism of both substrates (22). The emergence and spread of drug resistance depends, in part, on the number of mutations required to encode resistance and their effects on parasite fitness (24). Specific multiple point mutations is however very important in a gene make up of a resistant marker for an antimalarial drug (20, 25). Many adverse reactions are attributable to reduced CYP2C8 expression, but yet unreported during clinical trials. The expression often leads to any one or all of the following, viz; poor metabolizer phenotypes, hepatoxicity and a severe reduction in white blood cell count (22). The aforementioned may result in the risk of both mild and severe adverse clinical outcomes associated with AQ treatment (16). Furthermore, the presence of this defective allele in our population is suggestive of possible inter-population differences in clinical outcomes associated with ACT drugs.

CONCLUSION

Currently, the increasing knowledge in genomic revolution is significantly improving our understanding of reasons why individuals and populations differ in their susceptibility to multiple diseases. The occurrence of inter-ethnic differences in the frequencies of clinically relevant CYP450 variants is the real reason populations don't maintain the stable allele frequencies predicted by the Hardy-Weinberg equilibrium. A remarkable inter-ethnic differences in autosomal recessive CYP2C8*2 allele was observed. By implication, there appears to be a gradual incursion of genetic drift for poor CQ and AQ-Artemisinin metabolizers among the inhabitants. Further studies are required to evaluate the toxicological significance of this poor metabolizer in our settings and give insights to the adverse effect on the drug pharmacokinetics of CQ and AQ-ACT drugs.

AUTHORS CONTRIBUTION

OSH, OAB, OIA and MKS designed, did statistical analysis and contributed to the manuscript write-up, OAO supervised the collection of the samples, OBO and OF provided patients in their respective hospitals, OMA and MIF collected dried blood spot samples, AE, OAI and AO carried out the PCR and RFLP analysis. OIA edited the final manuscript.

COMPETING INTEREST

The authors of this study declare no competing interest. All authors partook in the design, implementation and the write-up.

REFERENCES

1. Jamison D, Feachem R, Makgoba M, et al.: The International Bank for Reconstruction and Development. Washington (DC): The World Bank 2006.
2. Petersen I, Eastman R, Lanzer M. Drug-resistant malaria: Molecular mechanisms and implications for public health. FEBS Letters 2011; 585(11): 1551–62.
3. Kumar SC. Drug Resistance in Malaria. In: Drug Resistance in Bacteria, Fungi, Malaria, and Cancer. Springer 2017: 429–47.
4. Antony HA, Parija SC. Antimalarial drug resistance: An overview. Tropical Parasitology 2016; 6(1): 30.
5. Paganotti GM, Gallo BC, Verra F, et al. Human genetic variation is associated with Plasmodium falciparum drug-resistance. J Infect Dis 2011; 204: 1772–8.
6. Gil JP, Gil BE. CYP2C8 and antimalaria drug efficacy. Pharmacogenomics 2007; 8(2): 187–98.
7. Parkinson A, Kazmi F, Buckley DB, Yerino P, Ogilvie BW, Paris BL. System-dependent outcomes during the evaluation of drug candidates as inhibitors of cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) enzymes: human hepatocytes versus liver microsomes versus recombinant enzymes. Drug Metabolism and Pharmacokinetics 2010; 25(1): 16–27.
8. Kalia S, Dutz JP. New concepts in antimalarial use and mode of action in dermatology. Dermatologic therapy 2007, 20(4):160-174.
9. Adehin A, Bolaji OO, Kennedy MA. Polymorphisms in CYP2C8 and CYP3A5 genes in the Nigerian population. Drug Metabolism and Pharmacokinetics 2017; 32(3): 189–91.

10. Marwa KJ, Schmidt T, Sjögren M, Minzi OMS, Kamugisha E, Swedberg G. Cytochrome P450 single nucleotide polymorphisms in an indigenous Tanzanian population: a concern about the metabolism of artemisinin-based combinations. Malaria Journal 2014; 13: 420.
11. Paganotti GM, Gramolelli S, Tabacchi F. Distribution of human CYP2C8*2 allele in three different African populations. Malaria Journal 2012; 11: 125.
12. Carrier Frequency Calculator (http://www.perinatology.com/calculators/Hardy-Weinberg.htm).
13. Lin JT, Juliano JJ, Wongsrichanalai C. Drug-Resistant Malaria: The Era of ACT. Current Infectious Disease Report 2010; 12(3): 165–73.
14. Adehin A, Bolaji OO, Kennedy MA. Polymorphisms in CYP2C8 and CYP3A5 genes in the Nigerian population. Drug Metabolism and Pharmacokinetics 2016; 30: 1–3.
15. Fernandez P, Zeigler-Johnson CM, Spangler E, et al.: Androgen metabolism gene polymorphisms, associations with prostate cancer risk and pathological characteristics: a comparative analysis between south african and senegalese men. Prostate Cancer 2012: 798634.
16. Bains RK. African variation at Cytochrome P450 genes: Evolutionary aspects and the implications for the treatment of infectious diseases. Evolution, Medicine, and Public Health 2013; 2013(1): 118–34.
17. Peko SM, Ntoumi F, Vouvoungui C. Distribution of the cytochrome P450 CYP2C8*2 allele in Brazzaville, Republic of Congo. International Journal of Infectious Diseases 2019, 85: 49–53.
18. White NJ. Malaria. Edinburgh, United Kingdom: Elsevier Science Limited 2003.
19. VandenBrink BM, Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. Evaluation of CYP2C8 inhibition in vitro: utility of montelukast as a selective CYP2C8 probe substrate. Drug Metabolism and Disposition 2011; 39: 1546–54.
20. Backman JT, Filppula AM, Niemi M, Neuvonen PJ. Role of Cytochrome P450 2C8 in Drug Metabolism and Interactions. Pharmacological Reviews 2016; 68(1): 168–241.
21. Greenwood BM, Fidock DA, Kyle DE, et al. Malaria: progress, perils, and prospects for eradication. Journal of Clinical Investigation 2008; 118(4): 1266–76.
22. Dai D, Zeldin DC, Blaisdell JA, et al. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. Pharmacogenetics 2001; 11: 597–607.
23. Gil JP, Gil Berglund E. CYP2C8 and antimalaria drug efficacy. Pharmacogenomics 2007; 8(2): 187–98.
24. White N. Antimalarial drug resistance and combination chemotherapy. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 1999: 354: 739–49.
25. Agomo CO, Oyibo WA, Sutherland C, Hallet R, Oguike M. Assessment of Markers of Antimalarial Drug Resistance in Plasmodium falciparum Isolates from Pregnant Women in Lagos, Nigeria. PloS One 2016; 11(1): e0146908–e0146908.

Acute Compartment Syndrome

Jana Cepková[1], Leoš Ungermann[2], Edvard Ehler[3,*]

ABSTRACT
Acute compartment syndrome occurs most frequently in connection with injuries, terminal or chemical damage of tissues, ischemia, the activity of toxins or in patients with tissue ischemia or muscle necrosis. Clinical findings have found pronounced pain, followed by paresthesias, pallor, and paresis. Decreased pulsation of arteries has also been a frequent finding. In severe forms decompressive fasciotomy has been indicated within the first 12–24 hours after diagnosis. In the following paper, the authors present the case report of a 68-year woman who swallowed 1500 mg of trazodone as an attempt at suicide. After 12 hours her husband found her lying on the carpet with compression of the left arm under the trunk. The patient was treated conservatively and followed clinically, examined by ultrasonography, EMG and finally MRI.

KEYWORDS
compartment syndrome; trazodone intoxication; ultrasonography; electromyography; magnetic resonance imaging

AUTHOR AFFILIATIONS
[1] Department of Neurology, District Hospital Pardubice, Czech Republic
[2] Department of Radiology, Faculty of Health-Care Study, Pardubice University, District Hospital Pardubice, Czech Republic
[3] Department of Neurology, Faculty of Health-Care Study, Pardubice University, District Hospital Pardubice, Czech Republic
* Corresponding author: Department of Neurology, Faculty of Health-Care Study, Pardubice University, District Hospital Pardubice, Czech Republic; e-mail: edvard.ehler@nempk.cz

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INTRODUCTION

Acute compartment syndrome (ACS) is defined as an increase of intracompartmental pressure followed by perfusion pressure decrease with an ischemia of tissues in the compartment (1). Perfusion decrease develops into tissue necrosis with function impairment (muscle paresis, contractures of muscles), up to a loss in the extremities and, in exceptional cases, death. Most frequently ACS develops in connection with traumas (fractures, crush syndrome), combustion or chemical damage of tissues, infections (Streptococcus), compression from an overly tight fixation of extremity segments (plaster cast), local activity of toxins (snake bites), and after a longer compression such as from a disadvantageous position during surgery (lithotomic position) or in patients lying without moving for many hours due to a disturbance of consciousness or with intoxication (2, 3).

This paper details a woman with severe ACS after trazodone intoxication who was hospitalized by the authors.

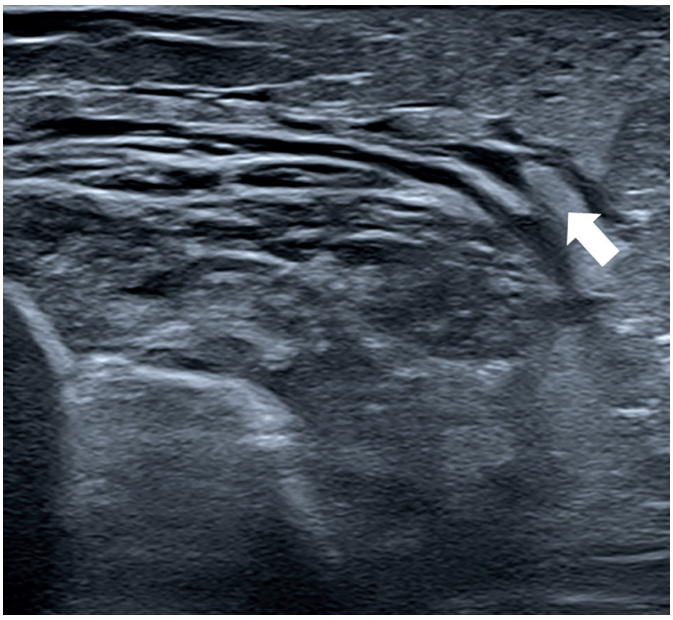

Fig. 1 Ultrasonography: Oedema and infiltration of left forearm.

CASE REPORT

A 68-year woman was admitted to a neurological ICU with somnolence, oedema, pain, and plegia of the left upper extremity. The previous evening she had had a quarrel with her husband and in the morning her husband found her lying motionless on the carpet near the radiator with her left arm underneath her. Retrospectively she admitted, that she had taken 1500 mg trazodone as an attempt at suicide. She was first examined at the traumatologic emergency room where she received an x-ray of the spine and upper left extremity, CT brain, and CT angiography of cerebral vessels. The findings were normal. However, she had a very painful oedema of the left upper extremity, pain which increased upon touching the skin, severe paresis of all segments (no active movement of fingers and hand, very restricted flexion in elbow and abduction of arm) and a creatine kinase 71 ukat/l (0.35–3.58), ALT 3.06 ukat/l (0.10–0.78), AST 5.26 ukat/l (0.05–0.72) which is characteristic for ACS. The surgeon did not decide to perform a fasciotomy. The patient was admitted to the neurological department and treated conservatively including rehabilitation. An ultrasonography of the left arm and forearm showed oedema of muscles with an infiltration of tissues while arterial stream courses in the brachial and radial arteries were normal (Fig. 1). Over the course of 3 days the movements of the upper left extremity appeared and improved stepwise. An electromyography (EMG) on day 6 after admission bore evidence for ACS. The compound muscle action potential of the median and ulnar nerves showed low amplitude and sensory nerve action potential for both nerves was only slightly lower (Tab. 1).

Case history. The patient has been treated for 15 years for asthma bronchiale, 10 years for hypothyreosis (Letrox 75 ug a day) and for depression (escitalopram 20 mg). Four years ago she cut the skin of her left wrist in a suicide attempt but without serious injury and without sequel.

During the patient's stay in the department of neurology her CK level initially increased (3rd day 248 ukat/l) and after 10 days it dropped to normal values. The patient successfully trained with the help of a physiotherapist. A psychiatrist evaluated her problems as a depressive disturbance with a reactive use of trazodone. After an 18 day stay in the hospital she was dismissed with a prescription of trazodone 150 mg in the evening, vortioxetine 10 mg, hydroxyzin 15 mg in the evening and her regular daily medication. She stayed in the rehabilitation institute for 6 weeks.

After her final check 3 months after ACS she was substantially improved - able to button up and eat with a knife and fork. The handgrip of the right hand was found to be 46 and 10 kPa in the left (normal value > 40). However, she still showed weakness of arm abduction, external rotation, wrist, and finger extension but was able to pinch her fingers together. No muscle changes with shortenings or contractures were found. Hypesthesia was found only on the dorsal aspects of the wrist, thumb, and index finger. An EMG showed motor and sensory conduction studies of

Tab. 1 EMG findings.

Muscle	Insertion activity	Fibrillation / Sharp waves	Frequency of MUP / amplitude	Tissue fluid
M. deltoideus sin.	Very low	0	0	Slowly leaks
M. biceps br. sin.	Slightly longer	0	3 / 0.6 mV	Leaks out
M. extensor digit. com. sin.	Prolonged	0	0	Leaks under pressure

MUP – motor unit potential

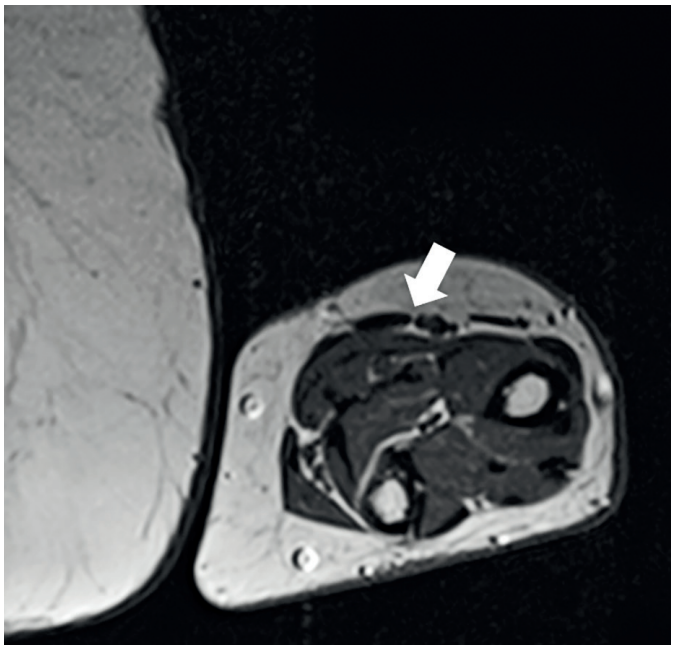

Fig. 2 Axial MRI T1 weighted: Atrophy of forearm muscles.

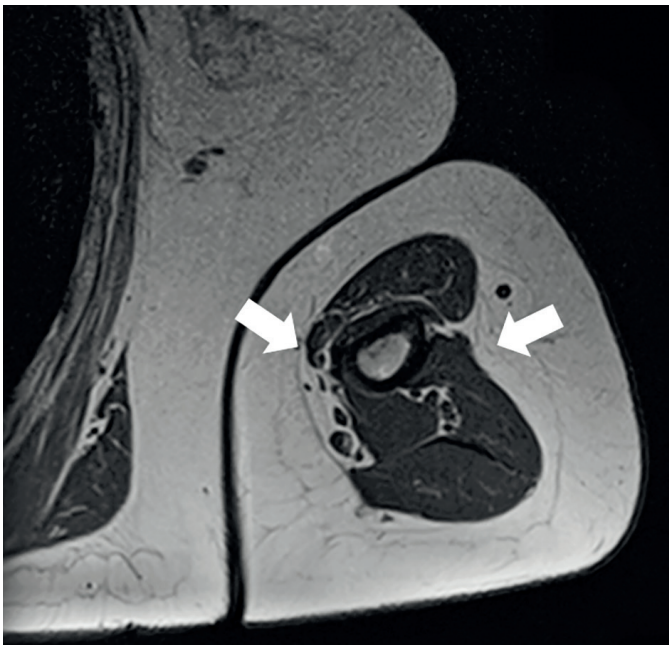

Fig. 3 Axial MRI T1 weighted: Atrophy of arm muscles.

nn. median, ulnar, radial to be normal. During a needle EMG investigation fibrillation, positive waves, small and short-duration MUP were found in all three muscles myotonic discharges. An MRI found atrophy of the muscles of the upper left extremity (Fig. 2, Fig. 3).

DISCUSSION

ACS develops due to tissue pressure increase in a space tightly surrounded with fascias in the compartment. Because of the vasodilation of arterioles and an increased permeability of vessel walls an increased plasma filtration occurs. The increased pressure in the compartment and subsequent collapse of veins creates a decrease in tissue fluid resorption. In every segment of the extremities there is a direct number of compartments. In the arm there are 2 compartments, in the forearm 3 and in the hand 10. The most frequent ACS occurs in the forearm, and according to bone fracture in 35% of cases due to pharmacological/drug depression of consciousness with a compression of tissue in 10%, and in paravenous application of medicaments 8% (4–6). Here the patient had a pharmacological decrease of consciousness during the time she was lying on the carpet near a radiator with her left arm under her trunk. Our patient suffered of acute compartment syndromes of her forearm, arm and shoulder, but the posterior forearm and the anterior arm were the most affected compartments.

In English literature there is a "rule of 5P" for ACS. This rule includes pain (out of proportion), paresis, pallor, paresthesias and pulseless arteries (3). The next step for diagnosis is measuring the tissue pressure – if the pressure in a compartment exceeds 30 mm Hg then fasciotomy is indicated. This should be done during the first 6 hours following diagnosis. The next possibility of diagnostics are the neuroimaging methods. In our patient there were clinical findings typical for ACS. Severe left upper extremity paresis made it necessary to exclude stroke. A brain CT and CT angiography did not find stroke or any other brain disorder. Tissue pressure was not measured. An ultrasonography of the arm and forearm disclosed tissue oedema but with a patency of arteries (5). Because of a long delay of admission (more than 12 hours since the beginning) and patency of arteries, the surgeon did not decide to perform a fasciotomy.

Electromyographical investigations on day 6 after admission showed a substantial decrease of compound muscle action potential for the median, ulnar, and radial nerves and slightly lower sensory action potential for all 3 nerves. In a needle EMG of 3 muscles pathological spontaneous activity was not found with an increase in insertion activity, no voluntary activity (MUP) in m. deltoideus and m. extensor digitorum communis and only 3 MUPS of normal parameters were found in m. biceps brachii. In a needle mark the tissue fluid leaked under pressure in m. extensor digitorum communis. The findings were characteristic for compartment syndrome with not severe muscle fibers damage.

At a check up after 3 months a significant increase of muscle strength was found. Muscle atrophy in arm and forearm was only moderate, hypesthesia persisted only for the radial nerve. In an EMG fibrillation and positive sharp waves, short and low-amplitude MUP were found, as well as myotonic discharges as an EMG parameter of direct muscle membrane damage. Muscle atrophy was found on the MRI. There were no muscle contractures and no deformities of the upper left extremity.

CONCLUSION

Acute compartment syndrome develops on the basis of an increased pressure in the compartment followed by the breakdown of tissue perfusion leading to the development of necrosis. Diagnosis is determined based on clinical findings measuring tissue pressure in the compartment. A fasciotomy is recommended for ACS meeting these criteria during the first 6 hours (7). Ultrasonography and MRI are

important as complementary investigations. EMG contributes to the diagnosis with the evaluating grade of muscle fibre damage and the evidence of damage of nerves passing through the compartment.

REFERENCES

1. Amato AA, Russel JA. Neuromuscular disorders. New York: McGraw Hill, 2008.
2. Allan D, Jones B. Compartment syndrome: A Forgotten Diagnosis. Lancet 2002; 359: 2248.
3. Von Keudell AG, Weaver MJ, Appleton PT, et al. Diagnosis and Treatment of Acute Extremity Compartment Syndrome. Lancet 2015; 386: 1299–1310.
4. Gustaffson P, Crenshaw AG, Edmudsson D, Toolanen G, Crnalic S. Muscle Oxygenation in Type 1 Diabetic and Non-diabetic Patients with and without Chronic Compartment Syndrome. PLOS ONE 2017, October 23.
5. Hill OT, Scoffield DE, Usedom J, et al. Risk Factors for Rhabdomyolysis in the U.S. Army. Military Medicine 2017; 182(7–8): e1836.
6. Saks CA. Compartment syndrome. NEJM 2017; 377: 19.
7. Guo J, Yin Y, Jin L, Zhang R, Hou Z, Zhang Y. Acute compartment syndrome: cause, diagnosis, and new viewpoint. Medicine (Baltimore) 2019; 28(27): e16260.

Iron Deficiency as Cause of Dysphagia and Burning Mouth (Plummer-Vinson or Kelly-Patterson Syndrome): a Case Report

Vladimíra Radochová[1,*], Radovan Slezák[1], Jakub Radocha[2]

ABSTRACT

The clinical presentation of iron deficiency can be very heterogeneous, including various oral and other mucosal problems. Here, in this case, we report the patient with burning mouth and dysphagia symptoms where iron deficiency was found to be the underlying cause after several months of investigations. This clinical syndrome is called Plummer-Vinson syndrome. It is sporadic with an incidence less than 0.1% of patients suffering from iron deficiency anemia.

KEYWORDS

iron deficiency; anemia; dysphagia; burning mouth

AUTHOR AFFILIATIONS

[1] Department of Dentistry, Faculty of Medicine in Hradec Králové, Charles University, and University Hospital Hradec Králové, Hradec Králové, Czech Republic

[2] 4th Department of Internal Medicine – Hematology, Faculty of Medicine in Hradec Králové, Charles University, and University Hospital Hradec Králové, Hradec Králové, Czech Republic

* Corresponding author: Department of Dentistry, University Hospital, Sokolská 581, 500 05 Hradec Králové,Czech Republic; e-mail: vladimira.radochova@fnhk.cz

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BACKGROUND

Iron deficiency anemia (IDA) is one of the most common anemias worldwide (IDA) and is frequently accompanied by other symptoms not typically associated with anemic syndrome (1). As a part of the clinical picture, patients with IDA may present with burning mouth syndrome, chronic glossitis, pale mucosal membranes etc. Various other symptoms include hair loss, nail changes, pica and rarely dysphagia (1). Dysphagia associated with IDA was described by Donald Ross Patterson and Adam Brown Kelly, both British laryngologists, in 1919. This clinical syndrome of IDA and dysphagia called Plummer-Vinson syndrome (PVS) is, however named after Henry Stanley Plummer and Porter Paisley Vinson, both physicians of Mayo Clinic, USA (2). PVS is a rare condition and consists of glossitis, atrophic oral mucosa, dysphagia and anemia. Strictures of the upper esophagus are frequently described as a part of PVS. The majority of reported cases were women (up to 89%) around 5th decennium (3). Here we present a case of a female patient presenting with dysphagia and burning mouth with long time unknown cause of both symptoms.

CASE PRESENTATION

A 65-year-old woman was referred for burning mouth to the examination to the Department of Dentistry, University Hospital Hradec Králové, Czech Republic. Her history of current complaints included one year of difficulty in swallowing of solid food, dry mouth, burning tongue especially on the tip of the tongue and angular cheilitis. She also reported chronic fatigue and shortness of breath on exertion and loss of weight of 3 kg per last year. No other symptoms were reported by the patient.

Her personal history includes arterial hypertension, hypercholesterolemia, gastroesophageal reflux caused by hiatal hernia and chronic pain caused by previous surgery of right femur 20 years ago. Her corresponding chronic medication included meloxicam, pregabalin, esomeprazole, atorvastatin, hydrochlorothiazide and metoprolol. She reported herself as nonsmoker without any allergies. She was retired at the time of examination and previously employed as an administrative office worker.

During previous investigations, she was seen by a number of specialists. She was repeatedly examined by otorhinolaryngologist with normal findings on clinical/physical examination six months before diagnosis. She was scheduled for barium contrast swallow test with the conclusion of hiatus hernia and corresponding inflammatory changes. She also underwent examination by her internal medicine specialist, and ultrasound of the abdomen was performed with no significant findings, and esophageal manometry was performed with normal results. She was scheduled for esophageal pH measurements. Until the dental examination, no laboratory examination was done.

Extraoral clinical examination revealed bilateral angular cheilitis with central rhagades with only skin and transition zone involved (Figure 1). Other skin findings on the skull and face were normal. Lymph nodes were not enlarged. Intraoral examination showed atrophic glossitis

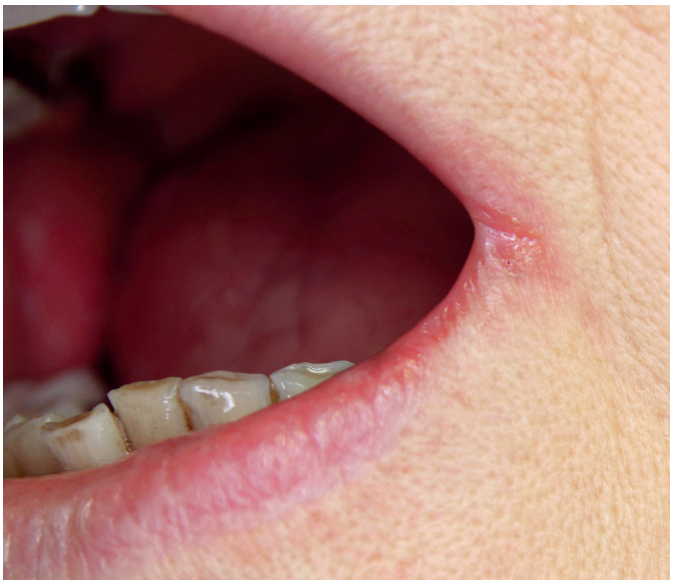

Fig. 1 Angular cheilitis.

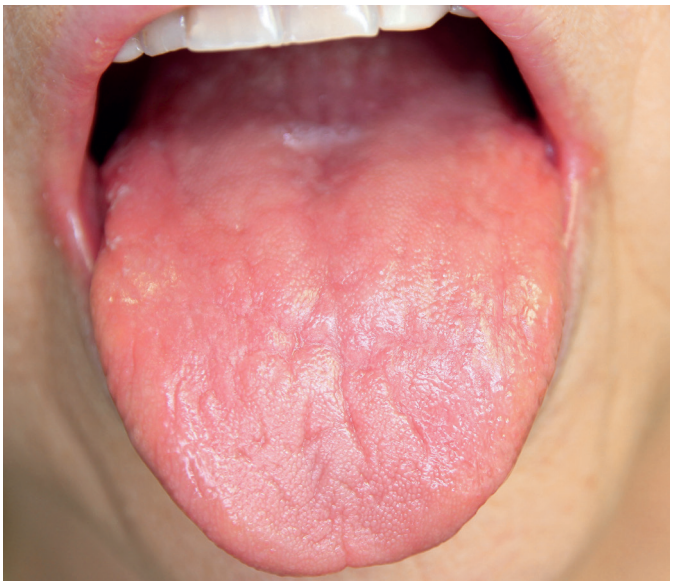

Fig. 2 Atrophic tongue mucosa.

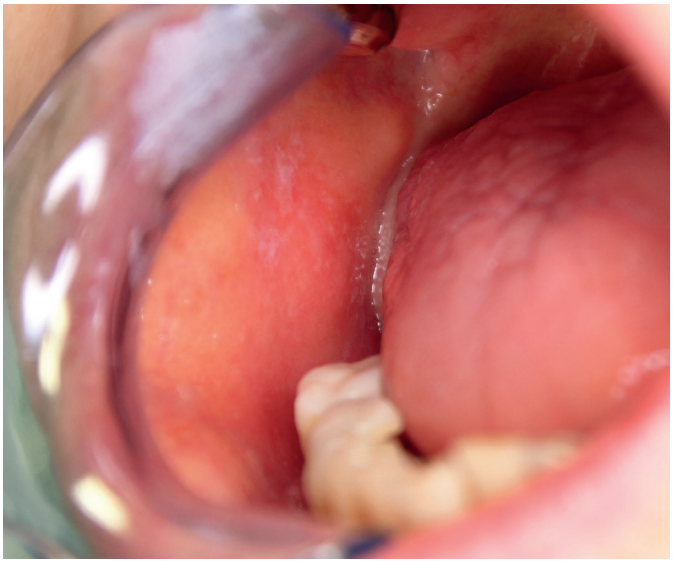

Fig. 3 Buccal erythema with candida overgrowth.

Tab. 1 Blood count values.

	At diagnosis	At + 6 months	Unit	Normal range
Leukocytes	7.92	6.39	× 10^9/l	4.00–10.00
Erythrocytes	4.46	5.4	× 10^{12}/l	3.80–5.20
Hemoglobin	76	140	g/l	120–160
Hematocrit	27.0	44.5	%	35–47
Mean cell volume	60.5	82.4	fl	82.0–98.0
Mean cell hemoglobin	17.0	25.9	pg	28–34
Mean cell Hb concentration	0.28	0.32	kg/l	0.32–0.36
Thrombocytes	439	368	× 10^9/l	150–400
Ferritin	7.0	38.8	ug/l	30–400

with loss of papillae (Figure 2). Discrete erythema with candida overgrowth (white patches) was present of both buccal mucosal surfaces (Figure 3). Other mucosal and tooth findings were unremarkable.

Complementary laboratory examinations were recommended based on history and clinical findings. Sum of resting (unstimulated) salivary flow and stimulated salivary flow (Škach's quantitative analysis of the saliva) revealed salivation of 8 ml / 2 × 15 minutes which is at lower normal margin. Full blood count with differential blood count; ferritin, serum iron, total iron-binding capacity, vitamin B12 and folic acid levels were measured. Tongue swab was sent for mycological cultivation. Significant results are shown in Table 1. Diagnosis of IDA was established. Topical treatment for angular cheilitis with hydrocortisone, natamycin and neomycin was recommended, and the patient was referred for hematology examination. Subsequent gastroscopy showed chronic gastric inflammation without atrophy on histology and previously known hiatus hernia. Colonoscopy was performed with normal findings. No blood losses were identified. Treatment with oral iron supplementation was initiated at a dose of 80 mg of elemental iron daily. The blood count normalized eight weeks after treatment, and iron supplies recovered six months after treatment (Table 1). Patient reported improving the symptoms eight weeks after treatment initiation and did not report any symptoms after six months (Figures 4–6).

DISCUSSION

Nutritional deficiencies, including iron, are also common and still represent a significant medical challenge even in the contemporary European population. Diet habits and socioeconomic status may contribute to the development of such problems (4). Potentially malignant lesions of the gut should be considered behind each iron deficiency cause (5). Mainly central European populations show extremely unfavourable incidence and prevalence pattern of colon cancer (6).

Burning mouth syndrome is one of the most frequent signs that bring patients to dental medicine clinics. It represents a wide variety of diseases ranging from benign to severe disorders. The exact etiology of burning mouth cannot be frequently identified, but the cause may be identified in many patients (7). Nutrition deficiencies and anemia represent the frequent and often reversible cause of burning mouth. Lin et al. described a decrease

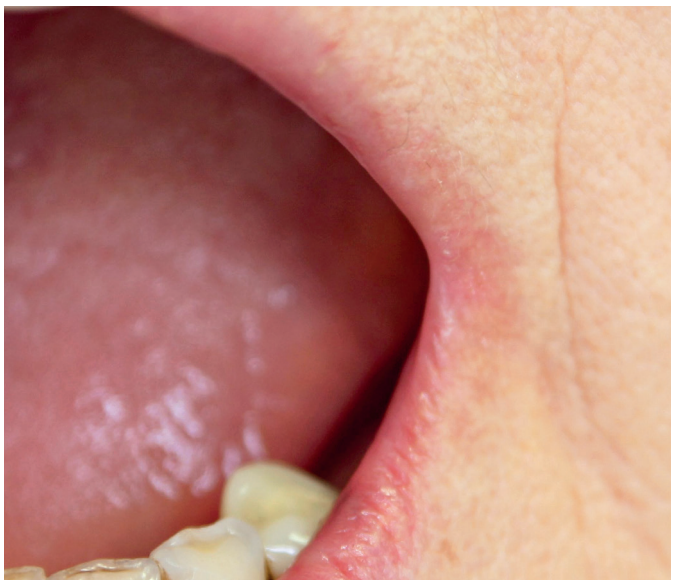

Fig. 4 Angular cheilitis after treatment.

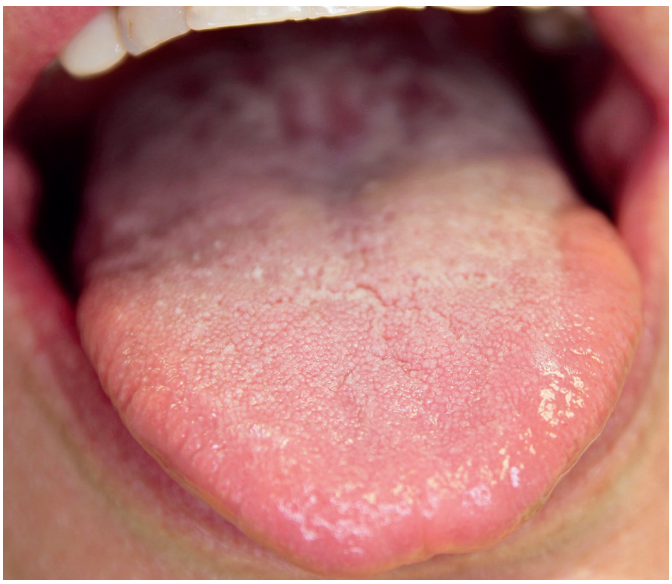

Fig. 5 Healthy tongue after treatment.

of hemoglobin in a cohort of 399 Asian patients with the burning mouth in 22.3%. 20.3% of these patients had iron deficiency, 2.5% vitamin B12 deficiency and 1.5% folic acid deficiency (8). According to Sun et al., patients with papillar atrophy more frequently have iron deficiency (26.7%) than vitamin B12 deficiency (7.4%) (9). Recently published data by Lu showed the prevalence of oral pseudomembranous and erythematous candidiasis around 40% of individuals in patients with iron deficiency (10). Atrophic glossitis may have a very similar clinical picture and could mask underlying deficiency (11). Reversal of symptoms by supplementation of missing nutrients has been shown to be effective in a cohort of 399 patients (5). The burning mouth disappeared within 5–10 months after supplementation of missing nutrient in virtually all affected patients, similar to our patient (12).

On the other hand, burning mouth with dysphagia is a sporadic clinical condition, and PVS is present in less than 0.1% of patients suffering from IDA (3). Typically, PVS is characterized by IDA combined with glossitis, atrophic mucosa, dysphagia and strictures of the upper esophagus (3). Pathogenesis of PVS remains unknown and how IDA contributes to the development of dysphagia has not been fully understood. Possible high turnover of the epithelial cells in upper esophagus dependent on iron storage remains a reasonable explanation (13). It has been proposed that genetic, immunologic and infectious factors may play a role (14). Strictures are based on the presence of esophageal webs that have not been seen in our patient. Our patient did not show any abnormalities in esophageal motility as well. Not all patients with symptoms compatible with PVS need to develop webs, and vice versa, not all patients with esophageal webs suffer from dysphagia and fulfil criteria for PVS (15, 16). Symptoms completely disappeared in our patient after treatment which does not always happen in all patients with PVS. The most frequents reasons for IDA in patients with PVS are menstrual blood loss, chronic gastrointestinal bleeding or hiatal hernia (as in our case). By far the most frequently, the association with celiac disease has been repeatedly reported (3). This fact may be explained either by the presence of refractory iron deficiency in some patients with the celiac disease caused by impaired nutrient absorption or by the contribution of autoimmunity to development of PVS. Other autoimmune disorders were also reported in patients with PVS such as autoimmune thyroiditis and rheumatoid arthritis. Burning mouth and dysphagia could also be a sign of underlying Sjögren syndrome, and diagnostic procedures should include sialometry as well as organ antibody testing. Prognosis of PVS is generally good with appropriate management with iron supplementation in the majority of patients. There have been reports about patients with PVS and strictures and stenosis of the upper esophagus who developed squamous cell carcinoma of the esophagus and presence of the webs probably represents a risk for developing pharyngeal cancer (17). Patients with PVS and esophageal webs and strictures should be therefore monitored for possible signs of carcinoma. Surveillance with repeated endoscopies has been suggested, but the preventive endoscopic evaluation is not confirmed (17).

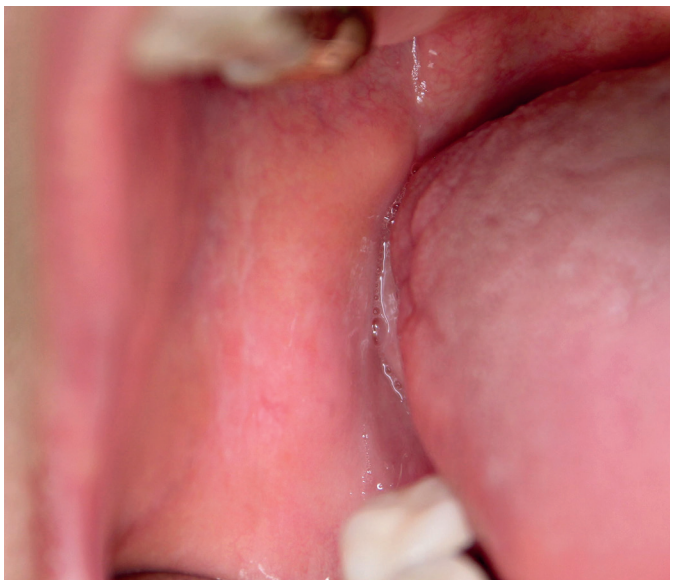

Fig. 6 Healthy buccal mucosa after treatment

LEARNING OBJECTIVES

Both dysphagia and burning mouth should bring attention to possible iron deficiency and other nutritional deficiencies. Dysphagia and burning mouth resolve after iron supplementation in the majority of patients.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet 2016; 387: 907–16.
2. Plummer HS. Diffuse dilatation of the esophagus without anatomic stenosis (cardiospasm): a report of ninety-one cases. J Am Med Assoc 1912; 26: 2013–5.
3. Novacek G. Plummer-Vinson syndrome. Orphanet J Rare Dis 2006; 1: 36.
4. Huffman FG, Vaccaro JA, Zarini GG, Biller D, Dixon Z. Inadequacy of micronutrients, fat, and fiber consumption in the diets of Haitian-, African- and Cuban-Americans with and without type 2 diabetes. Int J Vitam Nutr Res 2012; 82: 275–87.
5. Shine JW. Microcytic anemia. Am Fam Physician 1997; 55: 2455–62.
6. Zavoral M. Colorectal cancer screening in the Czech Republic. Z Für Gastroenterol. 2008; 46(Suppl 1): S29–30.
7. Sun A, Wu K-M, Wang Y-P, Lin H-P, Chen H-M, Chiang C-P. Burning mouth syndrome: a review and update. J Oral Pathol Med 2013; 42: 649–55.
8. Lin H-P, Wang Y-P, Chen H-M, Kuo Y-S, Lang M-J, Sun A. Significant association of hematinic deficiencies and high blood homocysteine levels with burning mouth syndrome. J Formos Med Assoc 2013; 112: 319–25.

9. Sun A, Lin H-P, Wang Y-P, Chiang C-P. Significant association of deficiency of hemoglobin, iron and vitamin B12, high homocysteine level, and gastric parietal cell antibody positivity with atrophic glossitis. J Oral Pathol Med 2012; 41: 500–4.
10. Lu S-Y. Perception of iron deficiency from oral mucosa alterations that show a high prevalence of Candida infection. J Formos Med Assoc 2016; 115: 619–27.
11. Terai H, Shimahara M. Atrophic tongue associated with Candida. J Oral Pathol Med. 2005; 34: 397–400.
12. Sun A, Lin H-P, Wang Y-P, Chen H-M, Cheng S-J, Chiang C-P. Significant reduction of serum homocysteine level and oral symptoms after different vitamin-supplement treatments in patients with burning mouth syndrome. J Oral Pathol Med 2013; 42: 474–9.
13. Okamura H, Tsutsumi S, Inaki S, Mori T. Esophageal web in Plummer-Vinson syndrome. The Laryngoscope 1988; 98: 994–8.
14. Tahara T, Shibata T, Okubo M, et al. A case of plummer-vinson syndrome showing rapid improvement of dysphagia and esophageal web after two weeks of iron therapy. Case Rep Gastroenterol 2014; 8: 211–5.
15. Gude D, Bansal D, Malu A. Revisiting Plummer Vinson Syndrome. Ann Med Health Sci Res 2013; 3: 119–21.
16. Nosher JL, Campbel WL, Seaman WB. The clinical significance of cervical esophageal and hypopharyngeal webs. Radiology 975; 117: 45–7.
17. Hoffman RM, Jaffe PE. Plummer-Vinson syndrome. A case report and literature review. Arch Intern Med 1995; 155: 2008–11.

Anomalous Course of Accessory Splenic Arteries in Gastrosplenic Ligament: Case Report and Clinico-Embryological Basis

Rajprasath Ramakrishnan[1], Dinesh Kumar Viswakumar[2,*], Bhavani Prasad Goriparthi[1]

ABSTRACT
Accessory splenic arteries in the gastrosplenic ligament constitute one of the extremely sub-component of abdominal vasculature variations and it is imperative to recognize this anomaly while planning for complex surgeries in the supra-colic compartment. We report the case of accessory splenic arteries in an approximately 50-year-old male cadaver encountered during routine educational dissection. One of them arising from left gastroepiploic artery supplies the spleen in addition to splenic artery. Another variant vessel bifurcated to enter greater omentum and anterior pole of spleen, as discrete branches. The anatomical vascular variation, if recognized during the imaging work-ups for elective surgical procedures could avoid potential iatrogenic blood loss.

KEYWORDS
abdominal vasculature; splenectomy; accessory splenic artery; left gastroepiploic artery

AUTHOR AFFILIATIONS
[1] Department of Anatomy, Pondicherry Institute of Medical Sciences, Puducherry, India
[2] Department of Anatomy, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India
* Corresponding author: Department of Anatomy, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India; e-mail: dinesh.88560@gmail.com

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INTRODUCTION

The splenic artery being one of the largest branches of the celiac trunk, supplies the structures of the foregut predominantly in the dorsal mesogastrium. It passes through the lieno-renal ligament and before reaching the hilum of spleen, usually gets divided into superior and inferior polar branches. The polar branches divides further into four or five segmental branches close to the hilum and supply splenic segments (1). In addition to the spleen, it also supplies pancreas via pancreatic branches, stomach via short gastric arteries and greater omentum via left gastroepiploic artery. Any additional or concurrent vessels supplying spleen, apart from the principal splenic artery can be termed as accessory splenic artery. An unexpected variant in splenic vasculature could lead to inadvertent blood loss during abdominal surgeries especially those involving splenic hilum.

CASE REPORT

During routine dissection of an approximately 50 years old formalin embalmed male cadaver in our institution, we encountered a variation in the vasculature of spleen (Fig. 1). While dissecting out the gastrosplenic ligament, we could find the left gastroepiploic artery arising from the splenic artery. Upon extending the dissection into greater omentum, we observed variation in the vasculature. One variant vessel, which sprouted as a direct branch from left gastroepiploic artery, entered the anterior pole of spleen in addition to the principal splenic artery located at the hilum of spleen (Fig. 2). Another vessel, from the left gastroepiploic artery, continued as a common trunk and got divided into two branches. One of the branch entered the greater omentum and supplied the greater curvature of stomach. Other branch terminated near the anterior pole of spleen (Fig. 2). Thus, we confirmed that there were two accessory splenic arteries arising from left gastroepiploic artery with one being a direct branch and other sprouting from a common trunk. Schematic representation of the origin and course of these variant vessels in this case is depicted in Fig. 3. The arteries were paralleled by their respective venous counterparts, which drained into left gastroepiploic vein. The spleen was of normal size and superior border presented eight notches of variable depth. We could not observe any other abnormalities or accessory spleens in the abdominal cavity. We couldn't observe significant abnormalities in other abdominal organs and their vasculature.

DISCUSSION

Even though accessory splenic artery is rarely reported, with an incidence of < 1.3%, recognition of this anomaly is crucial while planning complex surgeries in the supra-colic compartment (2). Based on the origin, Lipshutz (3) classified the splenic artery into four types: a) type 1: 75% of cases had splenic artery originating as a separate branch from celiac trunk b) type 2: in 15% cases, splenic artery was a branch of hepato-splenic trunk c) type 3: in 6% cases, abdominal aorta directly gave branch to splenic artery and d) type 4: in 4% cases, splenic artery was a branch of spleno-gastric trunk. Following another classification proposed by Michels (4), celiac trunk could be classified into 6 types with type 6 (coeliaco-mesentric type) having left gastric, splenic, common hepatic and superior mesenteric artery arising from a common trunk. The variation documented in the present study gains uniqueness because it doesn't fit into the purview of any classification.

Pandey SK et al. (2) documented that in 97% of cases, splenic artery divided into terminal branches before entering the splenic hilum i.e. in the lienorenal ligament itself. The branching pattern was further elaborated by Ashok K R et al. (5) who had studied the branching pattern in 42 cases

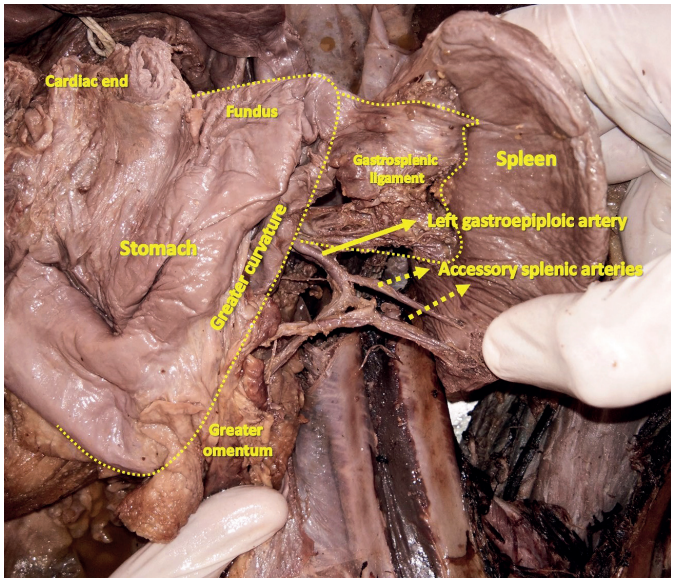

Fig. 1 Showing two accessory splenic arteries (dotted arrow) arising from left gastroepiploic artery (bold arrow).

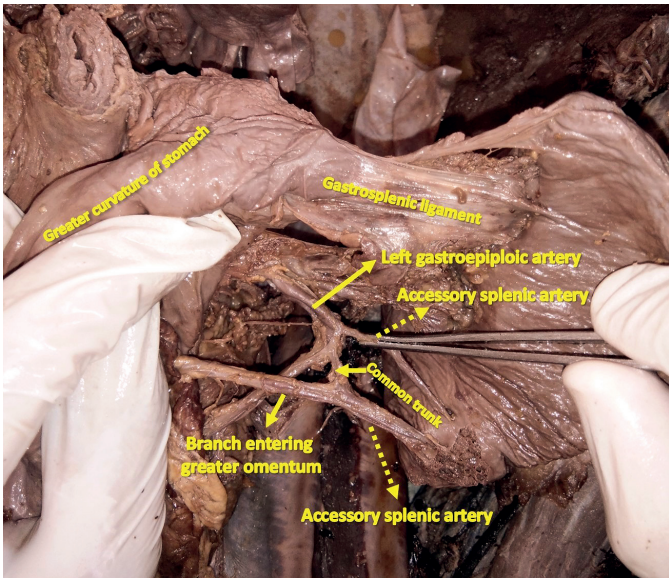

Fig. 2 Showing two accessory splenic arteries arising from left gastroepiploic artery; one as a direct branch and another from a common trunk with a branch to greater omentum.

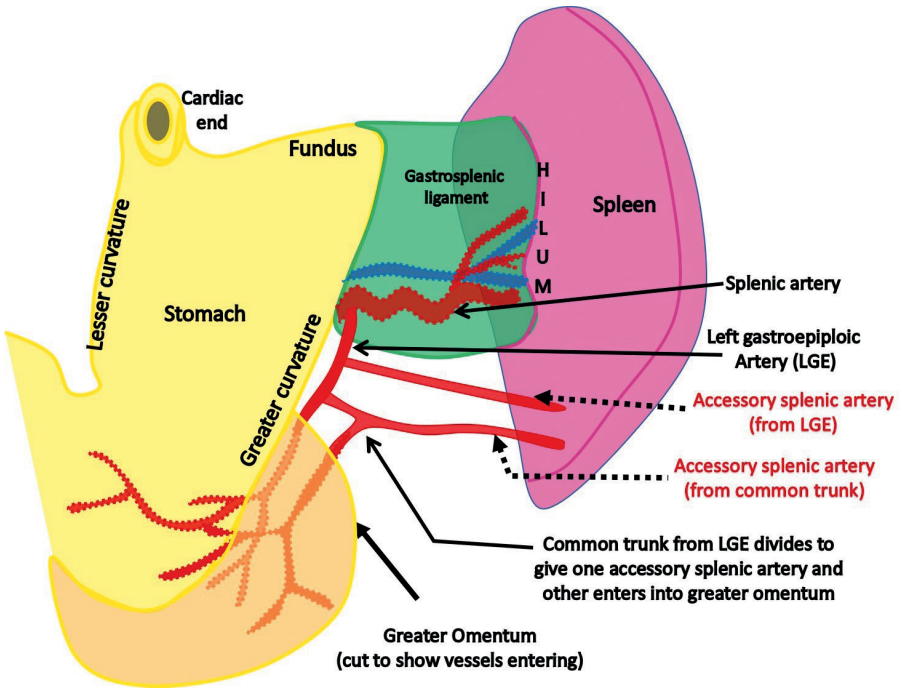

Fig. 3 Schematic representation of the origin & course of accessory splenic arteries.

and found three types of splenic artery termination: a) entering the hilum without branching (10.5%); b) distributed type – dividing away from hilum (55.3%) and c) bundled/marginal type – dividing at the hilum (34.2%).

Kumar N et al. (6) had documented a case report whereby splenic artery divides into two polar arteries 2–4 cm proximal to splenic hilum and accessory splenic artery sprouts from left gastroepiploic artery. Padmalatha et al. (7) and Kervancioglu et al. (8) had previously reported an accessory splenic artery emanating from left gastroepiploic artery and left gastric artery respectively. In the latter case, the accessory artery gave inferior phrenic arteries of both sides and left hepatic arteries. Geeta Anasuya et al. (9) observed a case accessory splenic artery from left gastroepiploic artery which terminated into smaller terminal branches at the lateral end of spleen. Patel SR et al. (10) documented accessory splenic artery in a patient with recurrent upper gastrointestinal bleeding. Upon visualizing using CT angiography, it was found that aberrant vessel originated from left gastric artery and supplied the stomach in addition to the upper pole of spleen.

The plausible embryological hypothesis for aberrant splenic artery could be the failure of regression or faulty fusion of third and fourth splanchnic arteries which forms the gut axial vasculature (11). Gut vasculature develops from the longitudinal anastomoses of branches of these vessels around primitive gut and mesogastrium. Holibkova et al. (12) suggested that the segmental branches of the spleen are involved in three types of anastomosis namely: extra-parenchymatous, intra-parenchymatous and sub-capsular. Juxtaposing the above said hypothesis, we could posit that the accessory splenic artery could be due to the retained anastomoses between inferior polar branches of splenic artery and left gastroepiploic artery. Investigation (13) of splenic artery in chinchilla showed the presence of common trunk, known as gastrosplenic artery, which supplied the dorsal spleen and greater curvature of stomach. In addition, the principal splenic artery supplied central and ventral spleens in the dorsal mesogastrium. This pattern is analogous to splenic and short gastric arteries in humans. In the present case report, the vascular pattern in dorsal mesogastrium is comparable to that of chinchilla.

The anatomical variation described over here is of profound clinical significance because due to its specific pattern of blood supply, spleen can be contextualized to be made of two distinct lobes and 3–5 segments (14). The inferior splenic pole is relatively more vascular and integrates significantly with related abdominal organs in the supra-colic compartment. Owing to this, autologous splenic implants involving inferior pole are being increasingly considered in grounds of traumatic injuries.

CONCLUSION

Even though, accessory splenic artery might be clinically asymptomatic under normal conditions, the knowledge about this would be of great help in salvaging the patient from being vulnerable to iatrogenic injuries particularly while mobilizing pancreatic tail and approaching splenic hilum. The present case report adds to the pool of available literature regarding the variations in the abdominal vasculature which is more relevant from an utilitarian perspective in the era of laparoscopy.

REFERENCES

1. Standring S. Gray's Anatomy: Anatomical Basis of Clinical Practice. 41st edition. New York: Elsevier Churchill Livingstone 2016, p. 1190.
2. Pandey SK, Bhattacharya S, Mishra RN, Shukla VK, Anatomical variations of the splenic artery and its clinical implications Clin Anat 2004; 17(6): 497–502.
3. Lipshutz B. A composite study of the coeliac axis artery. Ann Surg 1917; 65(2): 159–69.
4. Michels NA. Blood supply and anatomy of the upper abdominal organs, with a descriptive atlas. Philadelphia, PA: Lippincott 1955; 139–43.

5. Ashok KR, Kiran TV. Study of branching pattern of splenic artery. Int J Anat Res 2016; 4(1): 2073–5.
6. Kumar N, Patil J, Swamy RS, Guru A, Nayak SB. Atypical arterial supply to the spleen by polar branches of splenic artery and accessory splenic artery – a case report. J Clin Diagn Res 2014; 8(8): AD03–4.
7. Padmalatha K, Ramesh BR, Prakash BS, Balachandra N, Mamatha Y. Accessory splenic artery from left gastric artery. IJAV 2010; 3: 106–7.
8. Kervancioglu S, Yilmaz FG, Gulsen M, Kervancioglu P, Kervancioglu R. Massive upper gastrointestinal bleeding from an accessory splenic artery mimicking isolated gastric varices. Folia Morphol (Warsz) 2013; 72(4): 366–70.
9. Geeta Anasuya D, Jones Eben Raj, Sheela Grace Jeevamani. Unusual anatomical variation in the branching pattern of splenic artery. Int J Anat Res 2016; 4(3): 2809–12.
10. Patel SR, Lowe S. Accessory Splenic Artery: A Rare Cause of Upper Gastrointestinal Bleeding. Cardiovasc Intervent Radiol 2017; 40(7): 1115–7.
11. Zhou W, Qiu J, Yuan Q, Zhou W, Xiong J, Zeng Q. Successful treatment of aberrant splenic artery aneurysm with a combination of coils embolization and covered stents. BMC Surg 2014; 14: 62.
12. Holibkova A, Machalek L, Houserkova D, Ruzicka V. A contribution to the types of branching and anastomoses of the splenic artery in human spleen. Acta Univ Palacki Olomuc Fac Med 1998; 141: 49–52.
13. Özdemir V, Demirkan AC, Akosman MS. Subgross and macroscopic investigation of the coeliac artery in the chinchilla (Chinchilla lanigera). Folia Morphol (Warsz) 2013; 72: 258–62.
14. Liu DL, Xia S, Xu W, Ye Q, Gao Y, Qian J. Anatomy of vasculature of 850 spleen specimens and its application in partial splenectomy. Surgery 1996; 119: 27–33.

Symptomatic Hypokalemia in a 19-Year-Old Student

Sara Pereira[1,*], André Salgueiro[2], Paula Rosa[1], Carla Peixoto[1], Marta Ferreira[1], David Silva[1]

ABSTRACT

Primary hyperaldosteronism (PA) is the most common cause of secondary arterial hypertension and is frequently undiagnosed. It affects all ages but is more frequent between 20 and 60 years old. The clinical presentation is variable, and the diagnosis is based on screening and, in equivocal cases, confirmatory tests. A 19-year-old student presented with complaints of extreme fatigue, arterial hypertension, hypokalemia and metabolic alkalosis, raising a high index of suspicion for PA. Screening tests were performed and its expressiveness excluded the need of confirmatory tests. CT-scan showed a unilateral adrenal adenoma and the patient was submitted to laparoscopic adenectomy without complications. Prompt diagnosis and treatment are essential to avoid long term complications of PA.

KEYWORDS

primary hyperaldosteronism; hypokalemia; hypertension; aldosterone-producing adenomas; Conn syndrome

AUTHOR AFFILIATIONS

[1] Intermediate Care Unit – Centro Hospitalar do Médio Ave, Vila Nova de Famalicão, Portugal
[2] Bombarral Family Health Unit, Rua Doutor Arlindo de Carvalho, nº 27, 2540-073 Bombarral, Portugal
* Corresponding author: Intermediate Care Unit – Centro Hospitalar do Médio Ave, Rua Cupertino Miranda S/N, 4761-971 Vila Nova de Famalicão, Portugal; e-mail: saracp86@gmail.com

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INTRODUCTION

Primary hyperaldosteronism (PA) is the most common cause of secondary arterial hypertension and is frequently undiagnosed. The two most common causes of PA are aldosterone-producing adenomas (Conn syndrome) and bilateral idiopathic hyperplasia. Less common causes of PA include unilateral adrenal hyperplasia, carcinomas and ectopic tumors (Table 1) (1).

Tab. 1 Types of primary hyperaldosteronism.

Type of primary aldosteronism	Cases
Aldosterone-producing adenoma	30%
Bilateral idiopathic hyperplasia	60%
Primary (unilateral) adrenal hyperplasia	2%
Aldosterone-producing adrenocortical carcinoma	< 1%
Familial hyperaldosteronism (FH)	
Glucocorticoid-remediable aldosteronism (FH type I)	< 1%
FH type II	< 6%
FH type III (germline KCNJ5 mutations)	< 1%
FH type IV (germline CACNA1H mutations)	< 1%
Ectopic aldosterone-producing adenoma or aldosterone-producing carcinoma	< 1%

FH – Familial hyperaldosteronism

Patients of all ages may be affected, but the peak incidence is between 20 years and 60 years.

The most common presentation of the disease is normokalemic hypertension. The degree of hypertension is typically moderate but may be severe. In some cases, it may present as resistant hypertension or asymptomatic diastolic hypertension. Hypertension (as opposed to hypokalemia) is a prerequisite for PA diagnosis. Hypokalemia is only present in 9–37% of patients: in half of aldosterone-producing adenomas and in 17% of bilateral idiopathic hyperplasia. Thus, hypokalemia has low sensitivity and its absence has a low negative predictive value for the diagnosis of PA (2). Metabolic alkalosis is common.

Although in some cases the clinical presentation may be similar, patients with aldosterone-producing adenoma have higher aldosterone secretion rates, resulting in more severe hypertension and marked hypokalemia; patients with bilateral idiopathic hyperplasia have a milder disease with less hypersecretion of aldosterone and less hypokalemia. Also, patients with aldosterone-producing adenoma are generally younger (< 50 years) than those with bilateral idiopathic hyperplasia (3).

Hyperaldosteronism is associated with higher rates of cardiovascular and cerebrovascular morbidity and mortality as compared to patients with essential hypertension, matched for age, sex and blood pressure (1), so, early diagnosis is the key to prevent disease progression.

It is important to identify the high-risk population for PA. The screening must be considered if the patient meets one of the following criteria (2):

- sustained blood pressure greater than 150/100 mmHg on 3 measurements on different days,
- blood pressure > 140/90 mmHg resistant to 3 antihypertensive drugs (including a diuretic),
- need of 4 or more antihypertensive drugs to control blood pressure,
- hypertension and hypokalemia,
- hypertension and adrenal incidentaloma,
- hypertension and sleep apnea,
- hypertension and family history of early-onset hypertension or cardiovascular event under 40 years old, or first-degree relative diagnosed with primary aldosteronism.

The diagnosis is based on laboratory tests. The aldosterone-to-renin ratio (ARR) has higher sensitivity and lower variability than other measures, so it should be the first to perform. ARR is the ratio of plasma aldosterone and plasma renin activity or direct plasma renin collected in the morning (more than 2 hours after awakening), in sitting position for 5 to 15 minutes, with normal dietary salt intake (urinary sodium 100–200 mmol/24h), normal serum potassium level and without intake of angiotensin converting enzyme inhibitors or angiotensin receptor blocker for at least 2 weeks (4). If the ARR is higher than laboratory threshold, PA should be suspected.

In case of spontaneous hypokalemia, plasma renin below detection levels and plasma aldosterone concentration > 20 ng/dL, confirmatory tests are not needed (2). In the other scenarios it must be performed.

Nowadays, there are four confirmatory tests approved in America and Europe for the diagnosis of PA: oral sodium loading, saline infusion test, fludrocortisone suppression test, and captopril challenge (5, 6). There is not a gold standard confirmatory test, as each has advantages and disadvantages that should be assessed case by case. In Japan, the furosemide upright test is used, although it is not approved by Endocrine Society as a confirmatory test of PA (6).

All patients with suspicion PA should undergo adrenal computer tomography (CT) scan in the initial study to determine subtype (adenoma versus hyperplasia) and exclude adrenal carcinoma. When surgical treatment is indicated, adrenal venous sampling (AVS) should be performed by an experienced team, to distinguish between unilateral adenoma and bilateral hyperplasia. In some cases, AVS is not necessary, especially in younger patients (< 35 years), with spontaneous hypokalemia, high aldosterone levels, and unilateral adrenal lesions with radiological features consistent with a cortical adenoma on adrenal CT scan (2). In these cases, AVS is not routinely indicated as it is expensive, technically demanding and carrying a tiny risk of adrenal vein rupture, and so, unilateral adrenalectomy may be safely performed.

Treatment depends on whether it is unilateral or bilateral disease. In case of unilateral adenoma hyperplasia, curative surgery, such as laparoscopic unilateral adrenalectomy performed by an experienced surgeon, is considered

the treatment of choice. In case of bilateral disease, medical management with mineralocorticoid receptor antagonist is usually used as the first-line treatment modality.

CASE REPORT

We report a case of a 19-year-old student with arterial hypertension (first time documented in 2015: systolic blood pressure of 130–140 mmHg and 150 mmHg in 2018) and longstanding complaints of fatigue and headache.

He was referred to the Emergency Department by his Family Physician for assessment of symptomatic hypokalemia (K+ 1.9 mEq/L) detected in a routine blood test. He had no significant past medical history and was not taking any medications. On admission he denied headache and fatigue, was afebrile, a blood pressure of 156/91 mmHg and heart rate of 73bpm. The remainder of the physical examination was unremarkable.

Investigations (shown in Table 2) revealed hypokalemia (serum potassium of 1.4 mEq/L) and arterial blood gas analysis on room air showed alkalemia with metabolic alkalosis. The electrocardiogram showed normal sinus rhythm, a heart rate of 67 bpm and prominent U-wave in the precordial leads (Figure 1). During electrocardiographic monitoring, atrial and ventricular extrasystoles were noted.

Tab. 2 Laboratory data, blood gas analysis and hormonal examination.

Laboratory data	
Hemoglobin	16.9 g/dL (N 13–18)
Hematocrit	46.6% (N 40–52)
Platelets	281 × 10^3/uL (N 130–450)
Leucocytes	9.36 × 10^3/uL (N 4.5–13)
Creatinine	0.75 mg/dL (N 0.4–1.2)
Sodium	142 mEq/L (N 136–145)
Potassium	1.4 mEq/L (N 3.5–5.1)
Magnesium	2.2 mg/dL (N 1.8–2.5)
Reactive C Protein	< 0.1mg/dL (N 0.02–0.75)
Blood gas analysis	
pH	7.54 (N 7.35–7.45)
pO_2	91 mmHg (N 80–100)
pCO_2	51 mmHg (N 35–45)
HCO_3^-	43.6 mmol/L (N 22–26)
Sodium	143 mEq/L (N 136–145)
Potassium	1.6 mEq/L (N 3.5–5.1)
Lactate	1.7 mmol/L (N <2.0)
Hormonal examination	
Plasma aldosterone (supine)	32 ng/dL (N <15)
Plasma renin activity	< 0.5 ng/mL/hour (N 0.7–3.3)
ARR	65.44 (N < 30)

ARR – aldosterone-to-renin ratio; N – normal

Potassium chloride replacement therapy was started, and patient was transferred to the Intermediate Care Unit due to severe hypokalemia with arrhythmogenic potential.

On admission to the Intermediate Care Unit, patient was hemodynamically stable but with tendency to develop hypertension peaks and frequent atrial and ventricular extrasystoles.

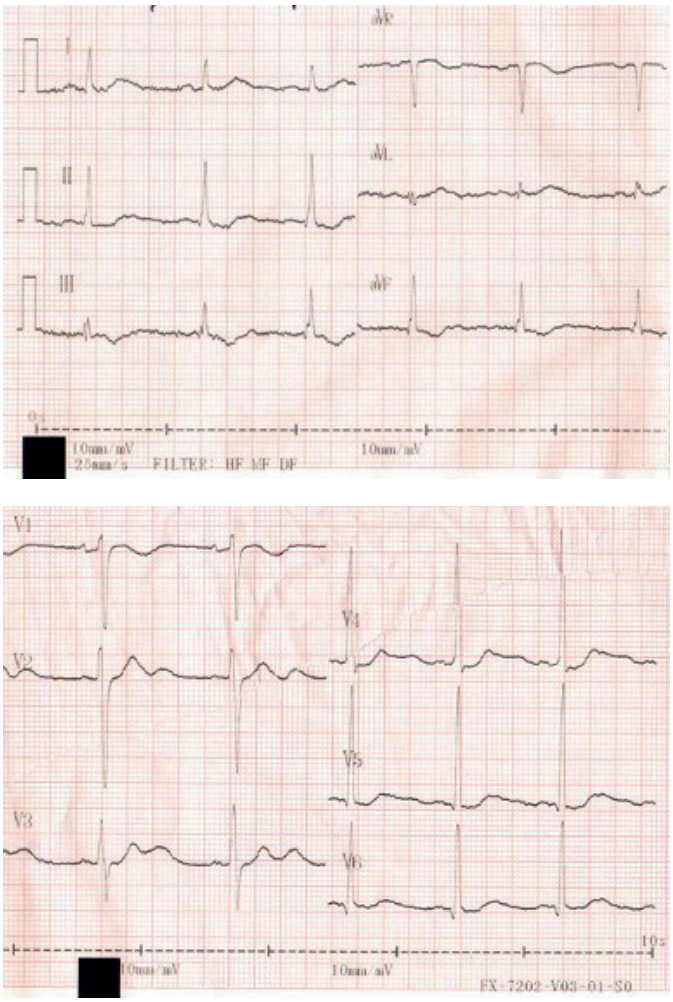

Fig. 1 12-lead Electrocardiogram.

Potassium chloride and magnesium sulphate were administered by subclavian central venous catheter. Serum aldosterone and Plasma Renin Activity (PRA) were evaluated once serum potassium level was corrected. Hypertension was managed with drugs that do not interfere with renin-angiotensin-aldosterone system.

The ARR was 65.44, excluding, in this setting, the need for confirmatory test of PA.; Urinary 24-hour excretion of metanephrine and normetanephrine were normal.

Non-contrast adrenal CT scan revealed a small regular hypodense nodule in the left adrenal gland, approximately 1.5 by 1.2 cm, with a CT attenuation of 11.3 Hounsfield units, suggestive of a benign cortical adenoma (Figure 2).

Following stabilization of hypokalemia and blood pressure profile, patient was started on spironolactone 300 mg/day, potassium chloride 600 mg 3id and amlodipine 5 mg/day, with frequent monitoring of potassium levels and dose adjustment. Amlodipine was suspended as soon as blood pressure control was achieved.

Following multidisciplinary team discussion, patient was referred for surgical treatment once stabilization of the potassium levels and blood pressure profile were achieved. The patient underwent a left laparoscopic adrenalectomy without complications. Histopathological examination confirmed features of adrenal adenoma.

Post operatively patient had recovery of his fatigue, his headaches became occasional and was not requiring

any antihypertensive drugs to control his blood pressure profile. Serum potassium and ARR on follow-up were normal.

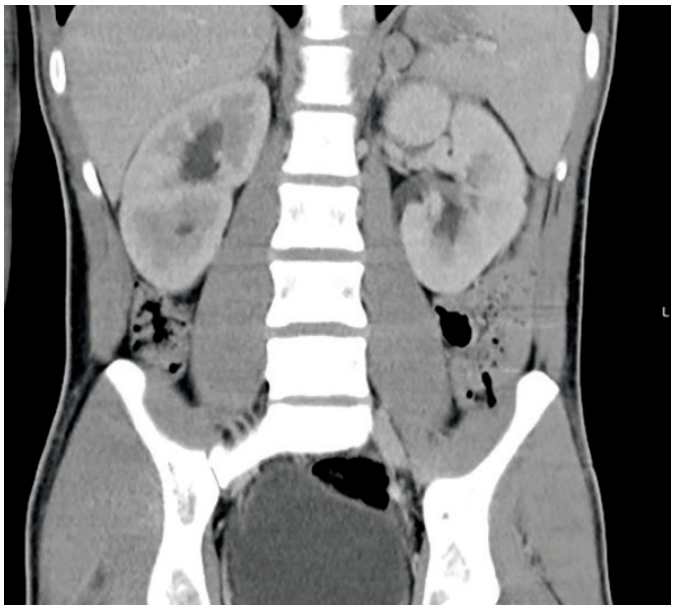

Fig. 2 Abdominal-pelvic CT scan showing a small regular hypodense nodule in the left adrenal gland (1.5 × 1.2 cm).

DISCUSSION

In PA there is an inappropriate (i.e. renin-independent) increase in aldosterone secretion. Under influence of high levels of aldosterone, there is an excessive sodium retention, volume expansion and plasma renin suppression, leading to hypertension. Thus, hypokalemia results from the urinary loss of potassium, in exchange for sodium in the distal renal tubule.

In this case, there was a high suspicion for the diagnosis of PA because the patient presented with hypertension, hypokalemia and metabolic alkalosis, typical presentation of aldosterone-producing adenoma subtype. He also exhibited neuromuscular symptoms related to severe hypokalemia, as well as longstanding complaints of extreme fatigue.

We followed a rational diagnostic approach. The confirmatory test for PA was omitted, due to the expressiveness of the screening test result (high levels of aldosterone and suppressed renin level), assessed in standard conditions, making the presence of adrenocortical tumor clear. Also, the confirmation of laterality by AVS was not performed before proceeding to the left adrenalectomy. The patient was young, clinically had a typical aldosterone-producing adenoma subtype, a marked aldosterone excess, as well as a unilateral adrenal lesion with radiological features consistent with cortical adenoma on adrenal CT scan, therefore not needing AVS (2).

The surgical treatment solved the symptoms and the arterial hypertension. Until now, he has not developed cardiac or cerebrovascular disease, namely new-onset diabetes mellitus, metabolic syndrome, stroke, atrial fibrillation, coronary artery disease, left ventricular hypertrophy or heart failure, which are more common in longstanding PA (1).

CONCLUSION

The diagnosis of PA is essential and should be made early in the course of the disease because proper treatment may prevent the progression of cardiovascular and cerebrovascular disease, offering patients a better quality of life, with less morbidity and mortality.

REFERENCES

1. Young W. Diagnosis and treatment of primary aldosteronism: practical clinical perspectives. Journal of Internal Medicine 2018; 285(2): 126–48.
2. Funder J, Carey R, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. Journal of Clinical Endocrinology & Metabolism 2016; 101(5): 1889–916.
3. Young W. Primary aldosteronism: renaissance of a syndrome. Clinical Endocrinology 2007; 66(5): 607–18.
4. Douillard C, Houillier P, Nussberger J, Girerd X. SFE/SFHTA/AFCE Consensus on Primary Aldosteronism, part 2: First diagnostic steps. Annales d'Endocrinologie 2016; 77(3): 192–201.
5. Reznik Y, Amar L, Tabarin A. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 3: Confirmatory testing. Annales d'Endocrinologie 2016; 77(3): 202–7.
6. Lee F, Elaraj D. Evaluation and Management of Primary Hyperaldosteronism. Surgical Clinics of North America 2019; 99(4): 731–45.

Metachronous Isolated Contralateral Lung Metastasis from Pulmonary Adenosquamous Carcinoma with EGFR Mutation

Hitomi Kawai[1], Kesato Iguchi[2], Norio Takayashiki[1], Shinichiro Okauchi[3], Hiroaki Satoh[3,*]

ABSTRACT

Lung metastasis and metachronous double primary lung cancer are both common and often present diagnostic challenges. We present a case of metachronous isolated contralateral lung metastasis from pulmonary adenosquamous carcinoma with EGFR mutation. A 75-year-old woman presented with left lung nodule on a routine follow-up chest radiograph. She had had surgery for pulmonary adenocarcinoma with EGFR Ex21 L858R mutation 6 years ago. She underwent surgical resection, and histologic findings revealed adenosquamous carcinoma with the same EGFR mutation. Re-assessment of the resected specimen of the primary tumor resected 6 years ago revealed the morphologically similarity to the left lung tumor. Based on morphological and genetic identity, final diagnosis was adenosquamous cell carcinoma and metachronous isolated contralateral lung metastasis. The diagnosis of metachronous isolated metastasis is difficult but important for appropriate management and prediction of prognosis. A careful pathological examination and evaluation of genetic abnormality are needed to make the correct diagnosis.

KEYWORDS

EGFR mutation; recurrence; lung adenosquamous carcinoma

AUTHOR AFFILIATIONS

[1] Division of Pathology, Mito Medical Center, University of Tsukuba-Mito Medical Center, Mito, Ibaraki, Japan
[2] Division of Surgery, Mito Medical Center, University of Tsukuba-Mito Medical Center, Mito, Ibaraki, Japan
[3] Division of Respiratory Medicine, Mito Medical Center, University of Tsukuba-Mito Kyodo General Hospital, Mito, Ibaraki, Japan
* Corresponding author: Division of Respiratory Medicine, Mito Medical Center, University of Tsukuba, Miya-machi 3-2-7, Mito, Ibaraki, 310-0015, Japan; e-mail: hirosato@md.tsukuba.ac.jp

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INTRODUCTION

Metachronous isolated contralateral lung metastasis from pulmonary carcinoma is not rare. However, establishing diagnosis of intrapulmonary metastasis or is not necessarily easy. The metastatic tumors cannot entirely be the same as the primary tumor morphologically and lead to misdiagnosis. Such misdiagnosis lead to the inappropriate treatment and worse prognosis if each clinical condition differs significantly. On the other hand, there are patients whose second cancer histologically resembles the first cancer. In these patients, we must be aware of mistakes in selection of treatment. Although the EGFR mutation is one of the most common gene mutations, there has been only one published case report which showed the important role of the EGFR mutation in diagnosis of metachronous isolated contralateral lung metastasis intrapulmonary metastasis or second primary lung adenocarcinoma (1).

In comparison with adenocarcinoma and squamous cell carcinoma of the lung, adenosquamous cell carcinoma is an uncommon tumor with distinct clinical behavior (2, 3). There have been many reports that patients with adenosquamous cell lung cancer have poorer prognosis than those with lung adenocarcinoma and those with squamous cell lung cancer (2, 3). We report a prolonged survival case of a metachronous isolated contralateral lung metastasis from pulmonary adenosquamous carcinoma with EGFR mutation, which was evaluated as second primary lung cancer at the time of surgical resection.

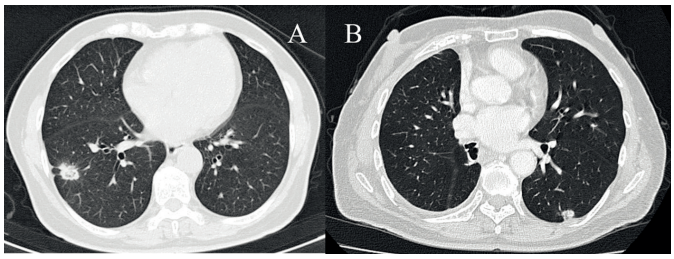

Fig. 1 Chest CT findings of the first tumor of the right lung (A) and the second tumor of the left lung (B).

CASE REPORT

A 69-year-old woman was admitted to our hospital due to an abnormal opacity in chest radiograph during mass-screening. She was a housewife and she never smoked. Chest CT scan revealed a nodule in the lower lobe of the right lung (Figure 1A). Hilar and mediastinal lymph node adenopathy was not observed. She was referred to our hospital for further examination and treatment. Physical examination and laboratory data were unremarkable. Right lower lobectomy with nodal dissection was performed, and the final pathological diagnosis was adenocarcinoma. Pathological tumor stage was proven to be IB. Mutation analysis of the tumor expressed epidermal growth factor receptor (EGFR) Exon 21 L858R mutation. She received postoperative adjuvant treatment with TS-1 for 5 years. Thereafter, she was followed up at our outpatient department without any additional adjuvant therapy. Six years after the surgery, a small nodule in the left lower lobe was detected on the chest CT scan (Figure 1-B). She received surgical resection to diagnose if this lesion was de novo tumor or possibly recurrence of lung cancer. Video-assisted thoracic surgery revealed a small nodule in the left lower lobe. Tumor size was 15 × 7 × 7 mm and surgical margin was free to the pleura. The component of the acinar type adenocarcinoma that proliferates in the glandular duct was main, but squamous cell carcinoma

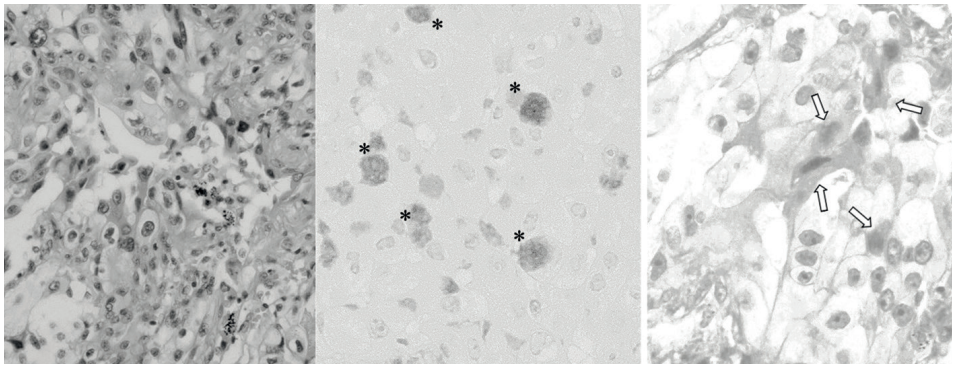

Fig. 2 Histological findings of the resected specimens of the second tumor. The resected tumor showed components of both adenocarcinoma and squamous cell carcinoma, with each comprising at least 10% of the tumor (Hematoxylin-Eosin staining) (A). There were tumor cells with positive for thyroid transcription factor-1 (B), cytokeratin 5/6 (C).

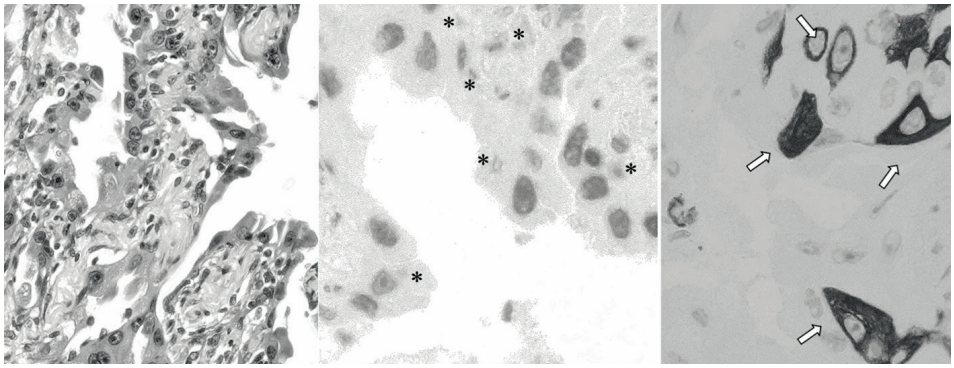

Fig. 3 Histological findings of the resected specimens of the first tumor. The resected tumor showed components of both adenocarcinoma and squamous cell carcinoma, with each comprising at least 10% of the tumor (Hematoxylin-Eosin staining)(A). There were tumor cells with positive for Alcian blue (B) and cytokeratin 5/6 (C).

components with intercellular bridges were 15% of the tumor (Figure 2-A). There were tumor cells with positive for thyroid transcription factor-1 (Figure 2-B), cytokeratin 5/6 (Figure 2-C), and Alcian Blue (Al-b). An EGFR Exon 21 L858R mutation was identified. Histologically, the nodule was diagnosed as stage IA adenosquamous cell carcinoma with EGFR mutation. At that time, these were evaluated as metachronous double primary tumors because they were different histological types of lung cancer. However, EGFR mutation type in both tumors were the same one, re-examination and comparison of these tumors were performed. Morphologically the cancer cells of the first tumor was very similar to those in the second tumor (Figure 3-A). There were tumor cells with positive for Al-b (Figure 3-B) and cytokeratin 5/6 (Figure 3-C). Based on these findings with same EGFR mutation, the second tumor was diagnosed to be a metastasis to the contralateral lung of the first tumor rather than de novo second primary caner. The patient received any additional therapy and is followed up for 3-year period with no recurrence.

DISCUSSION

Clinical history, radiographic findings and histologic features are most helpful in the differential diagnosis of pulmonary metastasis from the primary lung cancer and the second primary lung cancer. As a feature on CT images of lung metastatic lesions, typical features of hematogenous metastases include single or multiple, round to oval shaped, well-circumscribed radiographic masses without spiculations, calcifications, or tree-in-bud appearance. However, lung lesions in some patients show atypical radiographic features and a possibility of metastasis should be suspected in patients with a history of lung cancer, even if a long time has passed since the diagnosis and treatment of the primary lung cancer (4, 5). Kondo et al reported a patient who had a contralateral pleural tumor 14-year after undergoing complete resection of primary lung adenocarcinoma (1). Histologic and genetic analysis of the previously resected specimen and the samples obtained of a newly performed biopsy confirmed that deletion Ex 19 EGFR mutated adenocarcinoma, and the recurrence of lung cancer was determined (1). CT image of the patient was contralateral pleural thickening. Although the pleural thickening had positive positron emission tomography, however, it was evaluated not as an image that required distinguishing between lung metastasis and second primary lung cancer.

Whenever a well-circumscribed lung tumor identified in chest CT scan, the possibility of metastatic lung cancer should be considered and excluded. Genetic studies are at present mandatory for pathologic diagnosis if the primary lung cancer was known. EGFR is one of the most common and important gene mutations. As far as East Asians are concerned, EGFR mutation is expressed in about a half of adenocarcinomas (6–8). However, the positive rate of EGFR mutation in adenosquamous cell carcinoma is reported only as 0 to 5% (9–13). In our patient, morphologically, both the primary tumor and the second tumor developed contralateral side consisted of the components of adenocarcinoma as well as squamous cell carcinoma, each comprising more than 10% of the tumor. Therefore, the final pathological diagnosis was lung adenosquamous cell carcinoma. Exon 21 R858R EGFR mutation was identical in both tumors.

It has been evaluated that patients with lung adenosquamous cell carcinoma had poorer prognosis than those with lung adenocarcinoma and those with lung squamous cell carcinoma (2, 3). For patients presenting early-staged lung adenosquamous cell carcinoma, surgical removal of the tumor offers patient benefit. EGFR-TKI could be a useful treatment option for EGFR mutated patients with unresectable disease. However, it has not been established yet whether postoperative adjuvant treatment with EGFR-TKI for patients with completely resected lung adenosquamous cell carcinoma such as our patient will affect the prognosis or not.

In our patient, the possibility of "the second cancer" with "different" genetic background cannot be denied, but the possibility of second cancer having the "same" genetic background remains. Secondary cancer had some unclear part with the surroundings and had a pleural indentation as it located adjacent to pleura. But these findings did not suggest that the second tumor was the second cancer having the "same" genetic background. If blood vessels and lymph flow involved in metastasis could be identified, it can be regarded as metastasis, but it is difficult to confirm morphologically if the lesion enlarged. We evaluated that the possibility of pulmonary metastasis was higher than the possibility of second cancer having the "same" genetic background, but we must admit that there is room for discussion. The best approach for second primary lung cancer remains a subject of debate. For resectable second primary lung cancer, surgical intervention is feasible and potentially effective and good prognosis can be expected for these patients (14). On the other hand, if the lesion is assessed as distant metastasis, chemotherapy, a systemic therapy, is indicated for most patients with lung cancer. Their prognosis must be poor. We supposed that treatment strategy can be similar in EGFR gene mutation patients and in negative patients although there is no report that clearly clarified it. In this patient, EGFR-TKI administration might be indicated if it was evaluated to be a recurrence. However, surgical treatment was performed because of resectable oligometastasis after long-term interval. If it was evaluated as a resectable second lung cancer, it was appropriate to perform surgical resection.

To the best of our knowledge, this is the first report of metachronous isolated contralateral lung metastasis from pulmonary adenosquamous carcinoma with EGFR mutation. The patient has been survived with disease free at present for the 84 months following the diagnosis of the first lung adenosquamous carcinoma. It was not possible to differentiate the recurrence from the second lung cancer as a result in our patient, since the driver gene was the same. If we are not sure whether it is recurrence or secondary cancer, it is important to evaluate and compare each tumor resected. We do believe that driver gene evaluation including EGFR mutation may provide useful information in differentiating recurrence from secondary

cancer in some lung cancer patients. From this perspective, we emphasize the benefits of our experience for real-life practice.

CONFLICT OF INTEREST

None.

FINANCIAL SUPPORT

None.

REFERENCES

1. Kondo N, Takuwa T, Hashimoto M, et al. Gene Mutation Analysis in determining late recurrence of adenocarcinoma of the lung. Ann Thorac Surg 2015; 100(2): 711–3.
2. Filosso PL, Ruffini E, Asioli S, et al. Adenosquamous lung carcinomas: a histologic subtype with poor prognosis. Lung Cancer 2011; 74(1): 25–9.
3. Cooke DT, Nguyen DV, Yang Y, et al. Survival comparison of adenosquamous, squamous cell, and adenocarcinoma of the lung after lobectomy. Ann Thorac Surg 2010; 90(3): 943–8.
4. Miyazaki K, Ano T, Nakazawa K, et al. Late fatal recurrence in gefitinib-treated NSCLC patients. Tuberk Toraks 2007; 55(4): 400–3.
5. Kagohashi K, Nakayama H, Kagei K, et al. Thoracic radiotherapy for mediastinal nodal recurrence. Acta Medica (Hradec Kralove) 2009; 52(1): 23–5.
6. Jiang H. Overview of gefitinib in non-small cell lung cancer: an Asian perspective. Jpn J Clin Oncol 2009; 39(3): 137–50.
7. Armour AA, Watkins CL. The challenge of targeting EGFR: experience with gefitinib in nonsmall cell lung cancer. Eur Respir Rev 2010; 19(117): 186–96.
8. Dungo RT, Keating GM. Afatinib: first global approval. Drugs 2013; 73(13): 1503–15.
9. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 2005; 23(4): 857–65.
10. Sugio K, Uramoto H, Ono K, et al. Mutations within the tyrosine kinase domain of EGFR gene specifically occur in lung adenocarcinoma patients with a low exposure of tobacco smoking. Br J Cancer 2006; 94(6): 896–903.
11. Tsao MS, Sakurada A, Ding K, et al. Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. J Thorac Oncol 2011; 6(1): 139–47.
12. Miyamae Y, Shimizu K, Hirato J, et al. Significance of epidermal growth factor receptor gene mutations in squamous cell lung carcinoma. Oncol Rep 2011; 25(4): 921–8.
13. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012; 489(7417): 519–25.
14. Sato S, Nakamura M, Shimizu Y, et al. Impact of postoperative complications on outcomes of second surgery for second primary lung cancer. Surg Today 2020 Jun 1.