

ORIGINAL ARTICLE

Elevated serum IgE in recurrent aphthous stomatitis and associations with disease characteristics

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OBJECTIVES: To characterize demographic, clinical and serological parameters in recurrent aphthous stomatitis (RAS) patients and analyse their association with serum immunoglobulin E (IgE) levels.

SUBJECTS AND METHODS: Forty-nine patients with RAS responded to a questionnaire that included demographic background, stress status, smoking habits, history and course of RAS episodes. They were also subjected to relevant laboratory tests, including determination of serum IgE levels.

RESULTS: A familial history of RAS was reported by 47.9% of the patients, stress in the previous year by 51.1% and smoking by 18.4%. Non-Caucasian origin, familial history of RAS, stress and smoking were associated with increased severity of RAS episodes. Haematological deficiencies were observed in 18.7% of RAS patients. Average IgE levels were increased and were significantly associated with younger age, ≤12 years of schooling, female gender, RAS episode frequency of every 2 weeks, early onset of RAS episodes and elevated C reactive protein levels.

CONCLUSION: Immunoglobulin E levels may be considered as part of the RAS patient's work-up. Further research is needed to identify biological mechanisms that account for the observed associations.

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Keywords: recurrent aphthous stomatitis; immunoglobulin E

Abbreviations

CRP C reactive protein

ESR erythrocyte sedimentation rate

IgE	immunoglobulin E
IgM	immunoglobulin M
MCH	mean cell haemoglobin
MCV	mean corpuscular volume
RAS	recurrent aphthous stomatitis
VAS	visual analogue scale

Introduction

Recurrent aphthous stomatitis (RAS) is the most common recurring idiopathic intra-oral ulcerative disease in the USA, affecting over 100 million Americans (Baccaglini *et al*, 2011). RAS is more common in white non-smokers under the age of 40 years and is associated with higher socio-economic status (Jurge *et al*, 2006). Aphthae are located on non-keratinized mucosa; the ulcers have well-circumscribed margins, erythematous haloes and yellow or grey floors. They appear first in childhood or adolescence and heal naturally in 7–14 days (Scully and Porter, 2008). Aphthae are painful and interfere with daily oral behaviour such as eating, speaking and swallowing, consequently decreasing quality of life (Krisdapong *et al*, 2012).

The aetiology of RAS lesions is unknown, but several local, systemic, immunologic, genetic, allergic, nutritional and microbial factors have been proposed as causative agents (Akintoye and Greenberg, 2005; Chavan *et al*, 2012). An association has also been proposed between RAS and psychological stress and anxiety (Miller and Ship, 1977; McCullough *et al*, 2007; Huling *et al*, 2012; Picek *et al*, 2012).

Allergy has been suspected as a cause of RAS, and hypersensitivity to certain food substances, oral microbes such as *Streptococcus sanguis*, and microbial heat-shock proteins have been suggested as possible causative factors (Chavan *et al*, 2012).

Immunoglobulin E (IgE) is one of five isotypes of human immunoglobulins, IgG, IgA, IgM, IgD and IgE (Schroeder and Cavacini, 2010). It is central to the pathogenesis of many allergic diseases and is important in

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defence against parasitic diseases (Oliver *et al*, 2010; Stone *et al*, 2010). While IgE was classically considered important in allergic diseases, recent research has broadened its role as a potential biomarker involved in the pathogenesis of different diseases, including atherosclerosis, pulmonary hypertension, ischaemic reperfusion injury, male infertility, nociception, anxiety, Alzheimer's disease, auto-immune diseases, obesity and diabetes (Anand *et al*, 2012).

Alteration in serum and salivary levels of IgA and IgG (Sistig *et al*, 2002; Mohammad *et al*, 2013), and serum levels of IgD and IgE have been reported in RAS patients (Scully *et al*, 1983), and elevated serum IgE levels have been reported in Behçet's disease (Onat *et al*, 2007).

With increasing evidence supporting an association between elevated serum IgE levels and dermatologic, immune-mediated conditions, as well as stress and anxiety, an analysis of the association between IgE levels and clinical and demographic parameters associated with disease severity in patients with RAS is warranted. Consequently, the aim of the present study was to characterize demographic, clinical and serological parameters in RAS patients and to analyse their association with serum IgE levels.

Subjects and methods

Utilization of human subject data followed the approved protocol and requirements of the Institutional Review Board. Medical records of 49 patients suffering from RAS who were referred to the Department of Oral Medicine, Tel-Hashomer, Israel between 1 January 2008 and 31 December 2009 and who met the inclusion criteria were analysed.

Inclusion criteria included patients aged 18–50 years with a history of regularly recurring oral ulcerations of at least two episodes of RAS in the prior 6 months. Exclusion criteria included patients with HIV, hepatitis B or C, oral mucosal diseases including lichen planus, pemphigus vulgaris and mucous membrane pemphigoid, patients who chronically use medications associated with oral ulcerations and patients who were pregnant.

Recurrent aphthous stomatitis was evaluated according to a detailed questionnaire followed by relevant laboratory tests. Patients were interviewed in Hebrew at the first visit before medications were prescribed. The resultant data were recorded on a standard intake form. The questionnaire included demographic details (age, gender, education, country of origin and country of birth), smoking habits, familial history of RAS (at least one-first-degree relative) and age of onset of RAS.

The variables were defined as follows:

Age: below or above 21; gender: male or female; education: less or more than 12 years of schooling; Country of birth: Israel, 'Western' (North and South America, Western Europe, Australia, New Zealand), Africa, Asia, former Soviet Union (FSU). Country of origin: combined from the origin of each subject's father and mother according to the previous birth category.

Origin was further categorized as: Caucasian, including Western countries and the FSU; non-Caucasian, including subjects with at least one parent who originated from Africa and Asia.

History and course of RAS episodes were evaluated by four parameters: mean duration of RAS episodes (weeks), mean number of aphthae in one episode, mean number of RAS episodes in the last year and visual analogue scale (VAS) for pain assessment (0–10 scale). In addition, aphtha sites were mapped according to patients' self-assessment as well as according to clinical examination.

Basic self-assessment of stress included two questions: 1. Were you under stress in the last year? 2. Do you consider yourself a stress-prone person?

Patients were questioned about RAS-related diseases including:

- Inflammatory bowel disease or gluten-sensitive enteropathy with four questions: 1. Do you suffer from gastrointestinal diseases? 2. Do you suffer from chronic diarrhoea? 3. Do you suffer from abdominal pain? 4. Do you have bloody stools?
- Behçet's disease – according to the criteria of the International Study Group for Behçet's Disease's (1990).
- History of allergy.

Laboratory tests included: complete blood count with differential, folic acid, vitamin B12, ferritin, C reactive protein (CRP) and serum total IgE.

Statistical analysis

Numerical variables were presented as means and standard deviations, while categorized variables were presented as frequencies and percentages. A Pearson's correlation coefficient method was used. Differences between variables were subjected to univariate analysis by Pearson's chi-square test, and odds ratios (OR) and 95% confidence intervals (CI) were calculated. The statistical processing was by SPSS for Windows (version 19.0, SPSS Inc., Chicago, IL, USA) software. Statistical level of significance was chosen as $P < 0.05$.

Results

Demographic features

Distribution by gender, age, education, country of origin and country of birth is presented in Figure 1. Age ranged from 19 to 43 years with a mean age of 22.7 ± 5.92 years and median of 21 years. Most of the study population consisted of male patients aged 18–21 years with ≤ 12 years of education, native Israelis of non-Caucasian origin.

Male gender was positively associated with aphtha duration of over 3 weeks (Table 1). Non-Caucasian origin was positively associated with more than three aphthae per episode, with positive familial history of RAS and with a VAS score > 5 (Table 1).

Clinical features of RAS

History and course of RAS episodes in the study population are described in Tables 1 and 2 and Figures 2 and 3.

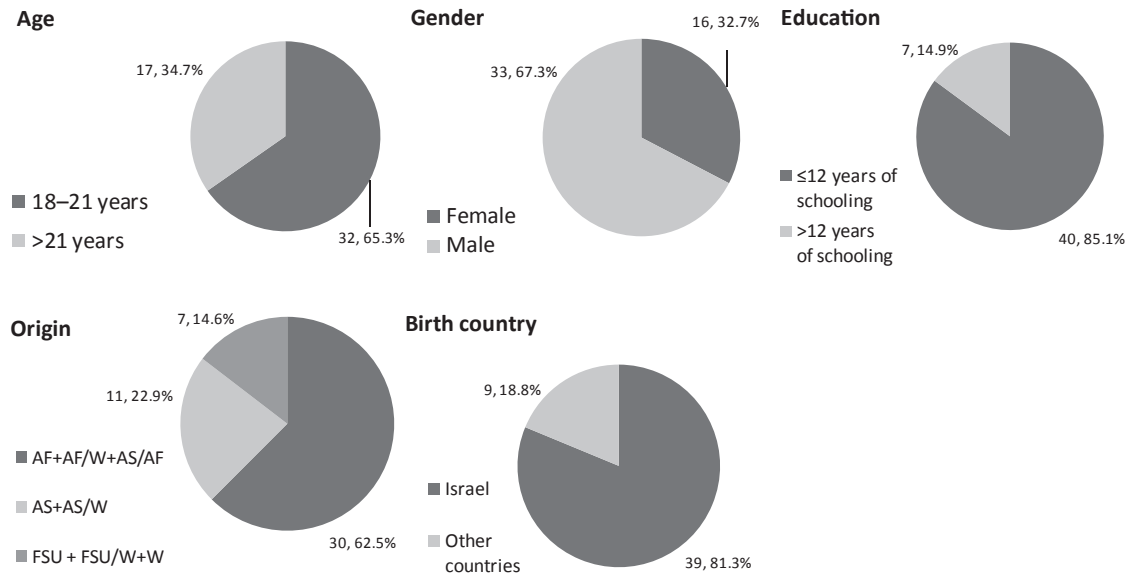


Figure 1 Characteristics of the study population according to age, gender, education, origin and birth country. AF, Africa; W, West; AS, Asia; FSU, former Soviet Union. Data are presented numerically and in percentage of patients

Table 1 The associations between various RAS-related parameters

	Variable positively associated	P	OR	95 % CI
Non-Caucasian origin	A familial history of RAS	0.035	8.12	1.01–73.84
	VAS > 5	0.02	1.86	1.40–2.48
A familial history of RAS	Young age at onset (5–10 years)	0.01	2.35	1.64–3.78
	Self-assessment as a stressful person ^a	0.037	4.05	1.06–15.48
	Reported deterioration in the previous year ^a	0.016	5.26	1.29–21.35
	Smoking ^a	0.047	1.54	1.21–1.95
	Male gender ^b	0.032	1.73	1.31–2.27
Longer duration of aphthae	A familial history of RAS ^a	0.019	6.43	1.20–34.41
	Stress in the last year	0.023	4.32	1.18–15.83
	Smoking	0.040	4.72	1.00–22.38
	Non-Caucasian origin	0.028	1.32	1.07–1.62

RAS, recurrent aphthous stomatitis; OR, odds ratios; CI, confidence intervals.

^aAphthae duration of over 2 weeks.

^bAphthae duration of over 3 weeks.

Table 2 Course of RAS episodes in the study population

	N	Mean ± s.d., Median, Range
No. of aphtha episodes in the last year	47	19.5 ± 11.3, 20, 4–40
No. of aphthae in one episode	48	2.4 ± 1.4, 2, 1–6
Mean duration of aphtha episode (weeks)	45	2.1 ± 1, 2, 1–4
VAS	23	6 ± 2.5, 6, 0–10
Mean age at onset (years)	49	12.6 ± 5.7, 12, 5–34

VAS, visual analogue scale; s.d., standard deviation; RAS, recurrent aphthous stomatitis.

The average age at onset was 12.6 years, with 36.7% and 73.5% of the individuals younger than 10 years and 15 years of age at onset, respectively.

A familial history of RAS was reported by almost half of the patients (Figure 3) with a positive association with young age at onset (5–10 years) and with aphthae duration of over 2 weeks (Table 1).

Stress in the last year was reported by a little over half of the study population (Figure 3). Of the 16 patients who considered themselves stress-prone, 11 patients (68.8%) also responded positively to having been under recent stress. Moreover, stress in the last year was positively associated with more than three aphthae in a single episode (Table 1). Patients who considered themselves stress-prone were positively associated with aphthae duration of over 2 weeks (Table 1).

Reported deterioration in the previous year of RAS episodes was positively associated with RAS episode duration of over 2 weeks (Table 1). These patients attributed the deterioration to stress (eight patients, 27.5%), decrease in oral hygiene (two patients, 6.9%), orthodontic treatment (two patients, 6.9%), change in diet (two patients, 6.9%), smoking cessation (one patient, 3.4%) and unknown aetiology (18 patients, 62%). Some of the patients reported more than one aetiology. Increased severity in the last year due to stress was positively associated with self-reported stress in the last year ($P = 0.001$).

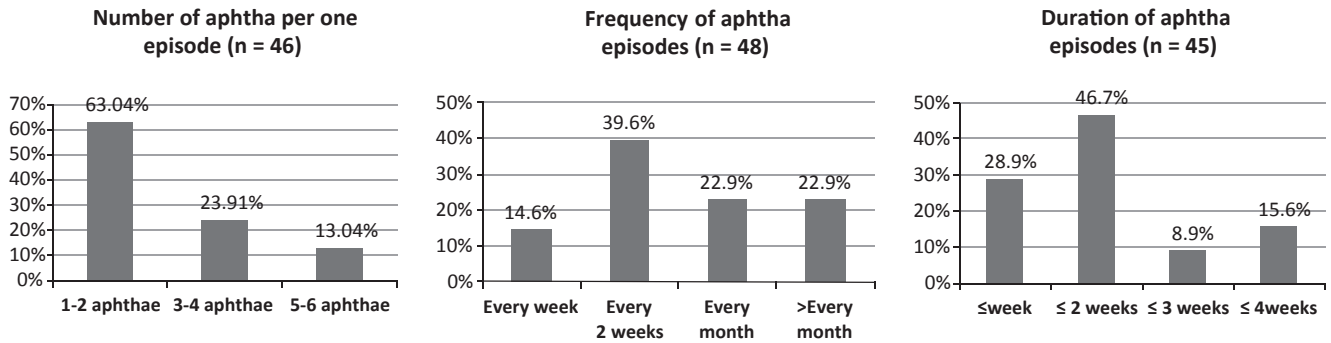


Figure 2 Severity of RAS episodes in the study population

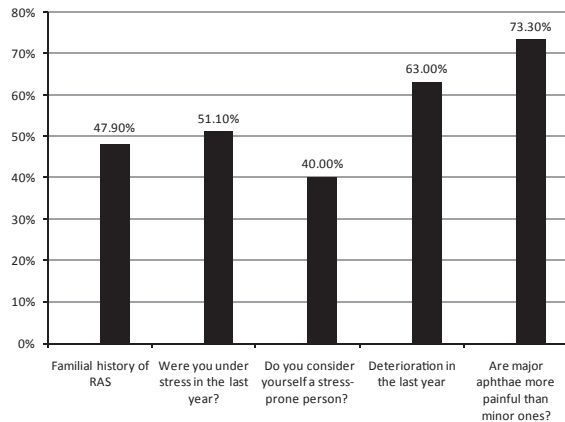


Figure 3 History of RAS episodes in the study population

The mean duration of the RAS episode was longer in men than in women ($P = 0.005$) and was positively correlated with the number of aphthae found in the clinical examination ($P = 0.036$, Pearson's correlation = 0.321). The mean VAS score was 6. VAS score was negatively correlated with age ($P = 0.018$, Pearson's correlation = -0.392) and positively correlated with the number of aphthae observed in the clinical examination ($P = 0.026$, Pearson's correlation = 0.383).

Minor aphthae were present in 71.4% of the study population (35 patients), a major aphthae was found in one patient (2.5%), major and minor aphthae were found simultaneously in 10.2% of the population (five patients), while nine patients (18.3%) presented without aphthae on the day of the clinical examination. Thirty-three patients (73.3%) answered positively to the question 'Are larger aphthae more painful than smaller ones?' (Figure 3). According to the patients, the most common ulceration sites in the oral cavity were the labial mucosa (71.4%, 25 patients) followed by the tongue (51.4%, 18 patients), buccal mucosa (48.6%, 17 patients) and mouth floor (20%, seven patients). The measure of agreement by Lambda score between the distribution of aphthae according to the clinical examination and the subjective assessment by the patient was below 0.6.

Medical history

The medical history of the study population is presented in Figure 4. Relevant medical history, as reported by

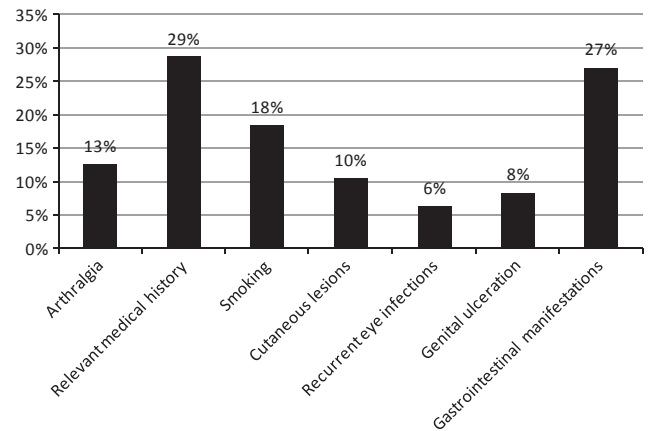


Figure 4 Medical history in the study population. Gastrointestinal manifestations: one positive answer to one of the four questions presented in Subjects and methods

patients in the health questionnaire, included: underweight (body mass index ≤ 18.5) (two patients, 4.1%), anaemia (two patients, 4.1%), history of allergy (eight patients, 16.3%) and gastro-oesophageal reflux disease (two patients, 4.1%). History of allergy as reported by the patients included allergy to dust (four patients), plant bloom (three patients), penicillin (two patients), cats (one patient) and guava (one patient). Some of the patients reported multiple allergens. No correlation was found between allergy history and IgE levels $>100 \text{ IU ml}^{-1}$ ($P = 1.000$) as well as IgE levels $>120 \text{ IU ml}^{-1}$ ($P = 0.785$) and $>150 \text{ IU ml}^{-1}$ ($P = 0.816$). Other medical conditions reported by the patients included: migraine (five patients, 10.2%), asthma (four patients, 8.1%) and hypothyroidism (two patients, 4.0%). No correlation was found between asthma and abnormal IgE levels $>100 \text{ IU ml}^{-1}$ ($P = 0.287$) as well as high levels of IgE: $>120 \text{ IU ml}^{-1}$ ($P = 0.562$) and $>150 \text{ IU ml}^{-1}$ ($P = 0.837$).

Smoking was positively associated with aphthae duration of over 2 weeks ($P = 0.047$, OR = 1.54, CI 1.21–1.95) and with over three aphthae in a single RAS episode ($P = 0.040$, OR = 4.72, CI 1.00–22.38).

Similar characteristics of RAS in the study population and Behçet's disease as reported in the literature are presented in Table 3. According to the inclusion criteria,

Table 3 Similar characteristics of Behçet's disease and RAS in the study population

Features	Incidence in Behçet's disease ^a (%)	Incidence in RAS study group (%)
RAS	100	100
Gastrointestinal manifestations	3–26	27.1
Arthralgia	45–60	12.5
Eye involvement	30–70	6.3
Cutaneous lesions	38–99	10.4
Genital ulceration	57–93	8.3
Non-Caucasian origin	Very common	81.2
Anaemia	Uncommon	4.1
Ferritin	Uncommon	6.5
Vitamin B12 deficiency	Uncommon	4.4
Folic acid deficiency	Uncommon	6.5
Elevated CRP	Very common	10.9

CRP, C reactive protein; RAS, recurrent aphthous stomatitis.
aAccording to Mendes *et al* (2009).

Table 4 Haematological deficiencies in the study population

Variable	Normal values	N (%)
HGB	Female-12–16 (g dl ⁻¹) Male- 14–18 (g dl ⁻¹)	Low 2 (4.1) Normal 46 (95.8)
MCV	80–97 (fl)	Low 3 (6.25) Normal 45 (93.7)
MCH	27–31 (pg)	Low 4 (8.3) Normal 44 (91.6)
Vitamin B12 deficiency	190–900 (ng ml ⁻¹)	Low 2 (4.4) Normal 43 (95.6)
Folic acid deficiency	3.1–20.5 (ng ml ⁻¹)	Low 3 (6.5) Normal 43 (93.4)
Ferritin	28–365 (ng ml ⁻¹)	Low 3 (6.5) Normal 43 (93.4)

HGB, haemoglobin; MCH, mean cell haemoglobin; MCV, mean corpuscular volume.

none of the patients fulfilled the criteria of the International Study Group for Behçet's Disease's (1990).

Haematological analysis

Haematological deficiencies and normal laboratory values are presented in Table 4. Overall, nine patients (five female, four male) with RAS (18.7%) showed at least one abnormal value for the following: haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin

Table 5 Total serum IgE levels according to gender in the study population

	Total serum IgE levels according to gender			
	Female	Male	Total	P-value
Number of patients	11 (32.4%)	23 (67.6%)	34 (100%)	
Total IgE level range (IU ml ⁻¹)	3.54–546	1–1287	1–1287	
Average IgE level (IU ml ⁻¹)	198.8	220.6	213.55	0.848
IgE level > 100 IU ml ⁻¹	9 (81.8%)	8 (34.7%)	17 (50%)	0.010
IgE level > 120 U ml ⁻¹	7 (63.6%)	6 (26.1%)	13 (38.2%)	0.035
IgE level > 150 IU ml ⁻¹	5 (45.5%)	5 (21.7%)	10 (29.4%)	0.156

The values in bold are the values that were statistically significant ($P < 0.05$).

IU ml⁻¹, international units (IU) per millilitre (ml); IgE, immunoglobulin E.

(MCH), vitamin B12, folic acid and ferritin. Of the nine patients with haematological deficiencies, one was deficient in ferritin, one was deficient in folic acid, one was deficient in vitamin B12 and the remaining six patients had combined deficiencies (one had haemoglobin and ferritin deficiency; one had MCV and MCH deficiency; one had MCV, MCH and ferritin deficiency; one had haemoglobin, MCV and MCH deficiency; one had MCH and folic acid deficiency; one had vitamin B12 and folic acid deficiency).

Of 46 subjects, CRP levels above 5.0 mg l⁻¹ were found in five patients (10.9%) and above 3.0 mg l⁻¹ in 11 patients (23.9%). CRP levels above 5.0 mg l⁻¹ occurred more often in females in the study group than males ($P = 0.017$).

Serum IgE levels according to gender in the study population are presented in Table 5. Average IgE levels were 213.55 IU ml⁻¹, median 100.25, s.d. 303.97 and range 1–1287 IU ml⁻¹, with 50% of RAS patients showing higher IgE levels than the normal values reported in the literature (0–100 IU ml⁻¹) (Stone *et al*, 2010; Dullaers *et al*, 2012). We also analysed the associations with the various clinical parameters not only for levels above 100 IU ml⁻¹, but also for serum IgE levels of above 120 IU ml⁻¹ and 150 IU ml⁻¹, thereby excluding 'borderline' antibody elevations (Tables 5 and 6; Figures 5 and 6).

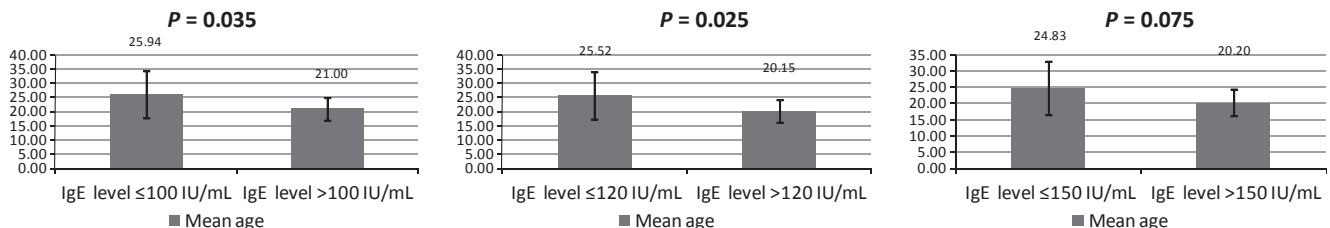


Figure 5 Total serum IgE levels according to age. Among RAS patients with IgE levels >100 U ml⁻¹, mean age was 21.00 ± 4.077 years and with IgE levels <100 U ml⁻¹, mean age was 25.94 ± 8.318 years ($P = 0.035$). Among RAS patients with IgE levels >120 U ml⁻¹, mean age was 20.15 ± 1.46 years and with IgE levels <120 U ml⁻¹, mean age was 25.52 ± 8.134 years ($P = 0.025$). Among RAS patients with IgE levels >150 U ml⁻¹, mean age was 20.20 ± 1.619 years and with IgE levels <150 U ml⁻¹, mean age was 24.83 ± 7.817 years ($P = 0.075$).

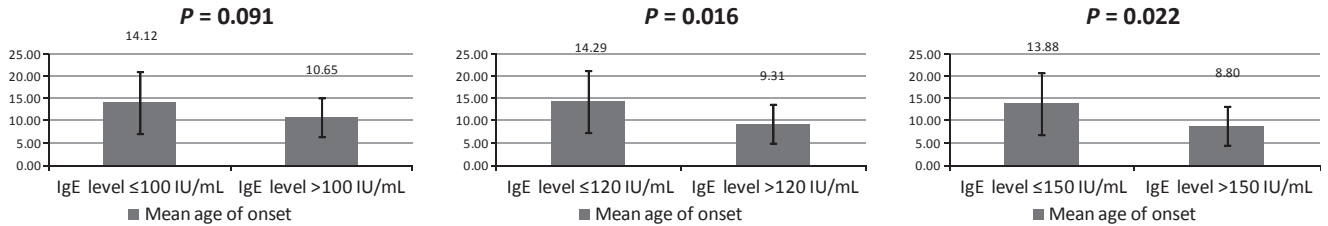


Figure 6 Total serum IgE levels according to age of onset of RAS episodes. Among RAS patients with IgE levels $>100 \text{ U ml}^{-1}$, mean age of onset of RAS episodes was 10.65 ± 4.35 years and with IgE levels $<100 \text{ U ml}^{-1}$, mean age was 14.12 ± 6.96 years ($P = 0.091$). Among RAS patients with IgE levels $>120 \text{ U ml}^{-1}$, mean age of onset of RAS episodes was 9.31 ± 4.04 years and with IgE levels $<120 \text{ U ml}^{-1}$, mean age was 14.29 ± 6.27 years ($P = 0.016$). Among RAS patients with IgE levels $>150 \text{ U ml}^{-1}$, mean age of onset of RAS episodes was 8.80 ± 2.57 years and with IgE levels $<150 \text{ U ml}^{-1}$, mean age was 13.88 ± 6.39 years ($P = 0.022$)

Table 6 Total serum IgE levels according to education, RAS frequency and CRP levels

		IgE level $>100 \text{ IU ml}^{-1}$		IgE level $> 120 \text{ IU ml}^{-1}$		IgE level $> 150 \text{ IU ml}^{-1}$	
		N (%)	P	N (%)	P	N (%)	P
Education (y)	≤ 12	16 (59.3)	0.034	13 (48.1)	0.019	10 (37)	0.055
	> 12	1 (14.3)		0 (0)		0 (0)	
RAS episodes every 2 weeks	No	7 (41.2)	0.221	5 (29.4)	0.226	2 (11.8)	0.017
	Yes	10 (62.5)		8 (50)		8 (50)	
CRP levels	High	4 (100)	0.003	3 (75)	0.098	3 (75)	0.044
	Normal	12 (42.9)		9 (32.1)		7 (25)	

The values in bold are the values that were statistically significant ($P < 0.05$).

y, years; CRP, C reactive protein; RAS, recurrent aphthous stomatitis; IgE, immunoglobulin E.

When IgE levels of $>100 \text{ IU ml}^{-1}$ were considered an interactive variable with all other variables, we found a significant positive association with female gender ($P = 0.010$) (Table 5), ≤ 12 years of schooling ($P = 0.034$) (Table 6), CRP levels $>5.0 \text{ mg l}^{-1}$ ($P = 0.003$) (Table 6) and younger age ($P = 0.035$) (Figure 5). When IgE levels of $>120 \text{ IU ml}^{-1}$ were considered an interactive variable with all other variables, we found a significant positive association with female gender ($P = 0.035$) (Table 5), ≤ 12 years of schooling ($P = 0.019$) (Table 6), younger age ($P = 0.025$) (Figure 5) and early onset of RAS episodes ($P = 0.016$) (Figure 6). Abnormal IgE levels ($>150 \text{ IU ml}^{-1}$) were positively associated with RAS episode frequency of every 2 weeks ($P = 0.017$) (Table 6), CRP levels $>5.0 \text{ mg l}^{-1}$ ($P = 0.044$) (Table 6) and early onset of RAS episodes ($P = 0.022$) (Figure 6).

Age at onset was negatively correlated with IgE levels as a continuous parameter ($P = 0.048$, Pearson's correlation = -0.341). Moreover, when IgE levels were dichotomized by 150 IU ml^{-1} and adjusted for positive familial history of RAS, age at onset was positively associated with elevated IgE levels ($P = 0.016$, OR = 0.751, CI 0.60–0.95). IgE levels were analysed relative to the other clinical and serological parameters examined in this study. No statistically significant associations were found ($P > 0.05$).

Multivariable logistic regression analysis for each IgE level (100 120 and 150 IU ml^{-1}) revealed that only early onset of RAS episodes remains a significant parameter associated with IgE levels $>120 \text{ IU ml}^{-1}$ ($P = 0.029$) and levels

$>150 \text{ IU ml}^{-1}$ ($P = 0.020$). None of the various parameters remained significant at IgE levels $>100 \text{ IU ml}^{-1}$.

Discussion

Recurrent aphthous stomatitis is a common oral disease that decreases patient's quality of life, but has no clear etiology. In this study, we analysed associations between various clinical parameters and total serum IgE levels in RAS patients.

Most cases were among young males (67.3%, 22.7 ± 5.92 years), consistent with previous studies (Ship *et al*, 2000; Chattopadhyay and Chatterjee, 2007; McCullough *et al*, 2007), and male gender was positively associated with longer duration of RAS episodes. In our study, 81.2% of the individuals were of non-Caucasian origin, which is 2.5-fold, their ratio in the general Israeli population corresponding to 28.7% in the age range from 15 to 39 years (<http://www.cbs.gov.il>). Moreover, non-Caucasian origin was positively associated with familial history of RAS, increased number of aphthae per episode and higher VAS score, supporting the notion of a hereditary component in RAS aetiology and severity. In addition, we found a positive family history association of RAS with young age of onset and longer duration of episodes as previously reported (Ship, 1972; Scully and Porter, 2008; Compilato *et al*, 2010).

Smoking was reported by 18.4% of the study population, which is lower than the proportion of smokers in the general population in Israel (24.3% with 29% of males

and 21% of females above 18 years of age) according to a report from the Israeli Ministry of Health for 2002–2003 (<http://www.old.health.gov.il/download/pages/isun2003.pdf>). Smoking was positively associated with longer duration of aphtha and increased number of aphthae per episode. Conversely, several studies have revealed a negative association between smoking and RAS, reporting that smokers experience an increase in mouth ulcers upon smoking cessation (Marakoğlu *et al*, 2007; Preeti *et al*, 2011).

Stress in the previous year was reported by 51.1% of our study population and was positively associated with more than three aphthae in a single episode. Moreover, 40% of the patients considered themselves stress-prone, and this was associated with aphtha duration of over 2 weeks. These findings suggest an association between stress and RAS severity, which is supported in the literature reporting that stresses – stemming from anxiety, job-related stress, a stressful life, emotional, physical, social and cognitive stresses – are related to RAS aetiology (Miller and Ship, 1977; McCullough *et al*, 2007; Albanidou-Farmaki *et al*, 2008; Gallo *et al*, 2009; Huling *et al*, 2012; Picek *et al*, 2012).

The most common sites of aphthae were the labial mucosa (71.4%), the tongue (51.4%), the buccal mucosa (48.6%) and the mouth floor (20%), as previously reported (McCullough *et al*, 2007).

Haematological deficiencies were found in 18.7% corresponding to previous reports (Jurge *et al*, 2006; Baccaglini *et al*, 2011). Interestingly, vitamin supplementation in some reports led to a decrease in aphtha severity (Carrozzo, 2009; Volkov *et al*, 2009), whereas no such reduction was found in others (Lalla *et al*, 2012). Natah *et al* (2004) found up to three times more ferritin deficiency in RAS subjects compared to healthy ones. In another study, 14% RAS patients were anaemic and 37.8% showed haematinic deficiencies (Burgan *et al*, 2006). The overall frequency of haematinic deficiencies in another study was higher: 56.2% in RAS patients vs 7% in controls (Compilato *et al*, 2010). Preeti *et al* (2011) attributed the contradictory findings in various studies to the association of haematinic deficiency and RAS to varying genetic backgrounds and dietary habits of the study population. In agreement with the literature, our findings support routine haematological screening of all patients with RAS to treat any underlying nutritional deficiency.

We believe that the most intriguing finding in our study was the positive association of increased IgE levels with RAS. Up to our knowledge, the present study is the first to analyse associations of elevated serum IgE with disease characteristics in RAS. Scully *et al* reported that IgE and IgD concentrations were substantially greater in patients with RAS than either in patients with other ulcerative oral disorders or the control subjects (Scully *et al*, 1983). Furthermore, serum IgD and IgE concentrations but not IgA, IgG or IgM were significantly greater in RAS than in controls (Scully *et al*, 1982).

Based on extensive literature from healthy population in the US (Gergen *et al*, 2009), Europe (Burney *et al*, 1997) and Israel (Hetman *et al*, 1988), normal IgE serum levels were set to 0–100 IU ml⁻¹. It should be noted that IgE expression is tightly regulated in the absence of disease

(Stone *et al*, 2010). Interestingly, in the present study, average IgE levels were 213.55 IU ml⁻¹, with 50% of the patients presenting with IgE levels >100 IU ml⁻¹, 38.2% with >120 IU ml⁻¹ and 29.4% with >150 IU ml⁻¹. Moreover, we also analysed the associations with the various clinical parameters not only for levels above 100 IU ml⁻¹, but also for serum IgE levels of above 120 IU ml⁻¹ and 150 IU ml⁻¹, thereby excluding ‘borderline’ antibody elevations. Multivariable analysis was performed and taken into account the influence of demographics, allergy and asthma history and RAS severity on serum IgE levels.

We found a positive association between increased IgE levels and younger age, ≤12 years of schooling, female gender, RAS episodes every 2 weeks, early onset of RAS episodes and CRP levels >5.0 mg l⁻¹. The positive association of increased IgE levels with younger age is expected as IgE levels increase gradually throughout childhood, peaking at 10–15 years of age (Stone *et al*, 2010). The positive association between increased IgE levels and ≤12 years of schooling may be related to the association with younger age.

Interestingly, in contrast to more males suffering from RAS, females with RAS showed a positive association with IgE level. This finding is supported by data which indicate a role for female oestradiol and progesterone in mast cell activity (Zierau *et al*, 2012).

Another finding was the positive association between frequent RAS episodes (every 2 weeks) and elevated IgE levels. Similarly, patients with chronic urticaria exhibit elevated serum IgE levels associated with disease severity and duration (Kessel *et al*, 2010). Consequently, it was suggested that IgE levels may serve as a marker to evaluate chronic urticaria, thereby potentially affecting patient management (Kessel *et al*, 2010).

High CRP levels (>5.0 mg l⁻¹) showed a significant positive association with increased IgE levels in RAS patients. The positive association between CRP and IgE levels supports the involvement of IgE in the inflammatory process. In agreement with the present study, Wang *et al* (2011b) found that interactions between CRP and IgE increased the risk of developing prediabetes and diabetes mellitus, even after adjustment for common diabetes risk factors, suggesting that IgE, like CRP, might serve as a biomarker of inflammation.

No association was found between abnormal IgE levels and history of allergies nor with asthma. In fact, recent research has revealed a broader pathophysiological role for IgE in diverse diseases, including metabolic diseases, auto-immune diseases, nociception and anxiety (Kessel *et al*, 2010; Parks *et al*, 2010; Shahzad *et al*, 2010; Wang *et al*, 2011a,b; Anand *et al*, 2012; Messingham *et al*, 2012; Pullen *et al*, 2012).

IgE functions through its FCεRI receptors on mast cells. Due to the synthesis and release of diverse types of inflammatory mediators, mast cells may produce pathophysiological changes in various organs, leading to the development of different diseases, as described above (Anand *et al*, 2012).

Relatively few studies have documented alteration of serum or salivary levels of Ig subtypes, for example, IgA, IgD, IgG and IgE, in RAS patients (Scully *et al*, 1983;

Brozović *et al*, 2001; Sistig *et al*, 2002; Mohammad *et al*, 2013; Saluja *et al*, 2012).

Bays *et al* (1977) identified IgE-bearing lymphocytes in the RAS lesions and peripheral blood of their patients. Natah *et al* (1998) showed an increase in mast cell number in a typical topographical pattern from RAS biopsies with signs of activation/de-granulation, suggesting active involvement of mast cells in RAS pathogenesis.

Several features of our study group resemble those of Behçet's disease. A recent systematic review concluded that although complex aphthosis is not a mild form of Behçet's disease, it is more likely to signify the subsequent development of full-blown disease (Baccaglini *et al*, 2011). Moreover, among 52 patients with Behçet's disease, 44% had high IgE levels ($>100 \text{ IU ml}^{-1}$), comparable to the average IgE levels in our study ($265.49 \pm 42.5 \text{ IU ml}^{-1}$ and $213.55 \pm 303.97 \text{ IU ml}^{-1}$, respectively) (Onat *et al*, 2007). This finding strengthens the similarity of Behçet's disease to RAS, and suggests that IgE level might be an independent diagnostic clue in Behçet's disease and RAS patients and may be involved in the unclear pathogenesis of both entities.

We discussed the strong association between RAS and stress. Mast cells and IgE have been previously shown to be involved in stress and anxiety. Stress may promote an increase in the inflammatory response, which is manifested as induction of mast cells resulting in the release of IgE to the skin and mucosa (Buske-Kirschbaum *et al*, 2002; Dhabhar, 2002).

Conclusions

This study analysed clinical, demographic and serological parameters in individuals suffering from RAS and supports common features of patients with RAS, such as non-Caucasian origin, male gender, young age of onset, familial history of RAS, stress and haematological deficiencies. Mutual characteristics were found between Behçet's disease and RAS in the study group. Elevated total serum IgE levels were observed in RAS patients, and when the different serum IgE levels were considered as an interaction variable with all other variables, we found a significant positive association with younger age, ≤ 12 years of schooling, female gender, RAS episodes every 2 weeks, early onset of RAS episodes and CRP levels $>5.0 \text{ mg l}^{-1}$.

These findings suggest that IgE level may be considered as part of the standard RAS patient work-up. More research is needed to identify biological mechanisms that account for the observed elevations in IgE levels as well as the association with clinical parameters.

Author contributions

G. Almozniño: designed study, analysed data, drafted paper. A. Zini: analysed data, drafted paper. Y. Mizrahi: designed study, analysed data. D.J. Aframian: designed study, analysed data, drafted paper.

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