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The association of tobacco and other factors with recurrent aphthous stomatitis in an US adult population

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OBJECTIVE: To determine point and annual prevalence of recurrent aphthous stomatitis (RAS).
SETTING: Reported prevalence of RAS in textbooks and much of the literature varies according to study location, patient selection and whether point prevalence (presence of lesions at examination) or period prevalence (history of lesions during a specified period) is reported. Many studies are based on non-probability samples and this may contribute to significant variation in reported prevalence and factors presumed to be associated with RAS.
METHODS: We analyzed data from the Third National Health and Nutrition Examination Survey, 1988–1994, a large United States probability sample, for RAS and covariates suggested in the literature using bivariate and multivariate logistic regression.
RESULTS: Oral mucosal examinations were performed on 17 235 adults 17 years and older. Of these, 146 (0.89%) had at least one clinically apparent aphthous lesion. For annual (reported) prevalence, Whites (20.87%) and Mexican-Americans (12.88%) had several fold higher prevalence of RAS than Blacks (4.96%). Adults younger than 40 years of age had almost twice the prevalence (22.54%) of those older than 40 years (13.42%).
CONCLUSION: Annual prevalence was significantly higher in whites and Mexican-Americans (compared with blacks), individuals 17–39 years of age, cigarette non-smokers, and those with recurrent herpes labialis history; while it was lower in males. Point prevalence was significantly higher in whites, Mexican-American, individuals 17–39 years of age, cigarette non-smokers, and males.
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Introduction

Recurrent aphthous ulcerative lesions are a frequent oral finding and have been described as the most common oral mucosal disease in America (Rees and Binnie, 1996). Lesions occur only in non-keratinized mucosa and are usually painful, shallow round ulcers with an erythematous halo covered by a yellowish-gray fibromembranous layer. The condition may be present in children (Kleinman *et al*, 1994) and may recur two to four times a year (Rogers, 1997).

Recurrent aphthous ulcerations are known as recurrent aphthous stomatitis (RAS), canker sores, minor aphthous ulcers, major aphthous ulcers, Sutton's ulcers, periadenitis mucosa necrotica recurrens, herpetiform ulcers, simple aphthosis, complex aphthosis, and other names. Lesions can occur by themselves (RAS) or in combination with other conditions or as part of a multiorgan syndrome (Rogers, 1997; Scully *et al*, 2003). Other conditions involving multiple organs associated with RAS have been reviewed elsewhere (Rees and Binnie, 1996; Scully *et al*, 2003).

The etiology of RAS is not understood although stress, hormonal factors, trauma, microorganisms, food hypersensitivity, immune dysregulation, family history, and a genetic predisposition may be factors in some cases (Rogers, 1997; McCarty *et al*, 2003; Scully *et al*, 2003). Epithelial cell death, yielding ulceration, results from the activation of a cell-mediated immune response in which T lymphocytes and other cells produce Tumor Necrosis Factor Alpha (TNFα) in addition to other cytokines (Field and Allan, 2003). Complement deposition in subepithelial vessel walls (contributing to vasculitis) has been reported (Reimer *et al*, 1983). The immuno-pathogenesis in RAS has been reviewed recently (Eversole, 1997; Field and Allan, 2003). The clinical ulcerative period may last 2 weeks and lesions may heal without leaving a scar (Rogers, 1997).

Other factors may be involved in the development of RAS. Some reports implicate the inheritance of HLA-B51 antigen (Shohat-Zabarski *et al*, 1992; Chang *et al*, 2001), vitamin B12 deficiency (Palopoli and Waxman, 1990; Weusten and van de Wiel, 1998; Piskin *et al*,

2002), recurrent herpes labialis (Ship *et al*, 1967; Katz *et al*, 2001), *Helicobacter pylori* (Birek *et al*, 1999), hepatitis C (Dalekos *et al*, 1998) and hypersensitivity to nickel (Pacor *et al*, 2003) among others. There have been some reports that suggest that tobacco plays a preventive role in RAS (Axéll and Henricsson, 1985a; Bittoun, 1991; Grady *et al*, 1992; Tuzun *et al*, 2000; Ussher *et al*, 2003).

Reported prevalence of oral aphthous ulcerations in textbooks and much of the literature varies according to study location, patient selection and whether point prevalence (presence of lesions at examination) or period prevalence (history of lesions during a specified period) is reported. Moreover, many studies are based on non-probability samples that may also account for the significant variation in prevalence reported by different groups. For example, in a study in Malaysia (Zain, 2000), the point prevalence was 0.5% while Axéll (1976) reported 2% in Sweden. Recently, a study of 7297 consecutive patients, examined at a dental school clinic in central Mexico over a period of 7.5 years, reported a RAS point prevalence of 0.45% for both sexes. In this study, the sample sex distribution was approximately two-thirds female and the point prevalence was 0.51% for males and 0.42% for females (Diaz-Guzman and Castellanos, 1991). Reichart (Reichart, 2000) reported on a cross-sectional sample of 2022 Germans, group-1 35–44 years old from the former East Germany and group-2 65–74 years old from West Germany. RAS point prevalence (both sexes) was 1.4% at examination and 18.3% on a 2-year prevalence (2YP) history (we assume a 2-year prevalence since the author compares his findings to those of Axéll, 1976) for group-1, while for group-2 it was 1 and 6.9%, respectively. In reviewing the literature, we failed to find any reports based on a large representative adult population of the USA. Thus, the purpose of this study was to determine point and annual prevalence for an US adult population, and determine relationships between RAS and reported associated factors contained in the national dataset based on a probability sample.

Materials and methods

The Third National Health and Nutrition Examination Survey (NHANES III) is a periodic survey conducted by the National Center for Health Statistics that was conducted between 1988 and 1994. Participants from 19 528 randomly selected households received a health interview, laboratory analysis of blood and urine, standardized oral mucosal evaluations by dentists, and physical examinations performed by physicians. A detailed description of the overall methodology is presented in Drury *et al*, 1996. Oral mucosal examinations were performed based on the World Health Organization's Guide to Epidemiology and Diagnosis of Oral Mucosal Diseases and Conditions (Kramer *et al*, 1980). A detailed description of the mucosal examination is presented in National Center for Health Statistics, 1996. Two measures of RAS prevalence were used: point prevalence (PP) from the oral mucosal examination and annual (reported) prevalence (AP) established from responses to the question: '[Have you] Ever had canker sores in past 12 months?' Similarly, recurrent herpes labialis (RHL) history was established from responses to the question: '[Have you] Ever had cold sores in past 12 months?'

In addition to data from the oral mucosal examination and from the interview (history), socio-demographic data: sex, age, and race/ethnic group were used. The race/ethnic group variable tested included non-Hispanic White, non-Hispanic Black, and Mexican-American groups. For analyses using race-ethnicity, 702 subjects who were classified as 'other' were excluded.

Income was measured by the poverty income ratio (PIR) that relates family income to the poverty level based on the subject's family size (National Center for Health Statistics, 1993). It is computed by dividing the midpoint of the reported family income category by the Census Bureau's poverty threshold for the calendar year the family was interviewed. PIR was categorized as low (0–1.3), middle (≥1.3–3.5), and high (>3.5). Low income families had low PIR levels.

RAS associated factors reported in the literature that were available in the NHANES III database were tested, and for completeness additional variables were included to provide a more complete hematological picture. The nutritional-hematologic factors tested were: serum values of vitamin B12 (B12) (available for only half the subjects) and folate, red blood cell folate (RBCF), ferritin, iron, hemoglobin, hematocrit, and mean corpuscular volume (MCV). Because of the close relation to B12, folate values were included and categorized based on serum folate and RBCF levels, MCV, and hemoglobin (Table 1). For the analysis, folate balance was coded as positive, normal, and negative. The mean and standard error (s.e.) were computed and a *t*-test for significant differences in means of continuous nutritional-hematologic factors for subjects with and without RAS was performed. Wald chi-square tests for association were performed and the results are summarized by superscripts in the prevalence column.

Tobacco use (cigarette smoking, cigar smoking, pipe smoking) was tested and we combined snuff and chewing tobacco into the smokeless tobacco variable. The number of cigarettes smoked per day was categorized as none, 1–9, 10–19, and ≥20; and cotinine (a long-lived metabolite of nicotine) levels were included. Reported RAS associated diseases tested were: RHL, *H. pylori* and hepatitis C.

Since the NHANES III employed a complex sampling design, SAS-callable SUDAAN version 8.0.2 was used to adjust for the sampling effect. Stratum-specific prevalence for each level of the previously mentioned associated factors (covariates) was determined and bivariate logistic regression was performed. A multivariate regression model using forward selection was fitted starting with the covariate with the strongest statistically significant ($P < 0.20$) association in the bivariate regressions. Covariates were added one at a time, removing those that did not meet the $P < 0.05$

Table 1 Classification of folate status*

	Serum folate (ng mL^{-1})	*RBC folate (ng mL^{-1})*	*Hemoglobin*	*Mean corpuscular volume*
Positive balance				
Stage II (excess)	> 10	> 400		
Stage I (early positive)	> 10	> 300		
Normal	≤ 10 ≥ 3	≤ 300 ≥ 200		
Negative balance				
Depletion				
Stage I (early negative)	< 3	> 200		
Stage II (depletion)	< 3	< 160 ≥ 120		
Deficiency				
Stage III (damaged metabolism)	< 3	< 120 ≥ 100		
Stage IV – male (clinical damage)	< 3	< 100	< 13	> 95
Stage IV – female (clinical damage)	< 3	< 100	< 12	> 95

*Adapted from Mahan and Stump (1999).

retention criterion. Next, 2-way and 3-way interactions were added where relevant and those that met our retention criterion were retained. Odds ratios (OR) and adjusted odds ratios (AOR) that are statistically significantly different (*t*-test for regression coefficient ≠ 0) are boldfaced in all tables. Adjusted odds ratios adjust simultaneously for the effects of other covariates in the multivariate model.

Results

Our results are summarized in Tables 2–5 and are grouped in three sections dealing with point prevalence, annual prevalence, and multivariate models.

Point prevalence

Oral mucosal examinations were performed on 17 235 adults 17 years and older representing 182 293 000 non-institutionalized adults. Of these subjects, 146 (0.89%) had at least one clinically apparent aphthous lesion. Table 2 shows the sample size, number of lesions found, RAS PP and 95% confidence interval (CI), Wald chi-square, bivariate OR and 95% Taylor series CI. Boldfaced ORs are statistically significant (*t*-test for regression coefficient ≠ 0) at $P < 0.05$.

Males (1.13%) had almost twice the RAS PP of females (0.67%). RAS prevalence of Mexican-Americans (1.14%) and Whites (0.90%) was more than twice that of Blacks (0.40%). Whites (OR = 2.24) and Mexican-Americans (OR = 2.83) had greater odds of RAS than Blacks. Adults younger than 40 years of age had higher RAS PP (1.20%) than those older than 40 (0.59%) and more than twice the odds of RAS (OR = 2.07) than older individuals. The association between income level (PIR) and RAS was not statistically significant.

The associations of RAS PP with cigarette smoking and with cotinine levels were significant. Moderate and heavy smokers had lower RAS PP than light or non-smokers. Cigarette non-smokers (OR = 13.20) and smokers of < 1/2 pack per day (OR = 10.63) had far greater odds of RAS than individuals who smoked > 1 pack per day. No other tobacco use (pipe, cigar, smokeless) was significantly associated with RAS.

The association of RAS PP with folate balance was significant. Individuals in positive folate balance had more than twice the odds (OR = 2.73) of RAS than those with normal folate balance. Neither of the RAS associated microorganisms-conditions available in our dataset (RHL, *H. pylori*, and Hepatitis C) was found to be significant. In summary, RAS PP was associated with race-ethnicity, age, cigarettes smoked per day, serum cotinine levels, and folate balance.

Annual prevalence

Table 3 shows the sample size, number of lesions found, RAS AP and 95% CI, Wald chi-square test, bivariate OR and 95% Taylor series CI. Males (16.33%) had a lower RAS AP than females (19.33%). Whites (20.87%) and Mexican-Americans (12.88%) had several fold higher RAS AP than Blacks (4.96%). Adults younger than 40 years of age had almost twice the AP (22.54%) than those older than 40 (13.42%). Whites (OR = 5.06) and Mexican-Americans (OR = 2.84) had greater odds of RAS than Blacks; and Whites had almost twice the odds of RAS than Mexican-Americans.

Individuals younger than 40 years of age had greater odds of RAS (OR = 1.88) than older individuals. The ORs for middle (OR = 1.45) and for high income (OR = 1.81) were significantly different from low income, the referent. Significant (chi-square) AP associations were found for gender, race-ethnicity, and age. Non-smokers had more then twice the odds of RAS AP (OR = 2.43) than individuals who smoked a pack or more of cigarettes per day. Similarly, individuals with low cotinine levels had more than twice the odds RAS AP (OR = 2.14). The associations between RAS AP and pipe smoking, cigar smoking, and use of smokeless tobacco were not significant. Individuals in negative folate balance had lower odds (OR = 0.80) of RAS than those with normal folate balance.

All three RAS associated microorganisms-conditions available in our dataset (RHL, *H. pylori*, and Hepatitis C) were found to be significant for RAS AP. Subjects

Table 2 Point prevalence for aphthous stomatitis, 95% confidence limits, and odds ratio

	Sample size	*Lesions*	*Prevalence (%)*	*Confidence limits 95%*	*Odds Ratio*	*Confidence limits 95%*
Gender	17 215	146	0.89[a]	0.68, 1.10		
Male	8074	72	1.13	0.72, 1.53	1.68	0.99, 2.84
Female	9141	74	0.67	0.46, 0.89	1.00	
Race-ethnicity	16 533	139	**0.85**[d]	0.65, 1.06		
Non-Hispanic White	6902	60	0.90	0.66, 1.15	**2.24**	1.32, 3.80
Mexican-American	4742	58	1.14	0.77, 1.50	**2.83**	1.53, 5.23
Non-Hispanic Black	4889	21	0.40	0.20, 0.61	1.00	
Age (years)	17 235	146	**0.89**[d]	0.69, 1.09		
17–39	7785	96	1.20	0.86, 1.55	**2.07**	1.29, 3.09
≥40	9450	50	0.59	0.37, 0.80	1.00	
Income level	17 235	146	**0.89**[a]	0.69, 1.09		
Low	5284	48	0.80	0.47, 1.14	1.00	
Middle	6872	60	0.91	0.60, 1.22	1.13	0.59, 2.19
High	5079	38	0.91	0.47, 1.35	1.13	0.50, 2.54
Cigarettes per day	17 200	146	0.89[d]	0.69, 1.10		
None	14 330	136	1.05	0.80, 1.31	**13.20**	1.79, 97.55
1–9	1139	6	0.85	0.30, 1.40	**10.63**	1.26, 89.63
10–19	1114	2	0.04	−0.02, 0.10	0.50	0.04, 6.30
≥20	617	2	0.08	−0.08, 0.24	1.00	
Cotinine (ng ml^{-1})	16 172	135	**0.87**[d]	0.66, 1.07		
<25	11 708	116	0.99	0.73, 1.25	1.19	0.34, 4.22
25–121	1412	8	1.09	0.00, 2.18	1.31	0.40, 4.29
121–328	2119	6	0.24	0.04, 0.44	0.27	0.04, 1.94
≥328	933	5	0.83	−0.17, 1.83	1.00	
Pipe smoker	16 009	141	0.94[a]	0.71, 1.17		
Yes	122	1	2.06	−2.00, 6.10	2.25	0.29, 17.57
No	15 887	140	0.93	0.70, 1.15	1.00	
Cigar smoker	17 235	146	0.89[a]	0.69, 1.09		
Yes	250	3	2.50	−.91, 5.90	2.95	0.69, 12.64
No	16 985	143	0.86	0.66, 1.06	1.00	
Smokeless tobacco	16 966	142	0.84[a]	0.64, 1.03		
Yes	508	3	1.84	−.37, 4.05	2.31	0.61, 8.69
No	16 458	139	0.80	0.60, 1.00	1.00	
Folate status	15 081	128	0.89[b]	0.67, 1.11		
Positive	735	13	2.06	0.68, 3.44	**2.73**	1.17, 6.36
Normal	11 857	92	0.77	0.53, 1.00	1.00	
Negative	2489	23	1.03	0.39, 1.66	1.34	0.70, 2.60
RHL in past year	17 228	146	0.89[a]	0.69, 1.09		
Yes	2679	37	1.09	0.66, 1.51	1.29	0.77, 2.13
No	14 549	109	0.85	0.61, 1.08	1.00	
H. pylori	7542	65	0.92[a]	0.56, 1.28		
Positive	3678	28	0.76	0.21, 1.30	0.75	0.31, 1.83
Negative	3799	37	1.00	0.54, 1.47	1.00	
Hepatitis C	16 178	137	0.88[a]	0.67, 1.09		
Positive	376	3	1.55	−0.81, 3.90	1.79	0.34, 9.43
Negative	15 802	134	0.87	0.65, 1.09	1.00	

Wald chi-square test: [a]N.S.; [b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$.
[d]Could not be estimated.
Odds ratios: bold indicates regression coefficient significantly different from 0 (*t*-test).

serologically positive for all three infections had significantly different RAS AP than serologically negative subjects; with those who reported RHL in the past year having greater odds of RAS (OR = 2.87) and those seropositive for hepatitis C and *H. pylori* having lower odds of RAS. In summary, RAS AP was associated with gender, low income, race-ethnicity, age, serum cotinine levels, cigarettes smoked per day, folate balance, exposure to hepatitis C, and *H. pylori*, as well as having RHL in the past year.

PP and AP of nutritional and hematological continuous variables

In Table 4 the mean values of PP and AP for nutritional and hematological continuous variables, for subjects with and without RAS, are shown side by side for easy comparison as well as the mean, s.e. and the results of a *t*-test for significant differences in means of continuous covariates (associated factors). There was no significant difference in the mean values of the hematological variables between individuals with and without clinically apparent RAS lesions (PP). Also, that there were statistically significant differences only for serum ferritin, serum iron, and RBCF between individuals who did and did not report having a RAS lesion in the past year (AP). The sample size of clinically apparent lesions for PP was less than that for AP and accordingly, the s.e. associated with AP are smaller than those of the PP.

Table 3 Annual prevalence of aphthous stomatitis, 95% confidence limits, and odds ratio

	Sample size	*Positive history*	*Prevalence (%)*	*Confidence limits 95%*	*Odds ratio*	*Confidence limits 95%*
Gender	17 215	2054	**17.90**[c]	16.68, 19.12		
Male	8074	837	16.33	14.73, 17.92	**0.81**	0.71, 0.93
Female	9141	1217	19.33	17.82, 20.85	1.00	
Race-ethnicity	16 514	1967	18.48[d]	17.13, 19.82		
Non-Hispanic White	6895	1186	**20.87**	19.32, 22.41	**5.06**	4.11, 6.21
Mexican-American	4734	559	12.88	4.12, 5.80	**2.84**	2.22, 3.62
Non-Hispanic Black	4885	222	4.96	10.96, 14.81	1.00	
Age (years)	17 215	2054	**17.90**[d]	16.68, 19.12		
17–39	7778	1185	22.54	20.58, 24.51	**1.88**	1.64, 2.15
≥40	9437	869	13.42	12.30, 14.53	1.00	
Income level	17 215	2054	**17.90**[d]	16.68, 19.12		
Low	5275	471	12.67	10.90, 14.44	1.00	
Middle	6862	829	17.36	15.78, 18.94	**1.45**	1.18, 1.78
High	5078	754	20.84	18.83, 22.86	**1.81**	1.49, 2.22
Cigarettes per day	17 180	2051	**17.90**[d]	16,68, 19.12		
None	14 312	1841	20.12	18.71, 21.52	2.43	1.64, 3.58
1–9	1138	66	9.51	6.81, 12.20	1.03	0.59, 1.77
10–19	1113	91	10.26	7.89, 12.62	1.08	0.63, 1.84
≥20	617	53	9.98	6.61, 13.36	1.00	
Cotinine (ng ml^{-1})	16 156	1953	**18.05**[d]	16.79, 19.31		
<25	11 696	1618	21.23	19.75, 22.70	**2.14**	1.52, 3.01
25–121	1410	118	13.55	10.44, 16.65	1.25	0.75, 2.06
121–328	2118	150	9.39	7.47, 11.31	0.82	0.54, 1.26
≥328	932	67	11.17	7.81, 14.54	1.00	
Pipe smoker	15 991	1924	18.24[a]	16.91, 19.56		
Yes	122	11	16.40	7.90, 24.90	1.14	0.57, 2.26
No	15 869	1913	18.25	16.88, 19.63	1.00	
Cigar smoker	17 215	2054	17.90[a]	16.68, 19.12		
Yes	250	27	15.53	9.91, 21.14	1.19	0.73, 1.93
No	16 965	2027	17.95	16.66, 19.23	1.00	
Smokeless tobacco	16 947	1999	17.79[a]	16.54, 19.04		
Yes	507	52	18.66	13.49, 23.82	0.94	0.66, 1.36
No	16 440	1947	17.76	16.50, 19.02	1.00	
Folate status	15 065	1822	**18.29**[b]	17.03, 19.54		
Positive	734	109	20.44	15.93, 24.94	1.12	0.84, 1.49
Normal	11 848	1454	18.68	17.43, 19.93	1.00	
Negative	2483	259	15.47	12.89, 18.05	**0.80**	0.66, 0.96
RHL in past year	17 214	2054	**17.90**[d]	16.68, 19.12		
Yes	2675	762	32.82	30.39, 35.24	**2.87**	2.47, 3.34
No	14 539	1292	14.53	13.19, 15.88	1.00	
H. pylori	7530	908	**17.98**[d]	15.95, 20.01		
Positive	3699	312	12.20	9.79, 14.61	**0.52**	0.40, 0.69
Negative	3831	596	20.84	18.40, 23.28	1.00	
Hepatitis C	16 163	1954	**18.03**[c]	16.80, 19.27		
Positive	376	32	8.79	4.37, 13.21	**0.45**	0.25, 0.81
Negative	15 787	1922	18.23	16.97, 19.49	1.00	

Wald chi-square test: bold indicates statistical significance. [a]N.S.; [b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$.
Odds ratios: bold indicates regression coefficient significantly different from 0 (*t*-test).

Multivariate models

Table 5 shows the multivariate models for RAS PP and AP. Males had greater odds of having a lesion at examination (PP: AOR = 1.91) than females but lower odds of having a reported lesion in the past year (AP: AOR = 0.82) than females. Whites (PP: AOR = 2.60) and Mexican-Americans (PP: AOR = 2.31) had more than twice the odds of having an aphthous lesion than Blacks while Whites (AP: AOR = 5.49) and Mexican-Americans (AP: AOR = 2.25) had greater odds of having RAS within the past year than Blacks. Individuals below the age of 40 (PP: AOR = 2.16) had greater odds than older individuals while the AP analysis indicates that individuals below the age of 40 (AP: AOR = 2.35) had greater odds than older individuals. Cigarette non-smokers and light smokers (PP: AOR 14.96 and 9.56, respectively) had substantially greater odds of having an aphthous lesion than heavy smokers while cigarette non-smokers (AP: AOR 2.99) had substantially greater odds of having an aphthous lesion in the past year than heavy smokers. Recurrent herpes labialis history was not significantly associated with RAS PP but individuals reporting RHL had greater odds of having RAS in the past year (AP: AOR = 2.63). In summary, gender, race-ethnicity, age, cigarette smoking were significantly associated with RAS PP while gender, race-ethnicity, age, cigarette smoking, and RHL history were significantly associated with RAS AP.

Table 4 Significance testing of nutritional and hematological continuous variables associated to aphthous stomatitis

	RAS point prevalence				*RAS annual prevalence*			
	n	*Mean*	*SE*	P *(t = 0)*	n	*Mean*	*SE*	P *(t = 0)*
Serum ferritin (ng ml^{-1})	16 424	124.50	1.818		16 408	124.54	1.810	
RAS	139	117.31	12.259	0.555	1984	106.58	3.762	**<0.001**
No RAS	16 285	124.57	1.816		14 424	128.50	1.846	
Serum iron (μg dl^{-1})	16 450	91.42	0.677		16 433	91.42	0.677	
RAS	137	90.66	4.651	0.870	1989	95.14	1.380	**0.005**
No RAS	16 313	91.43	0.680		14 444	90.60	0.642	
MCV (fl)	16 420	89.86	0.138		16 403	89.85	0.138	0.089
RAS	140	89.65	0.424	0.602	1980	89.64	0.165	
No RAS	16 280	89.86	0.138		14 423	89.90	0.146	
Hemoglobin (g dl^{-1})	16 421	14.15	0.029		16 404	14.15	0.029	
RAS	140	14.30	0.174	0.392	1980	14.10	0.060	0.258
No RAS	16 281	14.15	0.029		14 424	14.16	0.027	
Vitamin B12 (pg ml^{-1})	8355	477.81	6.139		8354	477.90	6.136	
RAS	72	556.55	49.463	0.114	1016	465.54	7.347	0.053
No RAS	8283	477.13	6.123		7338	480.54	6.623	
Serum folate (ng ml^{-1})	16 429	6.84	0.161		16 413	6.84	0.162	
RAS	138	7.05	0.705	0.757	1984	7.19	0.243	0.053
No RAS	16 291	6.84	0.161		14 429	6.76	0.166	
RBC Folate (ng ml^{-1})	16 431	198.66	2.591		16 414	198.65	2.591	
RAS	140	185.90	11.875	0.284	1976	205.69	4.440	**0.029**
No RAS	16 291	198.77	2.599		14 438	197.10	2.551	
Hematocrit (%)	16 419	41.89	0.080		16 402	41.89	0.080	
RAS	140	42.52	0.470	0.178	1980	41.68	0.151	0.067
No RAS	16 279	41.89	0.080		14 422	41.94	0.078	

Bold indicates statistical significance.

Table 5 Multivariate logistic regression for aphthous stomatitis

	Point prevalence		*Annual prevalence*	
Independent variable	*Adjusted odds ratio*	*Confidence limits 95%*	*Adjusted odds ratio*	*Confidence limits 95%*
Gender				
Male	**1.91**	1.06, 3.42	**0.82**	0.70, 0.95
Female	1.00		1.00	
Race/ethnicity				
Non-Hispanic White	**2.60**	1.55, 4.37	**5.49**	4.41, 6.82
Mexican-American	**2.31**	1.27, 4.22	**2.25**	1.75, 2.87
Non-Hispanic Black	1.00		1.00	
Age				
17–39 years	**2.16**	1.32, 3.54	**2.35**	2.04, 2.72
40+ years	1.00		1.00	
Cigarettes per day				
None	**14.96**	1.94, 115.60	**2.99**	1.97, 4.55
1–9	**9.56**	1.11, 82.62	1.11	0.64, 1.92
10–19	0.53	0.047, 6.56	1.09	0.62, 1.91
≥20	1.00		1.00	
RHL history				
Yes			**2.63**	2.27, 3.05
No			1.00	

Bold indicates statistical significance ($P < 0.05$).

Discussion

General remarks

Point prevalence is a reliable measure of disease present at examination but with episodic conditions, like RAS however it ignores events that have occurred outside the time of examination (Kleinman *et al*, 1991). On the other hand, AP is somewhat riskier since the patient may not have been aware of having the condition; may confuse the condition being studied with a similar condition; or may ascribe it to the wrong time period. Moreover, in NHANES III, laboratory analyte values were measured at the time of the examination, and were contemporaneous with the oral mucosal examination but were generally not contemporaneous with lesions that accrued over the past year. While there is no perfect prevalence measure, we feel that point prevalence is best.

When a condition with low prevalence is studied, relatively few cases are found. This makes multivariate analysis problematic since as the number of covariates

increases, the number of subjects in a stratum decreases, and concomitantly the s.e. increases. Consequently, the literature contains few multivariate studies of RAS PP. This is a critical deficiency since often restricting analysis to bivariate relationships ignores the possibility that 'a collection of variables, each of which is weakly associated with the outcome, can become an important predictor of the outcome when all are taken together' (Hosmer and Lemeshow, 1989). Moreover, many bivariate relationships that are statistically significant explain the same variation in the outcome as other covariates that are also statistically significant. Multivariate regression allows for simultaneous comparison of all variables involved and thus represents a more comprehensive analysis. Several variables found to be significant in bivariate analyses for PP (poverty level) and AP (ferritin, iron, RBC folate, poverty level, cotinine, folate balance, RHL history, *H. pylori*, and hepatitis C seropositivity) were not found to be significant in the multivariate model.

Nutritional related factors like folate, RBCF, vitamin B12, ferritin, and iron have been associated with RAS (Olson *et al*, 1982; Piskin *et al*, 2002; Thongprasom *et al*, 2002). In recent years considerable progress has been made in understanding the metabolic interrelationship between these factors. These factors were analyzed together with associated hematologic variables like MCV, hemoglobin and hematocrit to give a more complete hematologic picture. A functional classification of folate status is explained in Table 1. This classification takes into account the metabolic relationships between serum folate, RBCF, hemoglobin, and MCV to give a better interpretation of the folate-hematologic status.

The gender factor

Our analysis failed to show a statistically significant difference in PP between the males and females which we attribute to the large s.e. However, the AP showed a small but statistically significant lower prevalence for males. Interestingly, the multivariate model not only maintained this significance but also showed significance for the PP data.

Most published reports show a somewhat higher RAS prevalence for females than males (Donatsky, 1973; Axéll, 1976; Axéll and Henricsson, 1985b; Kleinman *et al*, 1994; Reichart, 2000; Garcia-Pola *et al*, 2002). Our study and the report from Diaz-Guzman (Diaz-Guzman and Castellanos, 1991) show the opposite with prevalence being somewhat higher on males. In previous studies, when statistical analysis was done it was bivariate analysis, while in others no statistical difference between males and females were reported.

Our multivariate analyses summarized in Table 5, show that males have higher PP (AOR 1.91) but lower AP (AOR 0.82) than females are perplexing at first impression. We assume that PP is the 'gold standard' and look to AP as the source of the discrepancy. Assuming the true prevalence is the same between genders, the gender difference between PP and AP may be due to reporting bias. Perhaps females are over reporting or males underreporting RAS experience, or a combination of both.

False positive responses occur when individuals who never had RAS reported that they did or individuals who had RAS more than 1 year ago reported that they had RAS within the year (forward telescoping). Forward telescoping is not uncommon with medical events such as Pap smears and low back pain (Carey *et al*, 1995). On the other hand, false negatives occur when individuals who have had RAS within the past year fail to report it, either because they did not realize they had a lesion, forgot about it, or recall the RAS lesions as being outside the 1-year window (backward telescoping).

Perhaps women are more inclined to notice small lesions at the time than men because they may have a lower pain threshold. In fact, studies of gender differences in pain perception suggest that females have lower pain thresholds than men (Chesterton *et al*, 2003) as well as less tolerance for noxious stimuli than men (Berkley, 1997).

The race-ethnicity factor

Non-Hispanic Whites and Mexican Americans had a significantly higher RAS PP and AP compared with the non-Hispanic Blacks. In the multivariate model, non-Hispanic Whites had significantly greater odds (PP: 2.60 and AP: 5.49) of having RAS, while Mexican-American ORs (PP: 2.31 and AP: 2.25) although lower, were significantly increased when compared with non-Hispanic Blacks. Thus, we conclude that race-ethnicity is a significant factor and that non-Hispanic Blacks are the least susceptible.

The age factor

Individuals 17–39 years of age had a higher RAS PP (1.20%) and AP (22.54%) than those 40 and older (PP 0.59%, AP 13.42%). These findings held true with the multivariate model as well. The 17–39-year age group had approximately twice the odds of RAS of the older group. Most reports in the literature indicate that younger individuals have a higher prevalence of RAS (Donatsky, 1973; Axéll, 1976; Axéll and Henricsson, 1985b; Reichart, 2000).

To compare our prevalence data with those reported by Reichart (Reichart, 2000) in a sample of more than three thousand Germans; we recomputed our data to match those age groups he reported on. Reichart grouped ages 35–44 and 65–74 years and reported for both sexes a PP of 1.4% and a 2YP of 18.3%. When recomputed for the 35–44-year group, our results for both sexes were 0.70% for PP and 17.96% for AP. For the 65–74-year group they reported 1% (PP) and 6.9% (2YP) while our values were 0.71% (PP) and 8.9% (AP). It is interesting to see some similarities in our results to those of Reichart. Thus, we conclude that younger individuals have a higher prevalence of RAS. It would be of interest to determine if the episodes of RAS in younger individuals decrease as they get older.

The poverty factor

While the bivariate analysis showed RAS AP to be significantly different between levels of income with ORs of 1.45 (middle) and 1.81 (high), these were not significant in the multivariate model. Thus, we hypothesize that statistically significant bivariate associations between income level and RAS reported in other studies may have been due to confounding by the effects of other variables such as race-ethnicity and age.

While the income was not significant in the multivariate model, there is some theoretical basis to suspect it to be a factor. Poverty induces stress. Evans and English suggest that poor children are exposed to more stressors (i.e. noise, crowding, and lower quality housing) than middle-class children and that those stressors are associated with differences in socio-emotional adjustment (Evans and English, 2002). Social conditions and self-management are more powerful determinants of health than access to care as shown by the fact that despite universal access to health care in England, disparities in health according to socioeconomic status widened (Pincus *et al*, 1998). The effects of growing up in poverty may predispose the individual to poorer health, and upward mobility does not reverse or mitigate the adverse effects (Poulton *et al*, 2002).

There is evidence that higher stress levels may be associated with higher morbidity. Psychosocial stress, specifically, economic problems within 5 years of diagnosis, has been found to be associated with the onset of rheumatoid arthritis (Koopman, 2001). In an early report Ship *et al* (1960) studied the prevalence of oral lesions in 1788 medical, dental, veterinary medicine, graduate nursing, and dental hygiene students. They concluded that of all the factors studied, emotional factors (one could read stress) and prevalence of RAS were strongly related. Ferguson *et al* (1984) failed to find any correlation between psychological indices and RAS in women.

In a later report Ship (1966), found a positive linear relationship between the percentage of positive RAS cases and Duncan's socioeconomic index where the higher socioeconomic status strata had more RAS. However, these studies were conducted on a sample of professional students, which one may argue are already under a great amount of stress. Perhaps the low socioeconomic status professional students Ship studied, while poor relative to the predominantly middle and upper socioeconomic status professional students in the late 1950s, were substantially more affluent than the low socioeconomic status individuals in the NHANES III sample. It should be noted that there is a substantial body of literature relating stressors associated with poverty to adverse health. If higher socioeconomic levels could be associated with stress, it might be reasonable to expect a higher incidence of RAS in these individuals. Psychological mechanisms linked by hormonal and nervous system signals have been shown to modulate immune responses (Bierhaus *et al*, 2003; Dooley and Hogan, 2003), and it could be hypothesize that this is the possible mechanism through which stress could affect the prevalence of RAS.

The tobacco use factor

Tobacco use by pipe smokers, cigar smokers, and smokeless products were not found to be significant in our sample. As regards to smokeless tobacco, our findings disagree with those of Grady *et al* (1992), who reported that smokeless tobacco users were less than half as likely to have RAS in a sample of 1456 men from seven professional baseball teams. Kleinman *et al* (1994) found that in a sample of over 40 000 children there was a significant RAS reduction in those that used smokeless tobacco. Our results for pipe smoking disagree with those reported by Axéll and Henricsson, who found that pipe smoking seem to provide the most reduction in ulcers in a group of patients with a history of RAS. However, since the prevalence of pipe smoking and smokeless tobacco use in the NHANES III sample was small (and the point prevalence of RAS low) our s.e. were large and our statistical power was low.

The odds of RAS AP were significantly higher in individuals with low cotinine levels but this association did not hold for RAS PP. This could be due to the lack of statistical power in models using PP since cell sizes are small.

The cigarette smoking factors

Our data demonstrate that there is a significant reduction in RAS in individuals who smoke. The prevalence, unadjusted and adjusted odds ratios for number of cigarettes smoked were significant in bivariate and multivariate models for PP and AP. This suggests a dose response effect. Our findings are in agreement with previous reports (Axéll and Henricsson, 1985a; Shapiro *et al*, 1970; Bittoun, 1991; Grady *et al*, 1992; Tuzun *et al*, 2000; Ussher *et al*, 2003). A reduction in RAS prevalence with increased blood levels of cotinine, a long-lived metabolite of nicotine, was also found to be significant for PP and AP. Our findings are in agreement with those of Atkin *et al* (2002).

It is not clear how cigarette smoking can reduce RAS prevalence. It is likely that immunological mechanisms are involved in the development of the lesion (Eversole, 1997; MacPhail and Greenspan, 1997), and that the cytokine Tumor Necrosis Factor Alpha (TNF$\propto$) plays an important role in the pathogenesis and severity of RAS (Eversole, 1997; MacPhail and Greenspan, 1997). The predominant leukocyte cell type in the lesion is the T-lymphocyte (Eversole, 1997). Nicotine has been shown to directly and indirectly affect the immune response in inflammatory conditions (Sopori, 2002; Floto and Smith, 2003). Nicotine may act through the central nervous system by activation of the hypothalamus–pituitary–adrenal axis to induce the production of glucocorticoids and activation of the autonomic nervous system to reduce the level of inflammation (Sopori, 2002). Directly, nicotine can activate the nicotinic acetylcholine receptors on macrophages reducing the production of TNF$\propto$ and interleukins 1 and 6 (Floto and Smith, 2003). Interestingly, smoking raises the number of T-helper lymphocytes in Caucasians and a lowers it in African-Americans (Tollerud *et al*, 1991)

and thus may be a contributing factor to ethnic differences observed in RAS prevalence.

In a preliminary trial, three non-smokers with a long history of RAS with no remissions, were given nicotine (2–8 mg) in a chewable tablet form for 1 month and then weaned off. In all cases there was remission of RAS until the treatment was discontinued (Bittoun, 1991). In a group of 174 smokers enrolled in a 7-week smoking cessation program statistically significant increases in common cold symptoms and mouth ulcers were observed (Ussher *et al*, 2003). A statistically significant increase in RAS associated to Behcet's disease after smoking cessation has been reported (Soy *et al*, 2000). We hypothesize that smoking, specifically the nicotine in smoke, may be the responsible agent for the reduction in RAS.

The nutritional/hematological factors

Folate balance was calculated as described in Table 1. The significant associations found in the bivariate regressions were not found in the multivariate analysis. Significance testing of continuous nutritional-hematologic factors for subjects with and without RAS (Table 4), failed to detect any RAS PP significance, while with RAS AP only serum ferritin, serum iron, and RBC Folate showed statistical significance. The clinical significance of these results is difficult to interpret. The preponderance of reports in the literature (Olson *et al*, 1982; Challacombe *et al*, 1983; Porter *et al*, 1988, 1992; Palopoli and Waxman, 1990; Barnadas *et al*, 1997; Weusten and van de Wiel, 1998; Piskin *et al*, 2002; Thongprasom *et al*, 2002) seems to point out that some patients with RAS may exhibit some abnormal nutritional-hematologic values. While there have been some reports of these associations in the literature, our study did not confirm them.

The associated disease factors: recurrent herpes labialis, Helicobacter pylori, *hepatitis C*

Recurrent herpes labialis, *H. pylori*, and hepatitis C disease conditions associated with RAS were significant for RAS AP and not for RAS PP although individuals positive for *H. pylori* and hepatitis C had lower odds of RAS AP. Although individuals with all three diseases had statistically significant bivariate odds of RAS AP, only those who reported RHL during the past year had statistically significant adjusted odds of RAS AP (AOR = 2.63) in the multivariate model. While the RHL history was not significant for PP RAS in either the bivariate or multivariate models, the direction of bivariate relationship (Table 2) is in the expected direction; with individuals with a positive annual RHL history having a PP of 1.09 (0.66, 1.51) while those with no annual RHL history having a PP of 0.85 (0.61, 1.08).

An association between RHL and RAS appears to be certain. Our data and reports by others (Ship *et al*, 1967; Embil *et al*, 1975; Katz *et al*, 2001) tend to substantiate that many patients who have RAS also have RHL. One of the reports found that the odds of a RAS patient having RHL was 6.88 for women and 12.37 for men (Katz *et al*, 2001); while in our study the AOR was 2.63 for both sexes.

While the RAS–RHL relationship is clear, the relationships between RAS and hepatitis C and *H. pylori* are less so. Only one of 45 patient with hepatitis C and receiving alpha-interferon therapy has been reported to have developed RAS (Dalekos *et al*, 1998). Alpha-interferon was also reported to have induced oral ulcerations in a patient being treated with chronic hepatitis (Qaseem *et al*, 1993). Thus, at this time it appears that more research is necessary before a relationship between hepatitis C and RAS can be established. A relationship of RAS with *H. pylori* has been suspected, however serum antibody levels were not significantly increased in RAS (Porter *et al*, 1997) nor was it found associated to ulcerated oral tissue by nested PCR (Mravak-Stipetic *et al*, 1998). However, in another study, when sampling RAS ulcers with swabs for PCR identification of *H. pylori* DNA, more than 70% of the samples were found to be positive (Birek *et al*, 1999). Recently it has been suggested that *H. pylori* infection may affect the absorption of micronutrients like iron and vitamin B12 (Annibale *et al*, 2002).

Limitations

Most RAS studies have analyzed homogenous population samples while NHANES III represents a heterogeneous population. While NHANES III is a robust, well-respected population-based data source that used trained examiners, it nonetheless has limitations (Wartenberg and Buckler, 2001). One limitation is inherent in its design. Since is cross-sectional, it can be used to explore associations only, not causality (Shulman *et al*, in press), thus the findings must be viewed carefully. Despite the large adult sample size afforded by the NHANES III data set, the fact that only 146 individuals had clinically apparent RAS limited the power of our multivariate model for PP. Consequently, some of the covariates that did not meet our inclusion criterion ($P < 0.05$) might truly be significant. The other limitation of our study is the lack of physiologic measures of stress. These data were not collected as part of the NHANES III effort. It must be kept in mind that epidemiologically significant relationships are the first step indicating that there should be further evaluation preferably using experimental interventional studies.

Conclusions

Bivariate analyses should be interpreted cautiously. Even in this study, several variables found to be significant in bivariate analyses for PP (income level) and AP (ferritin, iron, RBC folate, income level, cotinine, folate balance, RHL history, *H. pylori*, and hepatitis C seropositivity) were not found to be significant in the multivariate model.

Finally, PP and AP studies should not be given the same degree of credibility. Our multivariate models for PP and AP were consistent with respect to race-ethnicity, age, and cigarette smoking. Although, they

are in conflict with respect to gender, we defer to the results of the PP model, our gold standard, and conclude that RAS is more prevalent in males than females. We attribute the conflicting results in the AP model to reporting bias. While AP data are cheaper to collect and more amenable to multivariate modeling due to higher prevalence rates, data should be interpreted with caution, especially where there is some basis to suspect response bias. Our findings have provided data on the general prevalence of RAS and the possible effects of associated factors and should be the foundation for further study.

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