

Dietary intake of patients with acne vulgaris in Karachi: an observation study

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ABSTRACT

BACKGROUND & OBJECTIVE: The pathology of acne is vaguely understood to date, current epidemiologic evidence in Eastern populations indicates that consumption of chocolate, milk intake, fatty products, and glycemic load may be involved. Previously little research has been conducted highlighting the possible relationship between diet and acne among patients in Pakistan. The objective of this study was to identify the association of acne vulgaris and dietary intake patterns.

METHODOLOGY: An observational study enrolled 246 participants, both genders, 16 to 40 years of age, diagnosed with Acne Vulgaris recruited from a tertiary-care hospital out-patient clinic in Karachi. The structured questionnaire consisted of various methods for assessing nutritional parameters. Statistical analysis was carried out using SPSS, the association of Acne was tested with dietary habits using Pearson-Chi Square test. Food items with a p-value less than 0.05 in the Chi Square test were considered in Binary logistic regression for estimating the risk for Acne with a 95% confidence interval (C.I.).

RESULTS: Fifty-two percent of participants were found with mild acne, use of yogurt, ice cream butter, margarine, French fries, chocolate, bakery chips, white bread, vegetable, fruits, cake dates, and pickles give a significant association with severity of acne using Fisher's Exact test $p < 0.05$. Logistic regression showed that butter gave more risk for acne with an odds ratio of 1.11 and 95% C.I. (1.0, 9.2).

CONCLUSION: It can be stated that dairy products, high glycemic index, and fatty foods are associated with acne aggravation in our population. As patients are always concerned about dietary advice, suggesting that they reduce these products would help them maintain their skin.

KEYWORDS: Acne Vulgaris, Diet, High Glycemic Index, Cocoa Products, Pakistan.

INTRODUCTION

Globally, acne is known to be a commonly afflicted dermatological condition [1,2] impacting almost all age groups, but specifically individuals in their teenage years. As per the available data, rates are 50% to 95% [3]. Acne is a prolonged dermatological condition developed as a result of inflammatory changes which take place in the pilosebaceous glands of the skin, causing the development of cysts, comedones, nodules, papules, and pustules is known to be acne vulgaris, or commonly referred to as acne. It is usually associated with the bacterium *Cutibacterium acnes*. The estimated prevalence of the condition is recorded to be 9.4 million worldwide, making it the 8th most commonly occurring disease globally, whereas in Pakistan, its

prevalence is identified to be 5% nationally [2,3].

There are multiple elements contributing to the development of acne, for example psychological aspects, genetic factors, environmental and hormonal factors [1,3,4]. The environmental factors encouraging the pathogenesis of acne include dietary intake such as high fat diet, sweets, and chocolates by the patients [5]. Dairy Products: High dairy products are known to be an aggravating factor of acne. As per a study held in 2005 constituents of milk, excluding lipids, have insulin encouraging capacities [6]. One study detected an encouraging association among levels of IGF-1 and acne [7]. As per multiple research studies there is a positive relation among acne and intake of total milk and skimmed milk [5-7]. Many studies discovered that a low glycemic index (GI) diet enhanced acne prevention and an equivalent development

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in insulin compassion among young males with acne vulgaris. Researchers of two renowned cross-sectional studies [8,9] reported there were no cases of acne among people, individuals residing in the rural areas consuming low-fat and low-glycemic-index diets could be a contributing factor for the lack of the dermatological condition being present among that population [10]. Hyper-glycemic food induced hyperinsulinemia is proposed to lead endocrine responses that aggravate acne, and a high glycemic load diet has been shown to affect acne in epidemiologic studies and in randomized, controlled trials [11,12].

The effectiveness of fat and fatty acids on acne formation is not well understood. Diets extraordinarily high in saturated fats are shown to be linked with high IGF-1 levels, whereas food that is low-fat and high in fiber has been associated with lessened IGF-1 levels [13,14].

In one study, Investigators matched the outcomes to the same group's intake of chocolate bars with the group who didn't have cocoa enrichment as per the study; it was found that there was no major difference among the groups [15]. Chocolate is considered one of the major contributing factors involved in the worsening of acne, but presently there is inadequate data that can relate the negative impact of its use on the skin. The aim of this study is to investigate any association between diet and acne that exists among the Pakistani population. To the best of our exploration and understanding, our study is the first of its kind to investigate the relationship of diet and acne severity in Pakistan.

METHODOLOGY

The research design involves an observational cross-sectional study by enrolling 246 clinically diagnosed patients for the treatment of acne between November 2020 to December 2020. The setting of the study was in an outpatient clinic as a part of Dow University Hospital, a public-sector, tertiary-care hospital located in Karachi, an urban city in Pakistan. Institutional Review Board (IRB) approval was sought and approved prior to data collection in Dow University of Health Science (Ref: IRB-1824/DUHS/Approval/2020). The study followed a nonprobability consecutive sampling design, both males and females; aged between 16-40 years, who are coming to the out-patient department (OPD) for treatment. Patients with mild to moderate facial acne vulgaris were evaluated by a dermatologist using the Comprehensive Acne Severity Scale (CASS) Table-I [16] to enroll participants of either male or female gender. Participants who were pregnant and/or lactating, or with endocrine disorders, or those having severe acne undergoing taking retinoid therapy were excluded from the study. Written informed consent was taken from all participants prior to verbally administering the questionnaire. A structured questionnaire was verbally administered by a post-graduate trainee. Baseline demographic, socio-economic, dietary, and health-related data was collected by a trained interviewer, using a structured, pre-tested questionnaire that was translated into local languages taking approximately 20-30 minutes to administer. The study was conducted according to the guidelines laid own in the Declaration of Helsinki, and all procedures involving

human subjects were approved by the Ethics Review Committee of Dow University of health sciences, Karachi. The questionnaire was pretested for input on content, language clarity, and layout. Revisions were made accordingly for final data collection from the study. The sample size for this study was calculated by a statistician, which encompasses an incremental 5% for participants to account for the loss of follow-up and unwilling to participate in the study.

Anthropometric measurements were collected by trained dietitian following standard procedures regarding parameter of weight. The weight was measured to the nearest 0.1 kg without shoes using an electronic platform on a model weighing scale and height to the nearest 0.5 cm (portable Stadiometer). The Body mass index (BMI) was calculated as kg/m² [17]. Dietary data were collected using a 50-item food frequency questionnaire (FFQ). This was developed by a nationally known FAQ nutrition researcher based on the most frequently used Harvard FFQ format review of the available literature and information gathered through informal dietary survey to reflect underlying dietary habits of the population in Pakistan. A well-structured and extensive questionnaire using preliminary information was formulated by keeping in mind the objectives of the study. For each food item, subjects were asked how frequently they had consumed the food without specifying the portion size. Participants could specify the number of times per day, per week, or month. Food intake frequencies were then standardized to the number of times per day.

Sample size

Calculation of the potential sample size was done using the open epi calculator using a confidence interval of 95 % and with desired precision of 0.05. The anticipated prevalence of acne vulgaris among adults was taken as 5 % (13). After adjustment for 10% non-response rate sample size came out to be 102, which is raised up to n=120 participants. (Total sample of 150 patients encompassing 30 patients as dropout rate, for loss of follow-up and unwilling participants).

RESULTS

Table-II reports the baseline characteristics of studied samples. In the present study, there were two hundred and forty-six samples among them 54.5% having age 16–20 years, 73.6% were female, 60.6% were students, 88.2% were unmarried, 11.8% were married, 32.1% had graduation, 38.6% were Urdu speaking, 57.1% reported family size 6–10 members, 6.9% reported for co morbid, 22% samples were found with underweight BMI.

The prevalence of acne was 90.2% shown in Figure-I, 23.6% were found almost clear, 14.6% were with moderate cases of acne, and 52% were mild cases of acne. The results of the association of acne with dietary habits are presented in Table-III. The results shown as %age among moderate cases of acne after per day consumption of milk was 52.9, yogurt (42.9) , ice cream (38.9) , cheese (30.6), milkshake (36.1), skim milk (30.6), margarine (16.7) , French fries (38.9), margarine (44.4), bakery chips (36.1), packed chips

(36.1), white bread (25), vegetables (38.9), fruits (38.9), cold drink (44.4), cake (52.8), mangoes (44.4), dates (47.2), watermelon (41.7), whole fat (38.9), white rice (54.3), one tablespoon butter (25), one slice of pizza (36.1), one bar of chocolate (38.9%) and one paratha (52.8%). mangoes (44.4), dates (47.2), watermelon (41.7), whole fat (38.9), white rice (54.3), one tablespoon butter (25), one slice of pizza (36.1), one bar of chocolate (38.9%) and one paratha (52.8%). Among these food items, consumption of yogurt, ice cream, milkshake, butter, margarine, French fries, chocolate, bakery & packed chips, white bread, vegetables, fruits, pickles, cake, dates, and white rice gives significant association with the severity of acne using Fisher's Exact

test ($p < 0.05$). The results of the binary logistic regression model to estimate the risk of dietary habits with food items are shown in Table-IV and are found statistically significant in Fisher's Exact test, results showed samples found with the frequent consumer of yogurt, ice cream, milkshake, butter, chocolate, bakery chips packed chips, white bread, fruits, pickles, cake and dates were more likely to be found with acne and have higher risk as compared to non-consumer of these food items, whereas consumption of French fries, vegetables, and white rice gives negative association with acne, samples of these food items consumer will be at lower risk of acne as compared to the frequent users of these items.

Table-I: Comprehensive Acne Severity Scale (CASS).

Grade	Description
Clear 0	No lesions to barely noticeable ones; very few scattered comedones and papules.
Almost Clear 1	Hardly visible from 2.5 meters away; a few scattered comedones and a few small papules; and very few pustules, comedones, and papules.
Mild 2	Easily recognizable; less than half of the affected area is involved; many comedones, papules, and pustules.
Moderate 3	More than half of the affected area is involved; numerous comedones, papules, and pustules.
Severe 4	The entire area is involving; covered with comedones, numerous pustules and papules, a few nodules, and cysts.
Very Severe 5	Highly inflammatory acne covering the affected area, nodules, and cysts present.

Table-II: Baseline Demographics of Studied population n (%).

Characteristics	n=246 (%)	
Age Group	16-20	134 (54.5)
	21-25	67 (27.2)
	26-30	29 (11.8)
	>30	16 (6.5)
Gender	Male	65 (26.4)
	Female	180 (73.6)
Occupation	Professional	28 (11.4)
	Student	149 (60.6)
	Retired/Jobless	22 (8.9)
	Housewife	26 (10.6)
	others	21 (8.5)
Marital Status	Single	217 (88.2)
	Married	29 (11.8)
Educational	Uneducated / illiteracy	10 (4.1)
	Read-Only	4 (1.6)
	Primary (Up to 5 Year)	17 (6.9)
	Matric (Up to 10 Years)	44 (17.9)
	Intermediate (Up to 12 Years)	71 (28.9)
	Graduation Up to 14 Years	79 (32.1)
Ethnic Group	Post- Graduation (Up to 16 Years)	21 (8.5)
	Sindhi	58 (23.6)
	Punjabi	29 (11.8)
	Balochi	4 (1.6)
	Pashton	34 (13.8)
	Urdu Speaking	95 (38.6)
Family Members	Other	26 (10.6)
	≤5	78 (31.7)
	6-10	142 (57.7)
Comorbid	>10	26 (10.6)
	Yes	17 (6.9)
Body Mass Index	Underweight > 18.5	54 (22.0)
	Normal 18.5 - 22.9	125 (50.8)
	Overweight 22.9- 27	50 (20.3)
	Obese 27-29	17(6.9)

Table-III: Association of Acne with Dietary Habits.

Per Day Quantity	Comprehensive Acne Severity Scale									
	Clear		Almost Clear		Mild		Moderate		p-value	
	n	%	n	%	n	%	n	%		
Milk	1 cup	19	79.2	32	57.1	82	65.1	18	52.9	0.46
	2 cup	0	0.0	1	1.8	3	2.4	1	2.9	
Yogurt	1 cup	19	79.2	39	68.4	81	63.3	15	42.9	
	2 cups	0	0.0	1	1.8	4	3.1	4	11.4	
Ice-cream	1 cup	18	75.0	30	51.7	68	53.1	14	38.9	<0.01*
	2 cups	2	8.3	8	13.8	2	1.6	7	19.4	
	More than 2 cups	0	0.0	0	0.0	1	0.8	0	0.0	
Cheese	1 cup	13	54.2	26	44.8	63	49.2	11	30.6	0.39
	2 cups	1	4.2	4	6.9	5	3.9	1	2.8	
Milkshake	1 cup	14	58.3	32	55.2	78	60.9	13	36.1	0.01*
	2 cups	0	0.0	6	10.3	2	1.6	4	11.1	
Butter	1 tbsp	16	66.7	30	51.7	65	50.8	9	25.0	0.04*
	2 tbsp	0	0.0	2	3.4	5	3.9	3	8.3	
	More than 2 tbsp	1	4.2	4	6.9	2	1.6	1	2.8	
Skim milk	1 cup	14	58.3	23	39.7	59	46.1	11	30.6	0.16
	2 cups	0	0.0	1	1.7	1	0.8	2	5.6	
	More than 2 cups	1	4.2	0	0.0	4	3.1	0	0.0	
Margarine	1 cup	15	62.5	19	32.8	54	42.2	6	16.7	0.04*
	2 cups	0	0.0	5	8.6	10	7.8	4	11.1	
	More than 2 cups	1	4.2	1	1.7	1	0.8	1	2.8	
French fries	1 cup	16	66.7	31	53.4	79	61.7	14	38.9	<0.001*
	2 cups	2	8.3	10	17.2	8	6.3	3	8.3	
	More than 2 cups	2	8.3	0	0.0	1	0.8	1	2.8	
Pizza	1 slice	12	50.0	25	43.1	65	50.8	13	36.1	0.057
	2 slices	3	12.5	13	22.4	9	7.0	4	11.1	
	More than 2 slices	2	8.3	2	3.4	9	7.0	0	0.0	
Chocolate	1 bar	20	83.3	30	51.7	74	57.8	14	38.9	0.02*
	2 bars	1	4.2	9	15.5	12	9.4	4	11.1	
	More than 2 slices	0	0.0	3	5.2	2	1.6	0	0.0	
Margarine	1 cup	15	62.5	33	56.9	75	58.6	16	44.4	0.63
	2 cups	0	0.0	3	5.2	5	3.9	3	8.3	
Bakery chips	1 cup	16	66.7	35	61.4	77	60.2	13	36.1	<0.01*
	2 cups	0	0.0	2	3.5	5	3.9	5	13.9	
	More than 2 cups	1	4.2	0	0.0	0	0.0	0	0.0	
Packed chips	1 cup	18	75.0	34	58.6	83	64.8	13	36.1	<0.01*
	2 cups	1	4.2	3	5.2	6	4.7	6	16.7	
	More than 2 cups	1	4.2	0	0.0	0	0.0	0	0.0	

*Denotes significant association (p<0.05).

Table-IV: Association of Acne with Dietary Habits.

Per Day Quantity		Comprehensive Acne Severity Scale								p-value
		Clear		Almost Clear		Mild		Moderate		
		n	%	n	%	n	%	n	%	
White bread	1 bread	18	75.0	30	51.7	51	39.8	9	25.0	<0.01*
	2 breads	2	8.3	9	15.5	37	28.9	9	25.0	
	More than 2 breads	1	4.2	2	3.4	2	1.6	1	2.8	
Paratha	1	20	83.3	39	67.2	83	64.8	19	52.8	0.42
	2	1	4.2	4	6.9	6	4.7	4	11.1	
	More than 2	0	0.0	0	0.0	2	1.6	0	0.0	
Vegetables	1 cup	17	70.8	38	65.5	70	55.6	14	38.9	0.01*
	2 cups	4	16.7	11	19.0	25	19.8	12	33.3	
	more than 2 cups	2	8.3	0	0.0	2	1.6	0	0.0	
Fruits	1 cup	16	66.7	39	67.2	74	57.8	14	38.9	0.02*
	2 cups	3	12.5	6	10.3	25	19.5	8	22.2	
	more than 2 cups	2	8.3	0	0.0	1	0.8	1	2.8	
Cold drink	1 cup	19	79.2	32	55.2	69	53.9	16	44.4	0.17
	2 cups	2	8.3	4	6.9	11	8.6	4	11.1	
	more than 2 cups	0	0.0	2	3.4	0	0.0	1	2.8	
Pickles	1 tbsp	15	62.5	30	51.7	71	55.5	13	36.1	0.03*
	2 tbsp	1	4.2	2	3.4	5	3.9	2	5.6	
	more than 2 tbsp	2	8.3	0	0.0	1	0.8	0	0.0	
Cake	1 cup	22	91.7	34	58.6	79	62.2	19	52.8	<0.01*
	2 cups	0	0.0	5	8.6	7	5.5	2	5.6	
	more than 2 cups	1	4.2	0	0.0	0	0.0	0	0.0	
Mango	1 cup	17	70.8	30	51.7	65	50.8	16	44.4	0.09
	2 cups	2	8.3	2	3.4	21	16.4	4	11.1	
	more than 2 cups	2	8.3	5	8.6	6	4.7	1	2.8	
Dates	1 cup	17	70.8	34	58.6	81	63.3	17	47.2	0.051
	2 cups	2	8.3	2	3.4	2	1.6	4	11.1	
Watermelon	1 cup	19	79.2	31	53.4	66	51.6	15	41.7	0.32
	2 cups	0	0.0	1	1.7	12	9.4	4	11.1	
	more than 2 cups	0	0.0	3	5.2	2	1.6	0	0.0	
Whole fat	1 cup	16	66.7	24	41.4	66	52.0	14	38.9	<0.01*
	2 cups	0	0.0	2	3.4	2	1.6	1	2.8	
White Rice	1 cup	21	87.5	45	78.9	71	56.3	19	54.3	<0.01*
	2 cups	2	8.3	9	15.8	41	32.5	15	42.9	
	more than 2 cups	1	4.2	2	3.5	2	1.6	1	2.9	

*Denotes significant association (p<0.05).

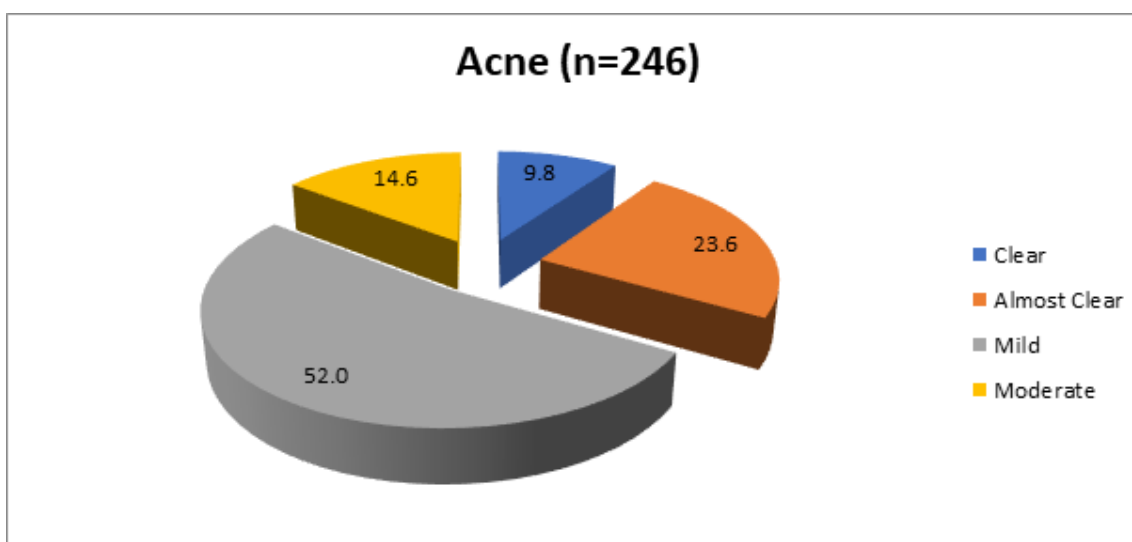


Figure-I: Frequency of Acne Vulgaris Patients according to Comprehensive Acne Severity Scale (CASS).

Table-V: Risk Estimation of Acne with Dietary Habits using Binary Logistic Regression.

Dietary Habits	n (%)	Beta	S.E.	Wald	Odds Ratio	95% C.I. for odds Ratio		p-value
						Lower	Upper	
Yogurt or curd	140(63.1)	0.024	0.44	0.003	1.024	0.42	2.44	0.95
Ice cream	140(63.1)	0.368	0.43	0.723	1.445	0.61	3.37	0.39
Milkshake	111(50)	0.511	0.44	1.333	1.667	0.70	3.96	0.24
Butter	84(37.8)	1.113	0.56	3.881	3.043	1.00	9.21	0.04*
French fries	153(69.5)	-0.062	0.47	0.017	0.940	0.37	2.37	0.89
Chocolate	141(63.5)	0.043	0.44	0.010	1.044	0.43	2.49	0.92
Bakery chips	111(50.2)	0.896	0.46	3.656	2.451	0.97	6.14	0.06
Packed chips	153(69.5)	0.489	0.43	1.241	1.631	0.69	3.85	0.26
White bread	158(71.5)	0.409	0.44	0.835	1.505	0.62	3.61	0.36
Vegetables	193(86.9)	-1.240	1.04	1.420	0.289	0.03	2.22	0.23
Fruits	197(88.7)	0.118	0.65	0.033	1.126	0.31	4.04	0.85
Pickles	75(33.8)	0.662	0.52	1.607	1.939	0.69	5.39	0.20
Cake	144(64.9)	0.446	0.43	1.061	1.562	0.66	3.65	0.30
Dates	120(54.1)	0.330	0.43	0.584	1.390	0.59	3.23	0.44
White rice	194(87.4)	-1.200	1.04	1.328	0.301	0.03	2.31	0.24

Dependent variable: Acne

Independent variables: Dietary Habits

*p<0.05 was considered statistically significant

DISCUSSION

The study aimed to investigate an association between acne vulgaris and various dietary foods among young adults in Karachi, Pakistan. Though research investigating the prevalence of acne vulgaris among various geographic regions of Pakistan has been conducted, to the best of our knowledge, this is the first study to examine the association of various dietary foods with the development and severity of acne vulgaris among a Pakistani population of under 30 years of age [18, 19]. The severity of acne was assessed using a standardized tool known as the Comprehensive Acne Severity Scale (CASS), which grades the progressive nature of acne on various parts of the body, although this study did not include participants in grade 4 or 5 category on the

CASS scale as they may already be consuming prescriptive medications for the acne severity (Table-I). Therefore, this study enrolled participants with an acne severity degree ranging between grade 0 (clear) and grade 3 (moderate). Based on the results, the overall prevalence of acne vulgaris within the present study was 90.2% and was mostly seen in the 16–20 year age group with 54.5%, and of the total 246 sample size majority, 60.6% of clinical examined acne vulgaris was among students. These findings are similar to a study conducted in Kuwait on school adolescents, with the self-reported prevalence of acne being 44.8% while clinical examination acne was reported to be 67.1% [20]. The prevalence rate of acne by clinical examination in the Kuwait study was lower to the prevalence of acne clinically reported as 90.2%, further categorizing them as 52% mild cases and 14.6% as moderate cases of acne among the participants in this study. The clinically presented acne prevalence was higher among the female gender with a reported 73.6% in

73.6% in this study, which is similar to the Kuwait study reporting 74.1% females with clinical and self-reported acne, and additionally, a Taiwan study which reported higher prevalence of acne among female school-going children, even after stratification by age [21].

Among various factors linked to the development of acne vulgaris, research has shown inconclusive findings regarding body mass index (BMI) as a modifiable factor associated with acne vulgaris. The majority of participants with acne in this study were categorized as having underweight (22%) or normal BMI (50.8%) classification for young adults.

These findings contrast from a Taiwanese study which found that school-going participants with higher BMIs had greater rates of acne. Similarly, a Kuwait study on young adolescents who were obese/ overweight was found to be more likely to have acne vulgaris in comparison to normal weight adolescents [20]. Out of the 246 young adult participants for this study, the CASS scale determined 52% participants with grade 3 or mild severity of acne, while 9.8% were categorized as grade 0 or having no lesions of acne visible on their body, while 23.6% of participants were determined to have “almost clear” or grade 1 of acne severity (Figure-I).

This study found that the frequency of yoghurt (1 cup), milk shake and ice cream intake had a significant association with development of grade 2 (mild) or grade 3 (moderate) severity of acne on CASS, which is similar to other reported findings of milk and ice cream intake having positive association with acne vulgaris occurrence among young adults from Malaysia, despite this study not having found a significant association of milk consumption with acne development based on CASS [22,23]. These results differ from a cross-sectional study conducted in South Korea, which indicated milk and dairy products intake was associated with the occurrence and progression of acne vulgaris [23]. Alkhabbaz and colleagues found neither the consumption of milk nor various other dairy products were associated with acne vulgaris in multivariable analysis, although intake of low-fat milk was associated with acne in univariable analysis. In contrast, skim-milk (low-fat milk) consumption was not found to be significantly associated with acne vulgaris within this study [20].

This study also found a statistically significant association between consumption of fatty products and acne vulgaris, such as butter ($p=0.01$) and margarine ($p=0.04$), which contrasts with the findings reported from a study in Turkey indicating the severity of acne did not progress as fat consumption increased [24].

High-glycemic foods such as French fries, bakery chips and packaged chips also known in Western culture to be junk foods, were all investigated to determine the impact of these products on the severity of acne vulgaris using the CASS tool. This study found a statistically significant association among French fries, bakery chips, and packaged chips in relation to the severity in acne vulgaris development (see Table-IV).

The impact of chocolate consumption was investigated and found to have a statistically significant association ($p<0.05$) with the severity of acne vulgaris among young adults in this study. Previous extended research has provided inconclusive findings regarding the intake of chocolate on acne development, severity and exacerbation [22-24]. A double-blind, placebo-controlled randomized, controlled trial was conducted to investigate the impact of milk chocolate intake in the majority of male participants with the diagnosed skin condition, which found eating chocolate led to progression in their acne severity [25,26]. In 2017, Ulvestad et al. evaluated that full fat dairy products were linked with moderate and severe acne whereas, one of the earliest study by Fulton et al, investigated acne and chocolate conducted on sixty-five subjects diagnosed with acne consumed milk chocolate bars as compared to subjects given a chocolate-less placebo bar containing vegetable fat, with results indicating no significant difference between the two groups, suggesting chocolate had no effect on acne development [27].

CONCLUSION

Dairy products, high glycemic index, and fatty foods are associated with flares of acne in Karachi. The patients should be recommended to avoid such food for long term maintenance of skin health.

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Sahar Soomro: Drafting the work or revising it critically for important intellectual content.

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Rabiya Khan: Collected the data and performed the analysis

Meeraal Kaikaus: Was responsible for the integrity of the study.