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Bacteria and Risk Factors of Acne Vulgaris in Northern West Bank, Palestine
Identificación de Bacterias y Factores de Riesgo del Acné en Cisjordania, Palestina

Abstract

Bacterial species and risk factors were examined in acne vulgaris patients in Northern West Bank. Samples were obtained from face and upper trunk lesions of 138 patients (age: 15-34 years) at Dermatology clinics. Samples were incubated in blood agar plates under aerobic and anaerobic conditions. Patients were asked to answer a questionnaire and all provided written informed consent. Bacteria were isolated from 88% of the patients including *Propionibacterium acnes, Staphylococcus epidermidis* and *Staphylococcus aureus*. Risk factors mentioned by patients included emotional stress, high temperature, sweating and local pressure. Chocolate, nuts and fatty food were mentioned as exacerbating, and vegetables and fruits as ameliorating foods for their acne, respectively. Results show high correlation between acne and bacteria with no relation between risk factors and specific bacterial strains.

Keywords: Acne vulgaris, Palestine, West Bank, risk factor.

Resumen

Se examinó bacterias y factores de riesgo asociados al acné en pacientes del norte de Cisjordania, Palestina. Se obtuvo muestras de la cara y el tronco superior de 138 pacientes (15-34 años) de clínicas dermatológicas, y se incubaron en condiciones aeróbicas y anaeróbicas. Los pacientes respondieron un cuestionario y firmaron un consentimiento informado. Se aisló bacterias en 88% de los pacientes, incluyendo *Propionibacterium acnes, Staphylococcus epidermidis* and *Staphylococcus aureus*. Los factores de riesgo mencionados fueron el estrés emocional, las temperaturas altas, el sudor y la presión local. El chocolate, las nueces y las grasas de la dieta se identificaron como factores que exacerban, y las frutas y verduras como alimentos que alivian el acné, respectivamente. Los resultados muestran alta correlación entre las bacterias y el acné, sin relación entre los factores de riesgo mencionados y alguna especie bacteriana específica.

Palabras claves: Acne vulgaris, Palestina, Cisjordania, factores de riesgo.
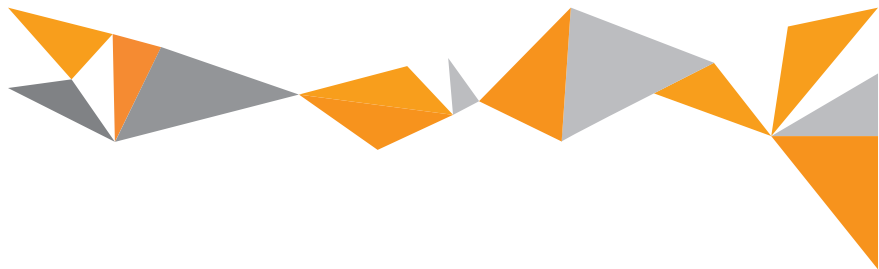
Introducción

Acne vulgaris is considered as a global disease. It is estimated that at least 70 percent of adolescents got acne (Marks *et al*., 2003). Skin lesions consist of white and black comedos, erythematous papules and pustules, and in severe untreated cases, deep pustules, and multiple nodules appear which may progress to scar formation (Layton *et al*., 2010).

The etiology of acne is multifactorial, which includes abnormal keratinization, hormonal function, bacterial growth, and immune hypersensitivity (Webster *et al*., 1995), the disease is limited to pilosebaceous follicles of the head and upper trunk (chest, shoulders, and back) because the sebaceous glands in these regions are particularly active, and at puberty, due to the arousal of sex hormones, there's an increase in sebum secretion, which lead to enlargement of these glands (Leyden *et al*., 2001), subsequently, inflammatory acne is the result of the host response to the follicular inhabitant *Propionibacterium acnes, Staphylococcus epidermidis* (*Staph. epidermidis*), and *Malassezia furur* which is a harmless, normal flora in the skin (Bojar *et al*., 2007). *Staph. aureus and Staph. epidermi-*

dis are gram positive, coagulase negative aerobes that presents as normal flora on the skin and appear to be involved in the pathogenicity, as they are aerobes their growth in the sebaceous unit is superficial and can't reside in the anaerobic condition in the infra-infundibulum portion of the sebaceous unit where the inflammation takes place. Whereas *Malassezia furfur* the filamentous forms are not usually associated in the pathologic condition and have no role in the disease (Burkhart *et al*., 1999).

Other risk factors might be considered as contributing factors to acne prevalence and severity including physiological factors such as the menstrual cycle, pregnancy, anxiety, and depression, and external factors such as hot and humid weather, mechanical skin irritation from local pressure and squeezing, daily ingested diet (chocolate, milk, nuts, high glycemic diet,…..etc) and excessive sweating (Stoll et al., 2001; Fung *et al*., 2001).

There are central factors that contributes in the pathophysiology of acne these are: the inflammatory response, bacterial colonization,

increased sebum production and hypercomification of pilosebaceous duct. Other risk factors might be considered are nutrition, genetics, and oxidative stress. (Suh *et al*., 2015).

Since it is found that there is a lot of variations of risk factors among *Acne Vulgaris*' patients in different countries where researches had been done, and there was a little published studies about these risk factors in West Bank, Palestine, it is worth to assess the prevalence of these risk factors and also the microbial agents colonized over the lesions in the study population.

This study will focus on the determination of the prevalence of microorganisms colonizing over the skin lesions with the development of the disease, since it is a matter of debate whether those acne lesions colonized with certain microorganisms or not, and if it is present, which is the most microorganism associated with acne lesions.

Also we will correlate the presence of the microorganisms with the risk factors that could be found in the same participants. That will enable

future studies to determine what is the best method to reduce the prevalence and severity of symptoms of acne either by proper knowledge about the sensitivity for these microorganisms, (if present), or life style modification for the associated risk factors.

Patients and Methods

Data Collection

Patients who attended dermatology clinics of northern part of The West Bank, Palestine, and were diagnosed of having *Acne Vulgaris* were targeted for the study. The investigators offered them a consent form describing the study goals, methods, and assuring the privacy and after the patients' acceptance to participate an interview questionnaire was done with the patients to determine the risk factors, and the patients were inspected for the type of the lesions, e.g. pustule, papule, nodule, comedons, and it's distribution, except for the female patients, they were only asked where the distribution of their acne lesions on their body,(in order to keep the patients' privacy, and for some social considerations), and that questionnaire was given a serial number to be linked with the culture.

After then, when the questionnaires were filled, the investigators informed the patients that they would swab one of the acne lesions from their skin, to be cultured at the lab, and after accepting that, a cotton swab, wet by 70% ethanol alcohol, was applied over the skin lesion to prevent contamination, then a sterile swap stick was applied gently over the lesion, then swapped over two culture media, one for aerobic and the other for anaerobic growth , and these two cultures were given a serial number similar to that of the questionnaire.

Ethics Statement

Before starting the study, a proposal was submitted to the IRB (Institutional Review Board) committee, and an acceptance to work on the study was given. Prior to enrollment in the study, a consent form was given to the participants which informs them about the goals and methods for the study then the research team introduced themselves and explained the goals, the methodology, and possible benefits from participating in the study. An Informed written consent was obtained from all of the participants.

Design and participants

This cross-sectional descriptive study was performed on 138 patients (15-34 years old), attending dermatology clinics in northern part of the West bank. And since a cross-sectional study was the study design, the smallest number of sample size that was used in the study was calculated according to the following equation N = Z2 X P (1-P) / D2. (Naing *et al.*, 2006).

Demographic Characteristics

In total, 138 valid questionnaires and skin swaps from faces were obtained from patients who have *Acne Vulgaris* of both female (66 patients) and male (72 patients), between 15-34 years old, who attended local dermatology clinics at the time of the study, and were residents in Palestine for more than 3 months, with absence of any other primary skin disease.

We obtained the characteristics of the participants using a self administered questionnaire. Personal information, including age, gender and family history of acne disease, and other questions regarding other risk factors were taken.

Patients who refused to sign the consent term or who have another types of Acne, e.g. Steroid acne, oil acne, chloracne were excluded from the study.

Laboratory Testing

Sterile swab samples were collected from one acne lesion of the participant's face skin then swapped over two culture media, one for aerobic and the other for anaerobic growth, and these two cultures were given a serial number similar to that of the questionnaire.

The culture media used in this study was (Blood Agar), which was used to culture acne lesion microorganisms in previous researches (Behrouz *et al.*, 2011).

After swapping the skin lesions, and inoculation into the two culture media, the samples were recruited daily to the microbiology lab, to be incubated under two conditions, aerobic at 37° C for one day, while anaerobic at 37° C for 5 days, then the isolated colonies from aerobic condition were identified by gram stain and biochemical tests (Catalase and Coagulase tests).

P. acnes was recognized as gram positive bacilli of colonies from anaerobic condition.

Statistical Analysis

The analysis of the data that have been got from the sampling was handled by using SPSS, and the data was coded and inserted, the descriptive analysis, was used for the continuous variables, where they were described by median and mean and (standard deviation), while the categorical variables were described by frequencies, tables, and charts, all of these were applied to demographic data, and known risk factors

that were mentioned in the literature as an exacerbating event to acne. The data was analyzed using Chi–square for categorical variables among association with known risk factors and microorganisms colonization, and T test for comparison of means.

P value less than 0.05 was considered as significant.

Results

Demographic and risk factors variations

138 acne patients, were selected in this study, with 52.2% (72) male, and 47.8% (66) female. The age of acne patients who were included in this study ranged from 15–34 years with a mean of 19.4 years.

Age at onset of acne ranged from 11–26 years for both sexes, but female patients developed acne at slightly earlier age than males: 16.8 years versus 17.0 years for males. Duration of acne ranged from 3 months to12 years with a mean of 2.46 years (2.6 years for males and 2.3 years for females).

Family history of acne in first degree relatives was present in 62.3% of acne patients (65.3% males and 59.1% females). P value calculated by Chi Square, was 0.486 for male and 0.32 for female which considered insignificant.

Papules only was found in 0.7% of acne patients, Pustules only in 15.9%, Nodules only in 1.4%, Comedos and papules 11.6%, Papules and pustules 34.1%, pustules and nodules 18.1%. Comedos and papules and pustules in 15.2%, pustules, papules and nodules 2.9%, as shown in Figure 1.

The face was the most common site of involvement of acne lesions in 95.8%, while shoulders 47%, the upper back 35.6%, and the chest 21.7%. Comedons, papules and pustules were distributed over all these areas, but nodules were seen on the back and the chest more than the face, and papules and pustules represent 34.1% mainly concentrated on the face as shown in Figure 2.

Figure 3 illustrates the factors that were claimed by acne patients as aggravating their acne condition

during the interview questionnaire. Emotional factors such as social, academic and emotional stress were mentioned by 60.1% of acne patients, while 2.2% claimed that there's a decrease in acne lesions during stress, and the rest (37.7%) claimed that there's no effect.

Also exposure to sunlight was reported to aggravate acne in 49.3% while the same percentage told that there's no effect, and excessive heat during summer time was pointed to aggravate acne in 46.4% of patients, while 15.9% of the patients stated that their acne became worse during winter time. Many acne patients, 30.4%, claimed that excessive sweating was an exacerbating factor for their acne, while 58% didn't, and 11.6% claimed that sweating improve their lesions.

43.5% of the patients claimed that local pressure and squeezing worsen their lesions, in the contrary, 55.1% denied any effect.

Premenstrual exacerbation of acne was experienced by 81.5% of female acne patients, while 13.8% of them had noticed no effect.

Among dietary factors, most acne patients stated that their acne was exacerbated by eating chocolate, nuts, fatty food, and spices on regular daily pattern.

Types of food that were most often believed by acne patients to aggravate their acne condition: nuts (60.9% of patients), chocolate (80.4%), fatty food (38.4%), milk and milk derivatives (24.6%), fruits (18.1%), spices (31.9%), juices and cola (26.8%), sweets (20.3%), red meat (15.9%). 81.9% of acne patients reported that their acne condition became better when they eat vegetables and fruits.
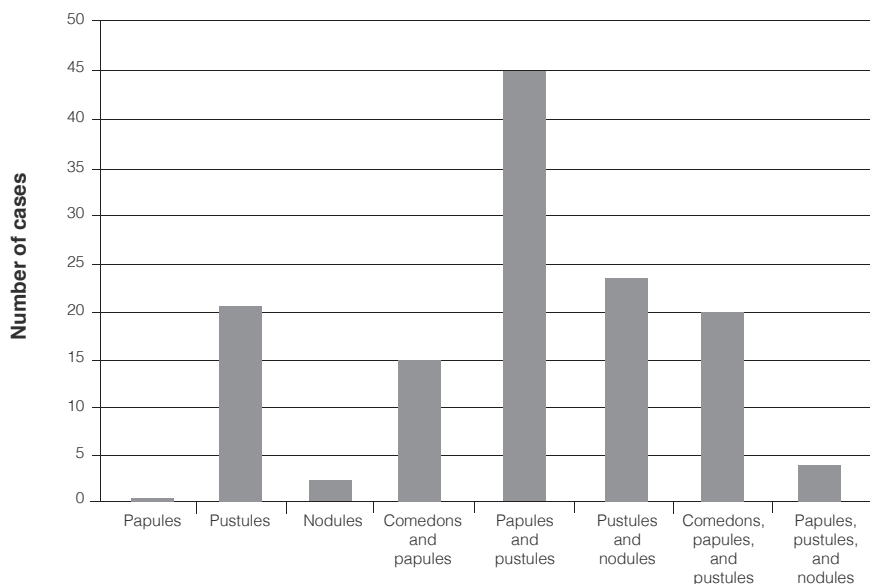
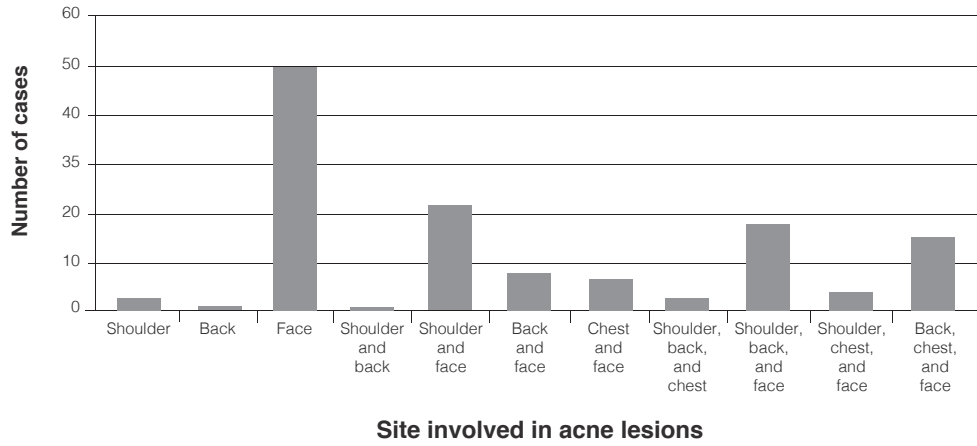## Fig 1. Types of acne lesions
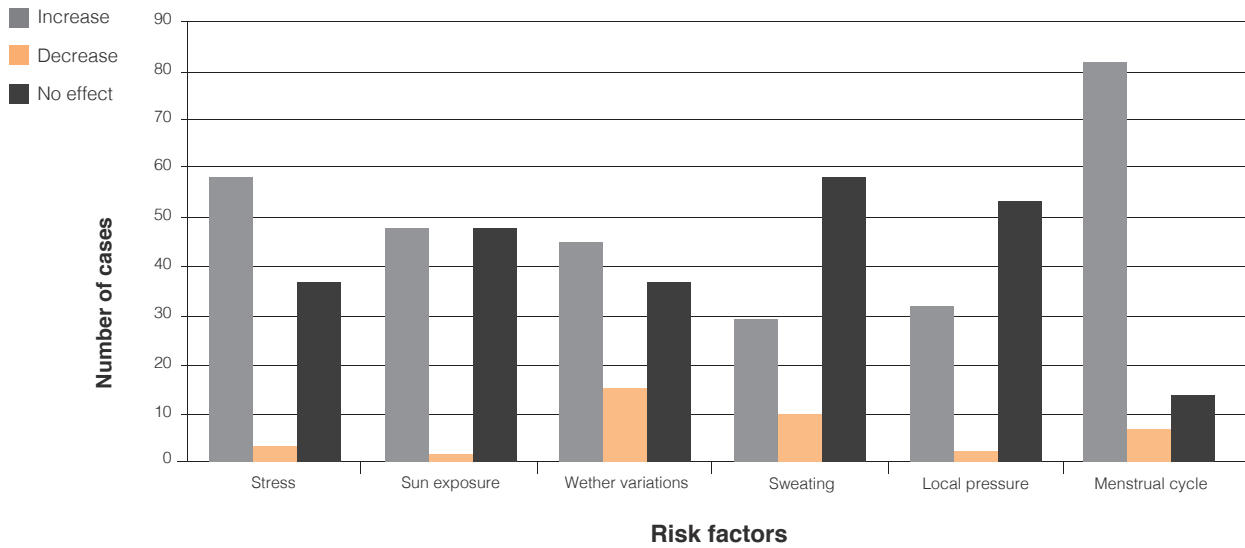
Fig 2. Distribution of acne lesions

Site involved in acne lesions

Fig 3. Risk factors affecting acne condition

Risk factors

Result of acne pathogens

Acne lesions had positive cultures in 87.7% samples. *P.acnes* was in 42.1%, (51 patients) and *Staph. epidermidis* in 23.1%, (28 patients), and *Staph. aureus* in 34.7%, (42 patients).

Association between known risk factors and microorganisms colonization

Table 1 illustrates the frequencies for each risk factor and specific microorganisms for comparison between relationship of risk factors among acne patients and the specific type of microorganisms.

Important points to view were positive family history which was associated with 36% of cases with *P. acne*. As for skin lesion types, comedoe skin lesions were correlated to *Staph. aureus*, papule, pustule and nodule with *P. acne*. Face, back, shoulders and chest lesions showed mainly growth of *P. acne* followed by *Staph. aureus* then Staph. epidermidis. When pointing on diet 50% of patients who consume sweets, juices and cola had lesions that were mostly colonized with *P. acne*. Worsening the condition with sunlight exposure increases with *P. acne* but not Staph. spp. local pressure and summer season appear to exacerbate the condition anyway.

Discussion

Demographic and risk factors variations. Among 138 acne patients enrolled in this study, and despite that the studies suggest a slight female predominance than male (Diao *et al.*, 2007), this difference in the predisposition to the disease is related to the androgen balance, increased estrogen levels is correlated to the disease (Unal *et al.,* 2015). In our study the prevalence of this skin disorder was slightly higher in male than female, with 52.2% (72) male, and 47.8% (66) female, this may be due to that the sampling techniques for this study is a non probability sampling, which includes all the acne patients presented to two clinics in northern West Bank, and may not reflect the real distribution between Palestinian males and females.

The age of acne patients who were included in this study ranged from 15-34 years with a mean of 19.4 years, +/- 3.38. Acne is not only a disease of adolescence, it also presents in adults of both sexes this could be to hormonal changes as high estrogen levels in pregnancy or stress conditions that affects the sebum secretion (Fung *et al.*, 2001). Late-onset acne, (acne that developed after age 21 years) has been shown to be the result of abnormalities in plasma androgens (Darley et al., 1982; Clark *et al.*, 1997).

In our work we found 64 of acne cases (46.3%) with 33 male patients (45.8%) and 31 female patients (47%), with late-onset acne which highlights the higher percentage of the adult population that experience this kind of disturbance in sex hormones.

The age at acne onset in Palestinian patients was found to be slight earlier in females (16.8 years) than males (17 years), which might reflect the earlier onset of puberty in females and again the hormonal effect of estrogen. These results were similar to those reported in the Turkish study of college students aged from 14 to 20 years. They found that the mean ages at acne onset in girls and boys were 13.4 and 14.1 years respectively (Aktan *et al.*, 2000).

Genetic factors are very important in determining individual susceptibility to acne. About (62.3%) of our patients mentioned a family history of acne, (65.3% males and 59.1% females). The role of genetic factors is also reported by other researchers (Cunliffe, 1989; Cunliffe *et al.*, 1999). Acne lesions comedos, papules, pustules and nodules were distributed all over the face (95.8%), the upper back (35.6%), the chest (21.7%) and the shoulders (47%) of our patients. These areas are known to be enriched with sebaceous glands that provide a lipid-rich environment for the proliferation of *P. acnes* bacteria, which contribute to the inflammatory process of acne (Marks *et al.*, 2003). Like other skin diseases, Acne might be influenced by the nutritional status of the patient. It has been found that shortage in the essential fatty acids, Linoleic acid and Linolenic acid, causes follicular hyperkerato¬sis in the pilosebaceous duct, and increases the transepidermal water loss in the skin of acne patients (Truswell *et al.*, 1985). This supports the suggestion that *Acne Vulgaris* might be aggravated by the consumption of a diet rich in saturated fats and monosaturated acids and low in polyunsaturated fatty acids as we found that patients reported acne exacerbation on eating, nuts (60.9%), chocolate (80.4%), fatty food (38.4%), while (81.9%) of acne patients stated that their acne condition became better when they eat fresh vegetables and fruits, which also contain vitamins that may be beneficial for improving and modifying acne (Ayres & Mihan, 1981).

Although, there exists convincing data supporting the role of high-glycemic-index foods in influencing hormonal and inflammatory factors, which can increase acne prevalence and severity (Ferdowsian & Levin, 2010), only (26.8%) for juices and carbonated drinks (normal sugar cola), and (20.3%) for sweets, of the patients reported acne exacerbation. Also, although some studies suggested that natural hormonal components of milk and/or other bioactive molecules in milk could exacerbate acne (Adebamowo *et*

Table 1. Correlation of microorganism presence with known risk factors

Variable		Microorganism culture results			
		No growth	S. epidermidis	S. aureus	P. acne
Gender	M	11.1%	23.6%	33.3%	31.9%
	F	13.6%	17%	27.3%	42.4%
Family history	Positive	14%	19.7%	30.2%	36%
	Negative	9.6%	21%	30.8%	38.5%
Type of skin lesions	Comedons	14%	18.9%	40.6%	27%
	Papule	14%	16.8%	33.7%	34.8%
	Pustule	10.9%	15%	28.6%	39.5%
	Nodule	6.4%	19.3%	25.8%	48.3%
Distribution of lesions	Face	12.3%	20.4%	32%	40.2%
	Back	12%	18%	30%	40%
	Shoulder	17.6%	17.6%	23.5%	41.3%
	Chest	12.5%	7%	27.5%	53%
Diet	Chocolate	13.5%	22.5%	27%	36.9%
	Milk	11.8%	26.4%	20.6%	41.2%
	Nuts	16.7%	14.2%	29.8%	39.3%
	Fruits and vegetables	12%	16%	32%	40%
	Fatty food	13.2%	22.6%	22.6%	41.5%
	Spices	15.9%	22.7%	27.3%	34.1%
	Juices and cola	8.1%	10.8%	32.%	48.6%
	Sweets	7.1%	14.3%	28.6%	50%
	Red meat	4.5%	18.2%	50%	27.3%
Sunlight	Increase	11.8%	25%	25%	38.2%
	No effect	13.2%	16.2%	35.3%	35.3%
Sweating	Increase	14.3%	21.4%	33.3%	31%
	No effect	13.8%	20%	25%	41.3%
Local pressure	Increase	5%	23.3%	33.3%	38.3%
	No effect	14.4%	17.1%	27.6%	36.8%
Variation in summer and winter	Summer	14%	12.5%	34.4%	39.1%
	Winter	9%	36.3%	27.3%	27.3%
Stress	Increase	14.5%	24.1%	25.3%	36.1%
	No effect	9.6%	13.5%	30.4%	36.5%

al., 2006; Adebamowo *et al.*, 2008), only (24.6%) of the patients stated acne exacerbation on ingestion milk and its derivatives.

As stress was reported in many studies to exacerbate acne lesions by increase of sebum production at the time of stressful events (Chen et al., 2007), our findings among the study patients support this concept by finding that emotional factors such as social, academic and emotional stress were mentioned by 60.1% of acne patients, as an aggravating factor for their lesions.

Acne is considered a multi-factorial etiology, where excessive exposure to sunlight and excessive summer heat exposure, excessive sweating, and local pressure and squeezing were considered as an aggravating events for acne(Abdel-Latif Nemr *et al.*, 2006; Thiboutot, 2009). And among our patients, the findings support this as 49.3% of the patients reported worsening of their acne on excessive exposure to sunlight, excessive summer heat exposure in (46.4%) of patients, excessive sweating in (30.4%), and application of local pressure and squeezing (43.5%) of the patients.

Hormonal status, especially those common changes in teenagers, pregnant women, and before menstruation have a close link between acne and its level, mainly Testosterone hormone which has been identified as a chief trigger of acne development, by stimulating pilosebacious gland for sebum secretion (Shalita, 2001; Grabbe *et al.*, 2007).

This is not exclusive to androgenic hormones, since a recent study prove that half of all women expe-

rience premenstrual flares of their acne in which, overall, 177 of 400 women (44%) reported premenstrual acne according to that study. In this study female acne patients reported a premenstrual exacerbation of acne by (81.5%), of total female patients, which supports the concept.

Acne pathogens

Acne is a chronic inflammatory disease of pilosebaceous unit, the involvement of microorganisms in the development of acne has a long and checkered history. It was suggested that microorganisms colonizing over skin lesions, especially, *P. acnes* was involved in the pathology of the disease (Bojar & Holland, 2004). However, a microbiological survey have been undertaken of comedoes isolated by micro-dissection, of closed comedoes 10.7% and of open comedoes 7.1% did not contain *Pityrosporum spp., Propionibacterium spp. or Staphylococcus spp.*, which is mostly associated with acne (Cunliffe *et al.*, 1985). In our study, swabs which were taken from acne lesions had positive cultures in (87.7%), including *Propionibacterium spp. or Staphylococcus spp.*, while those with no growth were 12.3%, which supports the data and suggests the involvement of microorganisms in pathogenesis of acne.

And since *P. acne* is the most common pathogen that had been suggested in participating in the pathogenesis of acne lesions (Ashbee *et al.*, 1997; Kim *et al.*, 2002; Białecka *et al.*, 2005), in our study among positive cultures, P. acnes was the most common isolated and cultured microorganisms form acne lesions in 42.1%, (51 patients), then Staph. epidermidis in 23.1%, (28 patients),

and S. aureus in 34.7%, (42 patients), which also colonized them, but the main pathogenesis was suggested with *P. acne.*

Association between common risk factors and microorganisms colonization

As illustrated in Table 1, which demonstrated the frequencies for each risk factor in the correlation with each, specific type of microorganism colonizing over acne lesions, we found that concerning *Staph. epidermidis*, it's colonized with those who had winter exacerbation with (36.3%) and milk ingestion with (26.4%), and *Staph. aureus* cultured mostly in comedos acne lesions in (40.6%), and the most common among male patients in (33.3%). While *P. acne*, the most common among female patients with (42.4%), and highly associated with high-glycemic diet, juices and cola in 48.6% and sweets in 50%.

But when testing the real association between those all known risk factors and the types of microorganisms using chi-square, we found that there's no significant association between the known risk factors used in this study with any of the specific types of microorganisms colonizing over the acne lesions, and due the lack of published data in this objective specifically (as the investigators best knowledge), we can't compare our results with previous ones.

The significance of this result came from the involvement in the treatment of *Acne Vulgaris*, as it makes no difference to consider any variable tested in this study, as a special landmark in the treatment of specific type of microorganisms.

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