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ORIGINAL ARTICLE

Cutibacterium Acnes Antibiotics Sensitivity: Can Basic Characteristics of Acne Vulgaris Patients Predict It?

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ABSTRACT

Background: Acne is a chronic inflammatory disorder affecting the pilosebaceous apparatus due to androgen-induced sebum formation, modified keratinization, inflammation, and bacterial colonization of hair follicles on the face, neck, chest, and back by *cutibacterium acnes* (*C. acnes*). The resistant *C. acnes* strains cause deep involvement of acne, failure in treatment or rapid relapse.

Methods: This study was conducted on 48 samples collected from 48 patients with acne vulgaris lesions (papules and pustules). *C. acnes* isolation, biofilm detection and antibiotic susceptibility testing (for both drugs: azithromycin and doxycycline) were applied on all samples then were compared to age, sex, severity of lesions, and hormonal disturbance. **Results:** Samples of all patients showed higher susceptibility to doxycycline than azithromycin. Statistically significant relationship was detected between Doxycycline sensitivity and age with more susceptibility below the age of 25. Another statistically significant correlation between Azithromycin and two parameters; hormonal changes and severity with more resistance in patients with hormonal disturbance and moderate to severe cases. **Conclusion:***C. acnes* is more susceptible to doxycycline than azithromycin. Regarding doxycycline susceptibility, it is inversely related to age. Regarding azithromycin susceptibility, it is less in patients with hormonal disturbance and those with severe disease.

Keywords: Acne vulgaris, *Cutibacterium acnes*, Antibiotics sensitivity, biofilm formation

INTRODUCTION

Acne vulgaris (AV) is a dermatological disease with many factors. Although it usually manifests during puberty and worsens throughout adolescence, it can occur at any age [1]. The Global Burden of Disease Study 2010 documented that acne vulgaris is the eighth most familiar skin disorder, with an estimated prevalence worldwide (for all ages) of 9.38% [2].Many factors point to acne severity as young age of onset of acne, and positive family history. Numerous factors could lead acne to exacerbate like hormonal changes, emotional stress, smoking and diet [3].

The pathogenesis has many factors mainly four primary elements as following: hyperseborhea, keratinocytes hyperproliferation, *C. acnes* colonization and inflammatory process mediated as an immunological reaction to *C. acnes*[1]. *C. acnes* has multiple virulence factors which contribute to antibiotic resistance. This occurs by multiple methods including mainly biofilm production and genetic changes [4].Antibiotics in management of acne had antibacterial and anti-inflammatory actions.

They decrease *C. acnes* counts, so prevent formation of inflammatory mediators [5]. Antimicrobial resistance among bacteria is considered the most significant health issue in the world [6]. Many studies correlate between antibiotic resistance and biofilm formation. It was found that biofilm formation could be a way for an organism to overcome unfriendly environmental effects including antibiotics and antimicrobial agents [7].

In this study we aimed to detect the effect of basic characters of patients on biofilm formation and antibiotics sensitivity of *C. acnes*.

METHODS

This study is a cross-sectional study performed on 48 patients who were established clinically as acne vulgaris patients.

Data Collection and Procedures:

History taking was performed regarding age, sex, age of onset and hormonal disturbance (regarding female patients). female patients with hormonal disturbance were diagnosed as polycystic ovarian syndromes (PCOs) depending on hormonal profile (LH\FSH ratio and free testosterone) and ultrasound.

Clinical evaluation of acne lesions concerning kinds; count, distribution, and severity had been documented. The degree of severity had been defined by the global acne grading system (GAGS) [8]. The overall severity score was developed from the addition of six regional sub-scores. Each is obtained by multiplying the factors-2 for the forehead, 2 for each cheek, 1 for the nose, 1 for the chin, and 3 for both chest and back by the most severe lesion within each region.

Sample collection:

Samples had been gathered from acne lesions in the face (papules and pustules) after disinfecting the skin with 70% alcohol. Sterile cotton swabs were used to obtain samples from the lesions. Considering closed papules, and pustules, the lesion had been nicked with a sterile needle under aseptic technique. Swabs had been positioned in a Thioglycolate media tube [9].

Isolation and detection:

Thioglycolate tubes containing inoculated sample swabs had been kept in an incubator at 37 °C for 7 days and then sub-cultured on blood agar anaerobically using OXOID jar enclosing Anaerogen kit (Oxoid- Germany) for 7 days at 37 °C. Detection of bacterial colony had been done regarding macroscopic appearance, Gram staining and biochemical outcomes [9]. Typical C. acnes colonies are grayish white, pinpointed to colonies of 0.5 mm in diameter; Gram-positive bacilli with different shapes, reactive indole, catalase, and nitrate reduction tests [10, 11]. Isolates were stored in 40% glycerol broth at -80°C [12].

Antibiotic susceptibility testing:

All strains were tested for antibiotic susceptibility to azithromycin and doxycycline. The minimal inhibitory concentration (MIC) had been evaluated by the broth microdilution technique according to clinical and laboratory standards institute guidelines [13]. The MIC had been determined as the least antibiotic concentration that inhibits observable outgrowth of a microorganism. The MIC below cutoff point value was defined as susceptibility. The cutoff point for azithromycin was $\leq$ 0.5 µg/mL and for doxycycline was $\leq$ 4 µg/mL [14].

Testing biofilm- developing capability of C. acnes:

Biofilm developing capability of *C. acnes* colonies was adapted from [15, 16]as follow:

A. A total of 5 ml of Reinforced Clostridial Medium (RCM) had been mixed with a loop of test organism from a growth on blood agar.

B. The turbidity had been then adjusted to an OD600 of between 0.08 - 0.1(equivalent to approximately 1 x 10^7 CFU/ml) and then diluted 1in 100.

C. Each well in the microtiter plate had been filled with 200 µL of microbial suspension; sterile RCM had been handled as a negative control and *Staphylococcus epidermidis* ATCC 35984 had been handled as positive control [17]. The plate had been

kept in an incubator statically for 72 hours at 37°C in anaerobic condition to help adherence of bacterial colonies to the plate and biofilm development.

D. The bacterial solution of each well had been carefully eliminated. The wells had been washed (three times) with 200 µL of Phosphate buffered saline to remove the unattached bacteria. After that, any biofilm present had been stabilized with 200 µL of 99% methanol for 15 min then the methanol suspension had been eliminated and the plates had been made dry by air.

E. Attachment of bacteria to the culture plate had been identified by crystal violet. 200 µL of 1% w/v crystal violet had been included in each well. The plates had been incubated at room temperature for 20 min. Additional dye had been washed with deionized water (3 times with 200 µL) and the plates had been left to dry by air for 20 minutes.

F. 200 µL of 30% v/v acetic acid had been included in the wells to solubilize incorporated dye. The ODs of the adherent bacteria had been established by a microplate reader using an absorbance wavelength at 590 nm.

G. The results were obtained according to [18]; Interpretation of the results as follows:

- Non-biofilm producer = OD ≤ODc
- Biofilm producer = OD >ODc

Tests had been performed in triple manner & the results had been expressed as means ± SD.

Ethical and administrative considerations: The protocol had been proposed and permitted by the Institutional Review Board (IRB). Printed approval had been given by each member. The study was carried out according to the code of Ethics of the World Medical Association (Declaration of Helsinki) for studies including humans.

STATISTICAL ANALYSIS

All results had been evaluated by SPSS 26.0. Quantitative results had been stated as the mean ± SD & median (IQR). Qualitative results had been stated as frequencies (number)& percentage. Chi-square test was made. P-value < 0.05 had been determined statistically significant (S), p-value ≥ 0.05 had been determined statistically non-significant (NS).

RESULTS

A group of 48 patients had been enrolled in this study; as shown in table (1) (41.7%) of cases ranged from 15 to 20 years old, while (33.3%) of cases ranged from 26 to 30 years old and (25%) of cases ranged from 21 to 25 years. Majority of cases (75%) were females and (25%) of cases were males.Regarding the age of onset of acne, more than half of cases (66.7%) were aged less than 25 years when the lesions started and about (33.3%) were more than 25 years old.Regarding hormonal changes, about (44.4%) of the female cases were positive polycystic ovary syndrome (PCOS). As illustrated in this table, nearly half of cases (54.2%) were graded as mild severity of the disease, (41.7%) of cases were graded as moderate and the rest (4.2%) of them were graded as severe cases. As regard antibiotic Susceptibility, cases showed higher doxycycline susceptibility (60.4%) than azithromycin susceptibility (25%).

Most cases were biofilm former (83.3%). But There had been non-statistically significant correlation between biofilm growth, and age, sex, age of onset, hormonal changes, and severity of disease (p>0.05) (Table 2).

On one hand, there was non-statistically significant relation between azithromycin sensitivity and age, sex, and age of onset (p>0.05) while there was statistically significant relation between Azithromycin and both; hormonal changes and severity with more resistance in patients with hormonal disturbance and those with moderate to severe disease (Table 3).

On the other hand, there had been non-statistically significant relation between doxycycline sensitivity, and sex, age of onset, hormonal changes, and severity (p>0.05), while there had been statistically significant relation between doxycycline sensitivity and age with more susceptibility below the age of 25(Table 4).

Assuming that the overall doxycycline sensitivity was significantly more than the overall azithromycin sensitivity in all samples, the detailed analysis of data showed

that the patients in age groups below 25, female patients, patients with PCO, and patients with moderate disease severity were more susceptible to doxycycline than azithromycin (Table 5).

Table (1): Basic characteristics of the studied group (patients with acne vulgaris).

Study group (n=48)			
Category		**No.**	**%**
Age	(15-20)	20	41.7
	(21-25)	12	25
	(26-30)	16	33.3
Sex	Female	36	75.0
	Male	12	25
Age of onset (Y)	<25 years	32	66.7
	>25 years	16	33.3
Hormonal changes (PCO) (n=36)	PCO +ve	16	44.4
	PCO -ve	20	55.6
Severity	Mild	26	54.2
	Moderate	20	41.7
	Severe	2	4.2
Biofilm	Former	40	83.3
	Non-former	8	16.7
Azithromycin	Sensitive	16	33.3
	Resistant	32	66.7
Doxycycline	Sensitive	29	60.5
	Resistant	19	39.5

Table (2): Relation between biofilm and different parameters among the studied group.

Variable			**Biofilm**		**tests**
			Former (n=40)	**Non-Former (n=8)**	**P value**
Age	(15-20)	**N**	18	2	0.487
		%	45.0%	25.0%	
	(21-25)	**N**	10	2	
		%	25.0%	25.0%	
	(26-30)	**N**	12	4	
		%	30.0%	50.0%	
Sex	Female	**N**	30	6	1.00
		%	75.0%	75.0%	
	Male	**N**	10	2	

		%	25.0%	25.0%	
age of onset	<25 years	**N**	28	4	0.273
		%	70.0%	50.0%	
	>25 years	**N**	12	4	
		%	30.0%	50.0%	
hormonal changes (PCO) (females only)	PCO +ve	**N**	12	4	0.230
		%	40.0%	66.7%	
	PCO -ve	**N**	18	2	
		%	60.0%	33.3%	
severity	Mild	**N**	22	4	0.744
		%	55.0%	50.0%	
	Moderate	**N**	16	4	
		%	40.0%	50.0%	
	Severe	**N**	2	0	
		%	5.0%	0.0%	

Table (3): Relation between Azithromycin sensitivity and different parameters among the studied group.

Variable			**Azithromycin**		**tests**
			Sensitive (n=12)	**Resistant (n=36)**	**P value**
Age	(15-20)	**N**	6	14	0.701
		%	50.0%	38.9%	
	(21-25)	**N**	2	10	
		%	16.7%	27.8%	
	(26-30)	**N**	4	12	
		%	33.3%	33.3%	
Sex	Female	**N**	10	26	0.441
		%	83.3%	72.2%	
	Male	**N**	2	10	
		%	16.7%	27.8%	
age of onset	<25 years	**N**	8	24	1.0
		%	66.7%	66.7%	
	>25 years	**N**	4	12	
		%	33.3%	33.3%	
hormonal changes (PCO) (females only)	PCO +ve	**N**	2	14	**0.047***
		%	20.0%	53.8%	
	PCO –ve	**N**	8	12	
		%	80.0%	46.2%	
severity	Mild	**N**	12	14	**0.001***
		%	100.0%	38.9%	
	Moderate	**N**	0	20	
		%	0.0%	55.6%	
	Severe	**N**	0	2	
		%	0.0%	5.6%	

***Significant difference.

Table (4): Relation between Doxycycline sensitivity and different parameters among the studied group.

Variable			Doxycycline		tests
			Sensitive (n=29)	Resistant (n=19)	P value
Age	(15-20)	**N**	13	7	**0.042***
		%	44.8%	36.9%	
	(21-25)	**N**	10	2	
		%	34.5%	10.5%	
	(26-30)	**N**	6	10	
		%	20.7%	52.6%	
Sex	Female	**N**	23	13	0.499
		%	79.3%	68.4%	
	Male	**N**	6	6	
		%	20.7%	31.6%	
age of onset	<25 years	**N**	19	13	0.679
		%	65.5%	68.4%	
	>25 years	**N**	10	6	
		%	34.5%	31.6%	
hormonal changes (PCO)	PCO +ve	**N**	9	7	0.221
		%	39.1%	53.8 %	
	PCO -ve	**N**	14	6	
		%	60.9 %	46.2 %	
severity	Mild	**N**	16	10	0.348
		%	55.2%	52.6 %	
	Moderate	**N**	11	9	
		%	37.9 %	47.4 %	
	Severe	**N**	2	0	
		%	6.9 %	0%	

*Significant difference

Table 5: Comparison between Azithromycin and Doxycycline sensitivity.

Variable			Azithromycin		Doxycycline		tests
			Sensitive (n=12)	Resistant (n=36)	Sensitive (n=29)	Resistant (n=19)	P value
Age	(15-20)	**N**	6	14	13	7	**0.027***
		%	50.0%	38.9%	44.8%	36.9%	
	(21-25)	**N**	2	10	10	2	**0.001***
		%	16.7%	27.8%	34.5%	10.5%	
	(26-30)	**N**	4	12	6	10	0.6
		%	33.3%	33.3%	20.7%	52.6%	
Sex	Female	**N**	10	26	23	13	**0.002***

		%	83.3%	72.2%	79.3%	68.4%	
	Male	**N**	2	10	6	6	0.08
		%	16.7%	27.8%	20.7%	31.6%	
age of onset	<25 years	**N**	8	24	19	13	**0.005***
		%	66.7%	66.7%	65.5%	68.4%	
	>25 years	**N**	4	12	10	6	**0.03***
		%	33.3%	33.3%	34.5%	31.6%	
hormonal changes (PCO) **(females only)**	PCO +ve	**N**	2	14	9	7	**0.009***
		%	20.0%	53.8%	39.1 %	53.8 %	
	PCO – ve	**N**	8	12	14	6	0.6
		%	80.0%	46.2%	60.9 %	46.2 %	
severity	Mild	**N**	12	14	16	10	0.27
		%	100.0%	38.9%	55.2%	52.6 %	
	Moderate	**N**	0	20	11	9	**0.001***
		%	0.0%	55.6%	37.9 %	47.4 %	
	Severe	**N**	0	2	2	0	0.2
		%	0.0%	5.6%	6.9 %	0%	

DISCUSSION

In the current study forty-eight patients of acne vulgaris were included. Most patients belonged to age ranged from 16 to 30 years old. Among 48 patients, 36 patients were females (75%), which can be due to more consciousness on the part of females as compared to males.Regarding antibiotic sensitivity, it was established that most *C. acnes* isolates showed higher susceptibility to doxycycline (60.4%) than azithromycin (25%). A previous report detected high susceptibility of *C. acnes* to doxycycline (73.5%), and more resistance of *C. acnes* to azithromycin (49%) which is the same in our results [20]. However, the accurate etiology of *C. acnes* resistance to antibiotics was unclear, the long course of the disease, drug administration method, recurrent consumption of antibiotics and bad treatment compliance are elements that participate in antibiotic resistance.

Concerning the antibiotic susceptibility of *C. acnes* in relation to age, this study found that there had been statistically significant relation between doxycycline and age as (44.8%) of cases sensitive to doxycycline were between 15 to 20 years. Also, Abdel Fattah *et.al*, [19] found a relationship between patientsage and antibiotic sensitivity.Althoughwe detected statistically significant relation between Azithromycin and disease severity as

moderate cases were usually resistant to Azithromycin, Nakase *et al.*[20] found that there was no significant relation between severity and antibiotic sensitivity. Regarding biofilm formation, this study stated that most isolates were biofilm former (83.3%) with no significant relation between biofilm formation and severity. This agreed with Kartika, *et al*.[21] study.

This study provides additional insight on the impact of basic characteristics and acne severity on antibiotic sensitivity in patients with acne vulgaris. *C. acnes* was more susceptible to doxycycline than azithromycin. Doxycycline susceptibility was inversely related to age. Azithromycin susceptibility was less in patients with polycystic ovary disease, age group more than 25 years and lesions with moderate severity.

CONCLUSION

C. acnes is more susceptible to doxycycline than azithromycin. Regarding doxycycline susceptibility, it is inversely related to age. Regarding azithromycin susceptibility, it is less in patients with hormonal disturbance and those with severe disease.

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