

Estimation of Demodex Folliculorum Infestation in Acne Vulgaris and Its Relation to Acne Severity

Thesis

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BY

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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LIST OF ABBREVIATIONS

16S rDNA	: 16S ribosomal deoxyribonucleic acid.
ACTH	: Adrenocorticotrophic hormone.
AD	: Anno Domini.
AIDS	: Acquired immune deficiency syndrome.
NOVA	: Analysis of variance.
ATP	: Adenosine triphosphate.
BMJ	: British Medical Journal.
CLSM	: Confocal laser scanning microscopy.
CMAJ	: Canadian Medical Association Journal.
CRH	: Corticotropin-releasing hormone.
CTP	: Cellophane tape preparations.
D.b.	: Demodex brevis.
D.f.	: Demodex folliculorum.
D	: Demodex.
dATP	: Deoxyadenosine triphosphate.
dCTP	: Deoxycytidine triphosphate.
dd H₂O	: Double distilled water.
dGTP	: Deoxyguanosine triphosphate.
DHEA. S	: Dehydroepiandrosterone Sulphate.
DHEA	: Dehydroepiandrosterone.
DHT	: Dihydrotestosterone.
DNA	: Deoxyribonucleic acid.
dNTPS	: Deoxynucleotide triphosphates.
dTTP	: Deoxythymidine triphosphate.
EGFR	: Epidermal Growth Factor Receptor.
EtBr	: Ethidium Bromide.
gDNA	: Genomic Deoxyribonucleic acid.
GnRH	: Gonadotropin-Releasing Hormone.
GTP	: Guanosine triphosphate.
H & E	: Haematoxylin and Eosin.
HBD-2	: Human Beta Defensin-2.
HIV	: Human Immunodeficiency Virus.
HLA Cw2	: Human Leucocytic Antigen.
HSD	: Hydroxy Steroid Dehydrogenase.

IgD	: Immunoglobulin D.
IGF-1	: Insulin like Growth Factor-1.
IGF-1R	: Insulin-Like Growth Factor-1 Receptor.
IL	: Interleukin.
Kb	: Kilobit.
LMMP	: Lab of Molecular Medical Parasitology.
MM	: Master Mix.
MSH	: Melanocyte Stimulating Hormone.
NK cells	: Natural killer.
nM	: Nanomole.
NPs	: Neuropeptides.
P value	: Probability value.
P. acnes	: Propionibacterium acnes.
P38 MAP kinases	: p38 mitogen-activated protein kinases.
PAPA	: Pyogenic Arthritis, Pyoderma Gangrenosum and Acne Con
PASH	: Pyoderma Gangrenosum, Acne and Suppurative Hidradenitis.
PCR	: Polymerase Chain Reaction.
POMC	: Proopiomelanocortin.
PPAR	: Peroxisome Proliferator-Activated Receptor.
RAPD-SCAR	: Random Amplified Polymorphic DNA- Sequenced Characterized Amplified Region.
RCM	: Reflectance Confocal Microscopy.
ROS	: Reactive Oxygen Species.
SD	: Standard Deviation.
SP	: Substance P.
SPM	: Skin Pressurization Method.
SSSB	: Standardized Skin Surface Biopsy.
TBE	: Tris-Borate-Edta.
Th	: Thelper cell.
TLR	: Toll-Like Receptor.
TNF	: Tumor Necrosis Factor.
U.V	: Ultra Violet.
α	: Alpha.
β	: Beta.

ABSTRACT

Background: Acne vulgaris is a disease of the pilosebaceous unit resulting from interplay of different factors as seborrhea, *P. acnes* colonization, hyperkeratinization of the follicular duct and inflammation. Demodex have been reported to cause blockage of follicles and tubules of sebaceous glands, reactive hyperkeratinization, and epithelial hyperplasia. In addition, they have also been demonstrated to act as mechanical vector for bacteria, cause host inflammatory response of chitin layer as foreign object, and create a humoral and cellular immune response thanks to their wastes.

Aim: The aim of the study was to compare the presence of demodex folliculorum between acne patients and normal subjects in order to clarify a possible association between demodex and acne vulgaris.

Patients and methods: The present study included 30 patients with acne vulgaris and 10 controls selected from the dermatology outpatient clinic, Kasr El Ainy hospital, Cairo University. The patients were divided according to the clinical severity of acne into three groups; group (A): mild acne, group (B): moderate acne and group (C): severe acne. Skin biopsies were taken from both patients and controls for pathological examination to detect pathological severity of acne lesions and possible detection of demodex intra or extra follicular as well as PCR examination for demodex folliculorum detection. Ten out of the thirty patients were randomly selected and given metronidazole 500mg twice daily for one month and were reevaluated clinically, pathologically and by PCR. Most of them improved clinically as well as pathologically following metronidazole therapy in spite the absence of demodex in these patients. This may be related to the anti-inflammatory effects of metronidazole.

Results: PCR revealed the presence of demodex in a similar percentage of both acne patients and control subjects (10%) with no significant difference ($P = 1$). Presence of demodex was higher in the mild acne group (group A) compared to other groups with significant difference ($P = 0.025^*$). The three acne patients in whom demodex were detected showed mild inflammatory infiltrate in pathological severity with no significant difference ($P = 0.087$). None of age, sex, course or duration had a significant effect on the presence of demodex ($P = 0.518, 1, 0.082$ and 0.174), respectively. Demodex folliculorum couldn't be detected in any of the patients or controls either intra or extra follicular. Most of patients improved clinically as well as pathologically following metronidazole therapy inspite the absence of demodex in these patients.

Conclusion: Based on the present research, Demodex folliculorum does not appear to have a significant association with acne vulgaris.

Keywords: Demodex, Acne vulgaris, PCR.

INTRODUCTION

Demodex is an ecto-parasite of pilo-sebaceous follicle and sebaceous gland, typically found on the face, the balding scalp, neck, ears and less commonly upper and medial region of chest and back (*Basta et al., 2002*).

Demodex folliculorum is more commonly localized to the face, while *D. brevis* is more commonly found on the neck and chest. Infestation with *D. folliculorum* is more common than with *D. brevis*, but the later has wider distribution on the body. *D. folliculorum* is usually found in the upper canal of the pilo-sebaceous unit at a density of \leq five/sq cm and uses skin cells and sebum for nourishment. *D. brevis*, on the other hand, burrows deeper into the sebaceous glands and ducts and feeds on gland cells (*Baima and Sticherling, 2002*).

Penetration of *Demodex* into the dermis or, more commonly, an increase in the number of mites in the pilo-sebaceous unit of $>$ five /sq cm, is believed to cause infestation, which triggers inflammation. Some authors consider the density of $>$ five mites per follicle as a pathogenic criterion (*Erbagci and Ozgoztasi, 1998*).

Infestation with this organism may play a role in many clinical entities, such as rosacea-like demodicosis, pustular folliculitis, papulo-pustular scalp eruptions, peri-oral dermatitis, and hyperpigmented patches of the face (*Dolenc-Voljc et al., 2005*).

Although Demodex infestation as a risk factor for rosacea has been confirmed, the association between acne vulgaris and Demodex infestation has not. Acne vulgaris is different from rosacea. They are two

distinct pathologies, with different symptoms, physiopathologies, causes and treatments (*Zhao et al., 2012a*).

Acne vulgaris is a multifactorial disease, originating in the pilosebaceous unit. The main symptoms include the microcomedone, comedone, papules, pustules, nodules, cysts and scars. However, until now, the etiology of acne vulgaris is still uncertain. It is currently considered as related to factors such as androgen, increasing sebum secretion, dyskeratosis of pilosebaceous duct, follicular orifice block up, proliferation of *Propionibacterium acnes*, or heredity. In recent decades, more and more clinical case-control studies have reported that *Demodex* infestation is associated with acne vulgaris, however much controversy persists (*William et al., 2012*).

AIM OF THE WORK

The aim of the study was to compare the presence of demodex folliculorum between acne patients and normal subjects in order to clarify a possible association between demodex and acne vulgaris and to detect the effect of treating demodex with metronidazole on demodex infestation and acne vulgaris.

ACNE VULGARIS

Acne is a chronic inflammatory disease of the pilosebaceous follicle that causes polymorph cutaneous lesions, among them comedones (as a primary lesion), papules, pustules, nodules and cysts which after regression may leave scars (*Semyonov, 2010*). The face, anterior trunk, and upper back are the most commonly affected areas due to their greater concentration of sebaceous glands. Acne is characterized by periods of exacerbation alternated with periods of stability [(*Schnop and Mempel, 2011*) and (*Huiying, 2015*)].

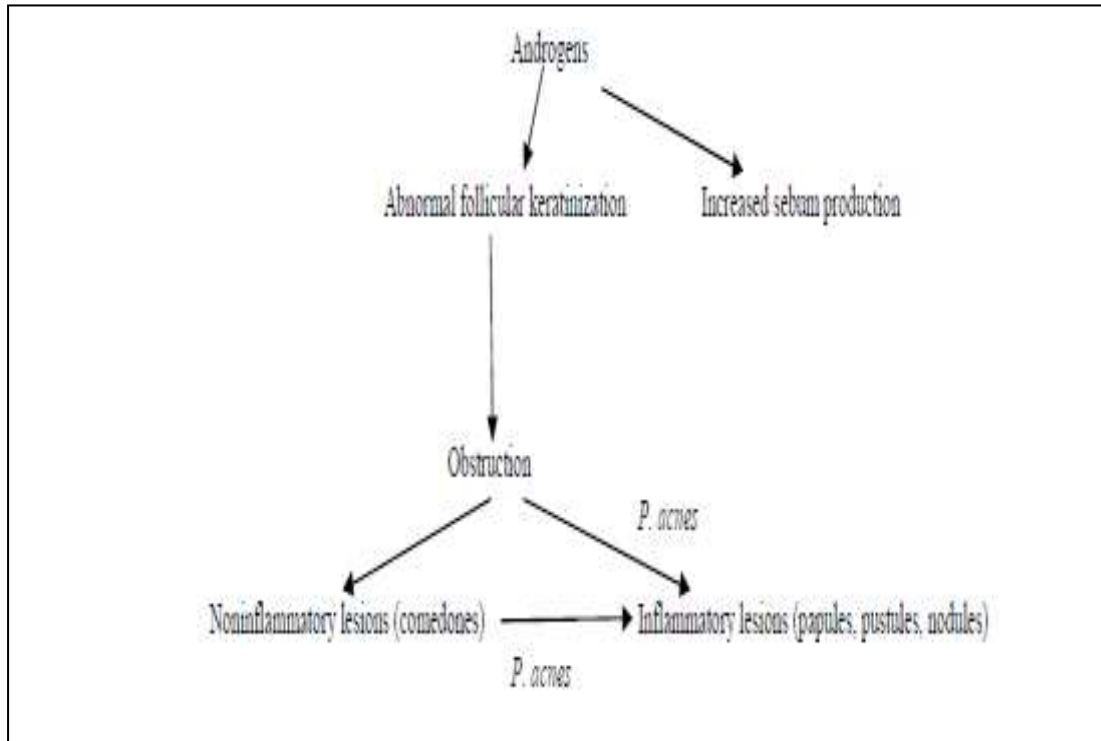
Acne is one of the most prevalent skin conditions, affecting more than 85% of teenagers. It is typically thought as a disease of youth but, 12% of femals and 3% of males may continue to have clinical acne in the third and forth decades of life [(*Yentzer et al, 2010*) and (*Friedlander et al, 2010*)]. Morbidity can be high and associated with disfigurement, pain, loss of confidence, and impairment of normal social and workplace function, with documented effects on quality of life including depression, dysmorphophobia, and even suicide (*Magin et al, 2010; Feton-Danou, 2010; Niemeier et al, 2010; Dunn et al, 2011 and Timms, 2013*).

Acne is a term derived from the Greek word “acme” in which the Greeks used this word to mean a point or a spot on the face. In the sixth century AD the term “acne” was first used by the emperor Justinian’s physician, Aetius Amidenus who translated it from Greek into Latin, and through these translations confusion arose regarding its original meaning (*Goodman, 2006*).

Aetiopathogenesis of acne:

The aetiopathogenesis of acne vulgaris is complex and is incompletely understood. At least four pathophysiologic events take place within the acne affected hair follicles as shown in [figure 1]: (1) Abnormal keratinization leading to follicular plugging (comedo formation), (2) Androgen-mediated stimulation of sebaceous gland activity (3) Proliferation of the bacterium *Propionibacterium acnes* (P.acnes) within the follicle and (4) Inflammation. In addition to these four basic mechanisms, genetic factors (*Longshore and Hollandsworth, 2003*), stress (*Zouboulis et al., 2002*) and possibly diet may influence the development and the severity of acne (*Davidovici and Wolf, 2010; Danby, 2010 and Bowe et al, 2010*).

Figure (1): Basic mechanisms involved in the pathogenesis of acne.



(Tahir, 2010).