INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous units and is characterized by seborrhea, the formation of comedones, erythematous papules and pustules, less frequently by nodules, deep pustules, or pseudocysts and in some cases, is accompanied by scarring. It is believed to be the most common disease of the skin. The condition usually starts in adolescence, peaks at the ages of 14 to 19 years and frequently resolves by mid-twenties ***(Adityan et al., 2009).***

Main etiologic factors for acne include hypercornification of the pilosebaceous duct, increased sebum production and colonization with Propionibacterium acnes (P. acnes) ***(Thiboutot, 2008).***

Lactoferrin is an iron binding glycoprotein which is known to have a role in decreasing inflammation and microbial infection. As an iron-binding protein, it sequesters iron that is essential for microbial growth, and it exhibits non-iron-dependent bactericidal activity by binding directly to the bacterial membrane and increase bacterial membrane permeability ***(Kruzel et al., 2007).***

Lactoferrin is considered a first-line defense protein involved in protection against a multitude of microbial infections and controlling the release of proinflammatory

cytokines. Therefore, Lactoferrin decreases skin inflammation due to its broad antibacterial and anti-inflammatory activities ***(Yalcin, 2006).***

Lactoferrin plays important role in modulating the inflammatory process by reducing production of some pro-inflammatory cytokines such as tumor necrosis factor (TNFα) or interleukins IL-1 and IL-6. And increased of anti-inflammatory interleukin IL-10 has also been reported in several cases ***(Håversen et al., 2002).***

Iron is essential as a catalyst for the production of reactive oxygen species. Therefore, lactoferrin can diminish the harmful influence of reactive oxygen species produced by leukocytes at the sites of inflammation ***(Ward et al., 2005).***

Few studies reported that many of acne patients had acceptable therapeutic response with a reduction of acne lesions with combined use of topical retinol and oral lactoferrin in mild and moderate acne ***(Gabriella et al., 2010)***. Oral lactoferrin in mild to moderate acne vulgaris is well tolerated and lead to an overall improvement in acne when administered as dietary supplement on a twice daily regimen ***(Edgar et al., 2011)***. Oral administration of Lactoferrin-enriched fermented milk ameliorates acne vulgaris with a selective decrease of triacylglycerols in skin surface lipids ***(Kim et al., 2010).*** However no studies directly assessed serum lactoferrin level in acne patients.

AIM OF THE WORK

The aim of this work is to assess the possible role of lactoferrin in acne patients which may offer a therapeutic option for those patients. Also relation of serum lactoferrin to acne severity will be assessed.

Chapter One

ACNE VULGARIS

Definition:

Acne is a term derived from the greek "acme" or prime of life, which is a common chronic skin disease involving blockage and/or inflammation of pilosebaceous units (hair follicles and their accompanying sebaceous gland), and can present as noninflammatory lesions, inflammatory lesions, or a mixture of both, affecting mostly the face but also the back and chest ***(Dawson et al., 2012).***

Epidemiology:

Acne is one of the most prevalent skin conditions, affecting more than 85% of teenagers. It typically starts at puberty and resolves slowly as the person reaches 20, although some people continue to have acne till 40 and 50 years. Men and women develop acne about equally. Persons of some races are affected more than others. Cystic acne is prevalent in the Mediterranean region ***(Ayer and Burrows, 2006).***

Pathogenesis:

Acne represents obstruction and inflammation of the sebaceous follicle. The primary and pathognomonic lesion of acne is the microcomedone, a microscopic lesion invisible to the naked eye, or lesions such as papule, pustule, or nodule ***(Bissonnette, 2011).***

The formation of a microcomedone requires a complex interplay of:

a) Follicular keratinazation.

b) Hyperplasia of sebaceous glands.

c) Propionibacterium acnes.

d) Inflammation ***(Knutsen-Larson et al., 2012).*** **Figure (1)**

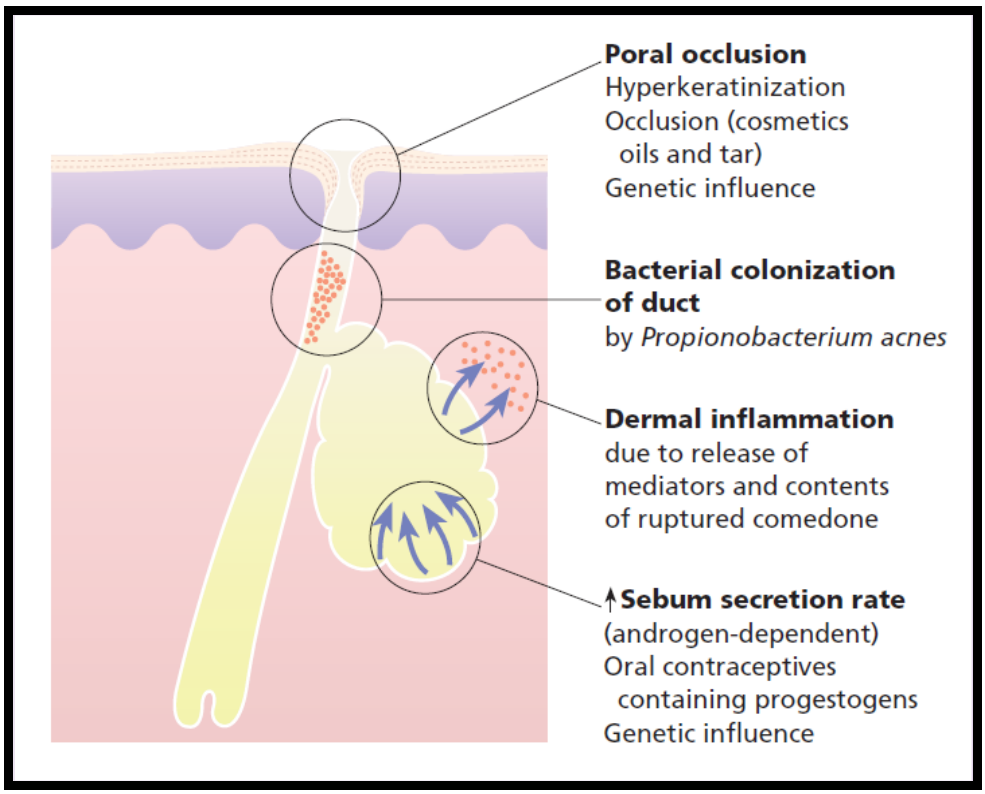

Figure (1): Pathogenetic factors causing acne ***(Cordain et al., 2002).***

a) Follicular Hyperkeratinization:

The primary change in the sebaceous follicle is the alteration in the pattern of keratinization within the follicle. Initial alteration is in the infra-infundibular portion where there is hyperproliferation. The excess keratin is also qualitatively altered as it tends to become densely packed along with monofilaments and lipid droplets ***(Gollnick, 2003).***

Comedogenesis occurs when abnormally desquamated corneocytes accumulate in the sebaceous follicle and form a keratinous plug. When the keratinous plug enlarges below a very small follicular pore at the skin surface, it becomes visible as a closed comedone (whitehead). An open comedone (blackhead) occurs if the follicular pore dilates. The small pore closed comedones are the precursors of inflammatory lesions ***(Cunliffe and Goulden, 2000).***

Subsequent proliferation of P.acnes generates inflammatory mediators and formation of inflammatory lesions. Immunohistochemical studies have shown an increase in the proliferation rate of the basal keratinocytes and abnormal differentiation of the follicular keratinocytes in the follicle wall of microcomedones and comedones ***(Jarrousse et al., 2007).***

An androgen-controlled defect may also contribute to abnormal proliferation through the 5-α reductase enzyme in the infundibulum. Additionally, a relative decrease in sebaceous linoleic acid was proposed. Follicular hyperproliferation is also

associated with abnormal lipid inclusions indicating abnormal differentiation ***(Thielitz et al., 2001).***

Cytokines are also involved in comdeogenesis. High levels of biologically active interleukin-1α (IL-1α) have been detected in comedones, believed to be expressed by follicular keratinocytes and triggered by changes in sebum composition and secretions. Also, IL-1α may compromise follicular barrier and thus induce inflammation ***(Guy et al., 1996).*** **Figure (2)**

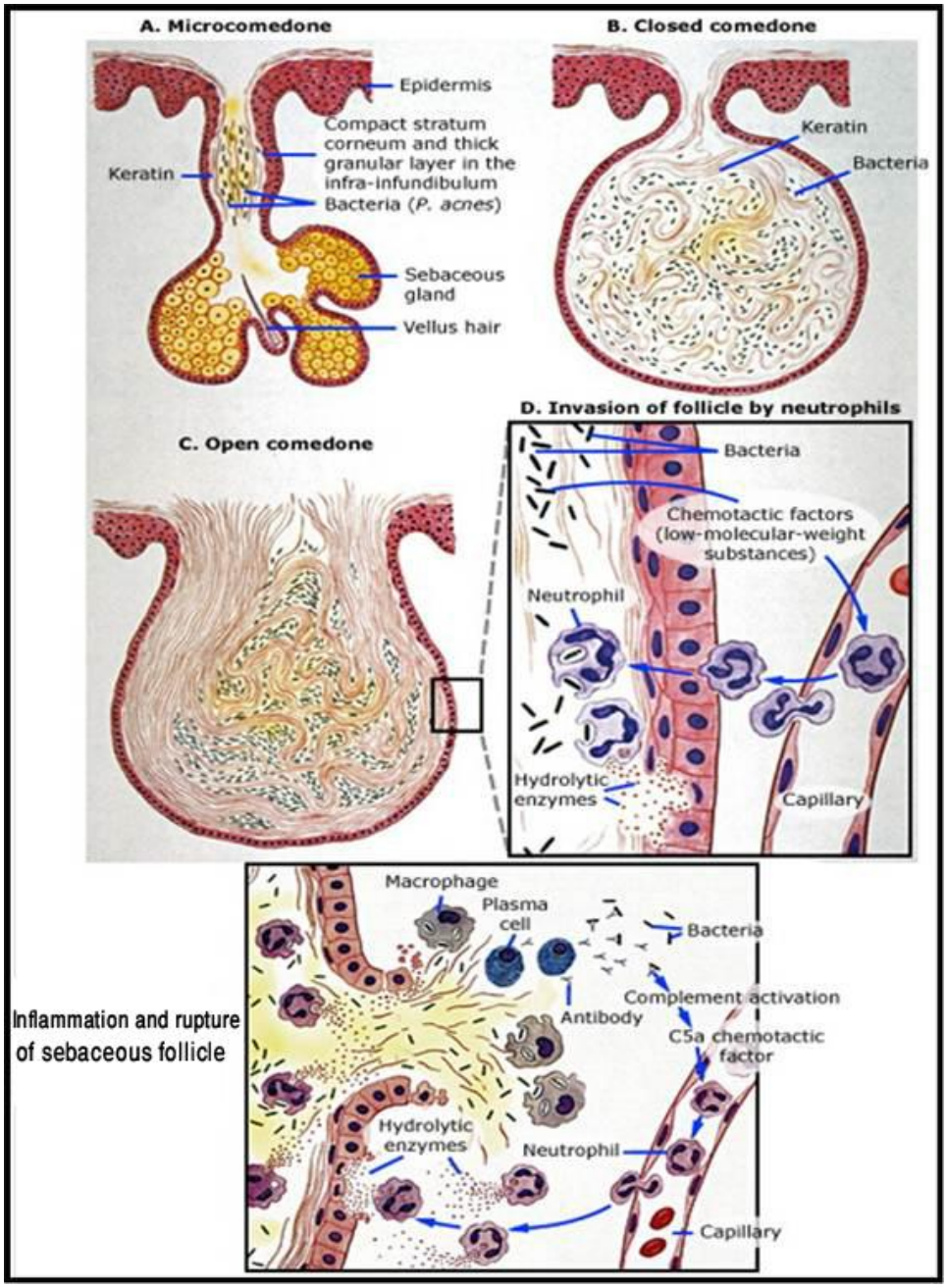

Figure (2): Microcomedone, open and closed comedone formation and follicular hyperkeratosis ***(Kurokawa et al., 2009).***

b) Seborrhea and sebaceous gland hyperplasia:

The sebaceous glands start to enlarge with androgenic stimulus at 7-8 years of age (adrenarche), with a net increase in sebum excretion. The overall size of the sebaceous follicles increase along with number of lobules per gland. Acne subjects, male and female, excrete more sebum than normal subjects, and the sebum excretion rate correlates well with severity of acne ***(Knutsen-Larson et al., 2012).***

Sebocytes and follicular keratinocytes are capable of metabolizing androgens through enzymes 5-α reductase (type 1), 3β and 17β hydroxy steroid dehydrogenase. These enzymes are present in undifferentiated basal sebocytes and with time the sebocytes differentiate and rupture releasing lipids into the sebaceous ducts. The differentiation of the sebocyte is initiated by androgen uptake into the cell and its coupling with the cytoplasmic androgen receptor which, in turn, stimulates gene transcription and differentiation ***(Fritsch et al., 2001).***

The increase in sebum production is mainly due to a difference in the response of the target organ (sebaceous follicle) to androgen and increase in circulating androgens, or both. The target-organ responsiveness is variable and may be the explanation for the bilateral asymmetry of acne ***(Deplewski and Rosenfield, 2000).***

Sebocytes from different areas of the body have different responses to dihydrotestosterone and testosterone. Sebocytes

from the leg have a lower response or do not respond, whereas those from the face show a dose-dependent proliferation ***(Andrea and Diane, 2008).***

c) Propionibacterium acnes:

Propionibacterium acnes is the relatively slow-growing, typically anaerobic, Gram-positive bacterium (rod) that is linked to the development of acne. This bacterium is largely commensal and part of the skin flora present on most healthy adult humans' skin. It is usually just barely detectable on the skin of healthy preadolescents. It lives primarily on, among other things, fatty acids in sebum secreted by sebaceous glands in the follicles.It is named after its ability to generate propionic acid ***(Bruggemann et al., 2004).***

Propionipacterium acnes shows orange fluorescence when exposed toWood's light, due to the presence of endogenous porphyrins. The bacterium can be killed by ultraviolet light. P. acnes is especially sensitive to light in the 405–420 nanometer range due to the presence of an endogenic porphyrin–coporphyrin III. A total irradiance of 320 J/cm² is found to inactivate this bacterium in vitro. This fact allowed the use of phototherapy in its eradication ***(Sakamoto et al., 2010).***

Colonization of the pilosebaceous follicle by P.acnes is a major factor for the inflammatory reaction in acne vulgaris, therefore, P.acnes has been a major target of therapy in inflammatory acne. Further evidence to support the hypothesis

that microorganisms are involved in acne was provided when antibiotics that reduced skin surface P.acnes (such as erythromycin and clindamycin) were shown clinically to improve acne and when the presence of resistant P.acnes strains was associated with reduced efficacy of these treatments ***(Dessinioti and Katsambas, 2010***; ***Ross et al., 2003).***

There are several mechanisms by which P.acnes may lead to the disruption of the follicular epithelium and subsequent inflammatory reaction ***(Dessinioti and Katsambas, 2010).*** **Table (1)**

Table (1): Mechanisms by which Propionibacterium acnes acts in acne pathogenesis ***(Dessinioti and Katsambas, 2010).***

1	P.acnes produces lipases, proteases, hyaluronidases, and neutrophil chemotactic factors.
2	P.acnes induces the production of TNF-α, IL-1α, and IL-8.
3	P.acnes induces the expression of proinflammatory cytokines IL-8, IL-1β, and TNF-α by human monocytes in acne patients and in controls.
4	Inflammation triggered through TLR2 is important in the pathogenesis of acne, and P.acnes was shown to induce monocyte cytokine production (IL-12, IL-8) through a TLR2-dependent pathway.
5	An increase in TLR2, TLR4, and MMP-9 expression by human keratinocytes occurred with incubation with P.acnes fractions.
6	P.acnes induces IL-8 and β-defensin-2 expression in keratinocytes via TLR2 and TLR4.
7	P.acnes induces keratinocytes growth in vitro.
8	P.acnes may be involved in the formation of the microcomedones.
9	P.acnes biofilm may lead to the increased cohesiveness of corneocytes seen in acne.

The proinflammatory cytokine IL-1α has been demonstrated in vitro to cause the formation of comedones in isolated pilosebaceous units. The addition of IL-1α resulted in hypercornification of the infundibulum similar to that seen in comedones, which could be inhibited by a IL-1 receptor antagonist. Thus, IL-1α has a central role in cutaneous inflammation and keratinocyte proliferation and may influence the evolution of acne lesions ***(Guy et al., 1996).***

d) Inflammation and immunological factors:

Inflammation is the key component of acne and the major reason for disease, post inflammatory hyperpigmentation and scarring. Inflammation, for a long time was believed to be a secondary process in the pathogenesis of acne. New data indicate that immunological events led by perifollicular helper T-cells in genetically predisposed individuals may in fact be a primary process, initiating comedogenesis through elaboration of IL-1. Further, inflammation may upregulate sebum production through production of inflammatory mediator leukotriene B4 that binds to receptors on the sebocytes ***(Jeremy et al., 2003).***

Sebum production is also influenced by neuropeptides - substance P and its degrading enzyme neutral endopeptidase - that may explain stress-induced aggravation of acne ***(Zouboulis and Bohm, 2004).***

Propionibacterium acnes may directly contribute to inflammation through generation of proinflammatory lipases, proteases, hyaluronidases, and chemotactic factors as shown previously. Lipases hydrolyze triglycerides to form free fatty acids which are comedogenic and primary irritants. At the cellular level, inflammation begins with an influx of CD4+ T-lymphocytes. Later, macrophages, Langerhans cells, and cells expressing HLA-DR are present. There is also angiogenesis and vascular adhesion molecule expression ***(Ganceviciene et al., 2009).***

Holland et al. (2004) have shown differences in the character of the cellular infiltrate and in the speed of evolution and resolution of inflammation. In sequential biopsies, it was observed that in patients who tended to scar, the immune response was milder initially and evolved slowly (compared to nonscarring patients), but it was more specific in cellular detail, and the inflammation persisted for a longer duration.

Dietary of effect:

Acne is a common and complex skin disease that affect individuals of all ages. Familial and ethnic factors are implicated in acne prevalence, this observation is complicated by the finding that incidence rates of acne have increased with the adoption of western lifestyles. These observations suggest that lifestyle factors, including diet, may be involved in acne pathogenesis ***(Smith et al., 2007).***

There are no systematic controlled studies on the relationship between acne and diet that dissociate diet from other confounding factors (particularly genetic ones). Several isolated observations and case reports, suggest that acne can develop in groups when a high–glycemic index diet is adopted ***(Wolf et al., 2004).***

It was suggested that the absence of acne in non-Westernized societies is attributable to environmental factors, mainly local diets, which have a substantially lower glycemic index than a Western diet. They believe that it is difficult to dissociate environmental factors, such as diet, from genetic factors in this study, especially in view of the fact that the people in these isolated regions live in close-knit and closed communities. They do admit, however, that "it remains possible that adolescents in Westernized societies may be repeatedly acutely hyper insulinemic due to their high-glycemic diet". Hyperinsulinemia in turn may initiate an endocrine cascade that affects the sebaceous gland and follicular keratinization ***(Cordain et al., 2002).***

Diet influences a number of hormones that regulate both keratinocyte proliferation and corneocyte apoptosis. The glycemic index is a relative comparison of the potential of various foods or combination of foods to raise blood glucose, based on equal amounts of carbohydrate in the food. In 2002 ***Liu and Willett*** reported that the concept of glycemic load (glycemic index the carbohydrate content per serving size) was

introduced to assess the potential of a food to raise blood glucose, based on both the quality and quantity of dietary carbohydrate ***(Liu and Willett, 2002).***

Numerous studies done established that chronic consumption of high glycemic load carbohydrates may cause long-term hyperinsulinemia and insulin resistance. Insulin influences circulating concentrations of free insulin like growth factor I (IGF-1) and insulin like growth factor binding protein 3 (IGFBP-3), which in turn directly regulate keratinocyte proliferation and apoptosis. Chronic and acute hyperinsulinemia simultaneously elevates free IGF-1 while reducing IGFBP-3 ***(Edmondson et al., 2003).***

Free IGF-1 directly stimulates basal keratinocyte proliferation, whereas IGFBP-3 inhibits basal keratinocyte proliferation irrespective of its IGF-1 receptor activity. Hence, elevations in the free IGF- 1/IGFBP-3 ratio promote keratinocyte proliferation ***(Boden and Shulman, 2002).***

Furthermore, IGFBP-3 is a ligand for the retinoid X nuclear receptor (RXR alpha) that along with other endogenous RXR alpha ligands (eg, trans retinoic acid and 9-cis retinoic acid) can operate in an additive manner to induce apoptosis. Hence, the therapeutic effect of pharmaceutical retinoids in acne patients may function in part by restoring the RXR signal that was reduced by diet/insulin mediated reductions in IGFBP-3 ***(Lee and Cohen, 2002; Cordain, 2005).***