Immunohistochemical Expression of Cyclo-oxygenase 2 and Liver X Receptor-α in Acne Vulgaris

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ABSTRACT

Introduction: Acne Vulgaris (AV) is a common inflammatory disease of pilosebaceous units. Liver X Receptor-α (LXR-α) is a ligand activated transcription factor. It controls transcription of genes involved in lipid and fatty acid synthesis. Cyclo-oxygenase 2 (COX2) is a rate limiting enzyme in prostaglandin synthesis. It plays important role in inflammation.

Aim: To evaluate the immunohistochemical expression of LXR-α and COX2 in acne vulgaris skin biopsies to explore their possible pathogenic role in this disease.

Materials and Methods: Sixty five subjects were included (45 cases with AV and 20 age and gender-matched healthy controls). Skin biopsies were taken from lesional and perilesional skin of cases and from site-matched areas of control subjects. The evaluation of LXR-α and COX2 was done using immunohistochemical technique. Data were collected, tabulated and statistically analysed using a personal computer with "(SPSS) version 11" program. Chi-square test was used to study the association between qualitative variables. Mann–Whitney test was used for comparison between quantitative variables. Student's t-test was used for comparison between two groups having quantitative variables. Spearman's coefficient was used to study the correlation between two different variables. Differences were considered statistically significant with p<0.05.

Results: COX2 was upregulated in lesional skin compared with peilesional and control skin both in epidermis and pilosebaceous units (p<0.001 for all). Higher epidermal COX2% was significantly associated with papulopustular acne (p=0.009) and higher acne score (p=0.018). Higher pilosebaceous units COX2% was significantly associated with papulopustular acne (p=0.04). LXR-α was upregulated in lesional skin compared with peilesional and control skin both in epidermis and pilosebaceous units (p<0.001 for all). Higher LXR-α % in epidermis and pilosebaceous units was significantly associated with papulopustular acne (p=0.01 for both) and higher acne score (p=0.03 for both). Significant positive correlation was detected between COX2% and LXR-α % in epidermis (p=0.001, r=0.87) and pilosebaceous units (p=0.001, r=0.65).

Conclusion: Both LXR-α and COX-2 play a role in the pathogenesis of acne vulgaris through their effects on cellular proliferation, inflammation and lipid synthesis. Research for new therapeutic modalities based on their inhibition is needed. More understanding of the interaction between LXR-α, COX2 and acne lesions may lead to effective interference, possibly directed toward specific cell types or steps within inflammatory pathways.

Keywords: Immunohistochemistry, Inflammation, Lipogenesis, Pilosebaceous unit

INTRODUCTION

Acne vulgaris is a common dermatosis of pilosebaceous units. It is characterized by seborrhea, comedones, papules, pustules, nodules and, sometimes, scarring [1].

It is a multifactorial disorder. Androgens, abnormal keratinocyte cornification, *Propionibacterium acnes* (*P. acnes*) colonization and proinflammatory mediators and cytokines all play roles in disease development [2].

LXR-α is a ligand activated transcription factor. It contains a central DNA-binding domain and a large ligand-binding domain with a lipophilic core that binds specific small-lipid molecules. After ligand binding, receptor undergoes a conformational change that promotes interaction with co-activator proteins that facilitate transcription of target genes [3].

LXR-α is recognized as sterol-responsive with the ability to bind to several oxysterolmetabolites. Many LXR-α target genes have been identified that implicate this receptor in a variety of physiological and pathological processes including cholesterol transport and metabolism, glucose metabolism and inflammation [4].

LXR-α is highly expressed in liver and is also found in adipose tissue, intestine, kidney and macrophages. It is also expressed in sebaceous glands, sweat glands and hair follicles [5].

Cyclo-oxygenase (COX) is a rate limiting key enzyme in the synthesis of Prostaglandins (PGs). It plays a role in certain biologic processes, including inflammation, angiogenesis, development and homeostasis [6].

There are two known forms of COX; COX1 which is present normally in all tissues and COX2 which is released by stimulatory cytokines during inflammation or infection [7].

COX2 is one of the downstream targets of NF-κB. It is induced in numerous processes such as cellular growth, differentiation, inflammation and tumourigenesis [8]. COX2 gene promoter contains several enhancer sequences and its expression can be induced through multiple signaling pathways [9]. The aim of this work was to evaluate the immunohistochemical expression of LXR-α and COX2 in acne vulgaris skin biopsies to explore their possible pathogenic role in this disease.

MATERIALS AND METHODS

This case-control study was carried out on 65 subjects. These included 45 cases with AV that were selected from the Dermatology outpatient clinic, Faculty of Medicine, Menoufiya University Hospital, Egypt, during the period between March 2014 to April 2015 and 20 normal skin specimens from non acneic, age- and gender-matched subjects from the Plastic Surgery Department as a control group.

The current work protocol was approved by The Ethical Committee of Menoufiya Faculty of Medicine.

A written consent form was obtained from every case and control subject before the study initiation. This was in accordance with the Helsinki Declaration of 1975 (revised in 2000).

All studied patients were subjected to complete history taking, general and dermatological examination. Clinical data describing patients' demographics (age and gender) and disease duration were all documented. Disease duration was calculated from lesion onset to time of the first visit. Selected cases were newly diagnosed with no history of acne treatment. Disease aggravation by food intake, menstrual cycle or sun exposure was assessed for every case. Aggravation was defined as an increase of more than 25% in acne lesions compared to after exposure to specific factor [10].

Biopsied acne lesions were categorized into comedonal, papulo-pustular and nodular.

Clinical assessment of disease severity was done according to Tan JKL into [11]:

Mild acne: Fewer than 20 comedones or fewer than 15 inflammatory lesions or a total lesion count lower than 30.

Moderate acne: 20-100 comedones or 15-50 inflammatory lesions or a total lesion count of 30-125.

Severe acne: More than five nodules or comedones count greater than 100 or a total inflammatory count greater than 50 or a total lesion count greater than 125.

Scoring of AV lesions [12]

Grade 1: Comedones and few papules;

Grade 2: Comedones, papules and a few pustules;

Grade 3: Larger inflammatory papules, pustules and a few cysts; a more severe form involving the face, neck and upper portions of the trunk;

Grade 4: More severe, with cysts becoming confluent.

Exclusion Criteria

Any case or control subject with one or more of the following was excluded:

1. Dermatological disease other than AV.
2. Systemic autoimmune or inflammatory diseases.
3. Local or systemic infections.

Biopsies

Skin biopsy samples were taken under 2% lignocaine local anesthesia from every case (from acne lesions and from perilesional skin) and control subject. Biopsies from cases and controls were site-matched. All specimens were fixed in 10% neutral-buffered formalin and subjected to routine tissue processing that ended with paraffin-embedded blocks ready for sectioning at the Pathology Department, Faculty of Medicine, Menoufiya University, Egypt.

Immunohistochemical (IHC) Staining for COX2 and LXR-α

5-μm-thick sections were cut from the paraffin-embedded blocks with subsequent steps of deparaffinization and dehydration in xylene and graded series of alcohol, respectively. Antigen retrieval was performed by boiling in 10 ml citrate buffer (pH 6.0) for 20 minutes, followed by cooling at room temperature. The slides were incubated overnight at room temperature with:

— Anti LXR-α concentrated Rabbit polyclonal antibody (ab106464) raised against LXR-α antigen, (Abcam Inc., Cambridge, USA).

— Anti COX2 concentrated Rabbit polyclonal antibody (RB-9072-PO) raised against COX2 antigen, (Thermo Fisher Scientific, USA).

Diaminobenzidine (DAB) was used as a chromogen. Slides were then counter-stained with Mayer's hematoxylin.

Interpretation of LXR-α and COX2 immunohistochemical Results

A brown nucleo-cytoplasmic and cytoplasmic stain was considered positive for LXR-α [5] and COX2 [13] respectively in lesional, perilesional and control specimens.

Evaluation Included

Epidermis and pilosebaceous units were assessed for the following:

1. Expression either: Positive or negative
2. Percent of positive cells: The percentage of the positive cell was assessed at 200X magnification field [14].
3. Histo-score (H- score): H score was calculated to all positive specimens according to the following equation:

   H score = +1x% of mildly stained cells + 2x% of moderately stained cells + 3x% of strongly stained cells [15].

4. Distribution was categorized as either:
   — Patchy: irregular or not uniform distribution;
   — Diffuse: uniform distribution.
5. Thickness pattern either:
   — Partial thickness;
   — Whole thickness.

Dermis was assessed for:

   Inflammatory cell Expression: positive or negative.

STATISTICAL ANALYSIS

Data were collected, tabulated and statistically analysed using a personal computer with "(SPSS) version 11" program. Chi square test was used to study the association between qualitative variables. Mann–Whitney test was used for comparison between quantitative variables. Student's t-test was used for comparison between two groups having quantitative variables. Spearman's coefficient was used to study the correlation between two different variables. Differences were considered statistically significant with $p<0.05$.

RESULTS

Clinical data of selected cases are summarized in [Table/Fig-1]. Immunohistochemical expression of COX2 in studied groups:

COX2 Expression in Lesional Skin

All cases showed positive COX2 expression with variable distribution in epidermis and pilosebaceous units. Dermis showed positive expression in inflammatory cells in all cases [Table/Fig-2,3].

COX2 Expression in Perilesional Skin

All cases showed positive COX2 immunostaining with variable distribution both in epidermis and pilosebaceous units. Dermis showed positive expression in inflammatory cells in 97.8% of cases [Table/Fig-2,4].

COX2 Expression in Control Skin

All examined sections showed positive expression with variable distribution in epidermis and pilosebaceous units. Dermal expression was negative in all examined control samples [Table/Fig-2,5].

Comparison between COX2 Expression in Studied Groups

Higher COX2 %, higher H score and diffuse distribution were significantly associated with lesional skin compared with control in epidermis and pilosebaceous units (p<0.001 for all). Whole

thickness staining (p<0.001) in epidermis was significantly higher in lesional skin compared with control skin [Table/Fig-2].

Positive inflammatory cell expression (p<0.001) was significantly higher in lesional skin compared with control in the dermis [Table/Fig-2].

Higher COX2 % and higher H-score, were significantly associated with lesional skin compared with perilesional skin in epidermis (p=0.02 for both) and pilosebaceous units (p=0.004, p=0.005 respectively). Whole thickness staining (p=0.02) in epidermis was significantly higher in lesional skin compared with perilesional skin [Table/Fig-2].

Higher COX2 %, higher H-score and diffuse distribution, were significantly associated with perilesional skin compared with control in epidermis and pilosebaceous units (p<0.001 for all). Positive dermal inflammatory cell expression (p<0.001) was significantly higher in perilesional skin compared with control [Table/Fig-2].

Relationship between COX2% in Lesional Skin and Clinical Data of selected cases

Higher epidermal COX2 % was significantly associated with papulopustular acne (p=0.009) and higher acne score (p =0.018). Higher pilosebaceous units COX2 % was significantly associated with papulopustular acne (p=0.04) [Table/Fig-6].

Immunohistochemical Expression of LXR-α in Studied Groups

LXR-α expression in lesional skin: All cases showed positive LXR-α expression with variable distribution in epidermis and pilosebaceous units. Dermis showed positive expression in inflammatory cells in all cases [Table/Fig-7,8].

LXR-α expression in perilesional skin: All cases showed positive LXR-α immunostaining with variable distribution both in epidermis and pilosebaceous units. Dermis showed positive expression in inflammatory cells in all cases [Table/Fig-7,9].

LXR-α expression in control skin: All examined sections showed positive expression with variable distribution in epidermis and pilosebaceous units. Dermal expression was negative in all examined control samples [Table/Fig-7,10].

Comparison between LXR-α Expression in Studied Groups

Higher epidermal and pilosebaceous units% (p<0.001 for both), higher H-score (p<0.001 for both), diffuse distribution (p=0.004 for both) and whole thickness distribution (p<0.001 for both) were significantly associated with lesional compared with control skin. Positive expression in dermal inflammatory cells (p<0.001) was significantly higher in lesional compared with control skin [Table/Fig-7].

Higher epidermal and pilosebaceous units% (p<0.001 for both), higher H-score (p<0.001 for both) and whole thickness distribution (p=0.011) in epidermis were significantly associated with lesional skin compared with perilesional skin [Table/Fig-7].

Higher epidermal and pilosebaceous units H score (p=0.002 for both), diffuse distribution (p=0.004 for both) and whole thickness staining (p=0.04 for both) were significantly associated with perilesional skin compared with control. Positive expression in dermal inflammatory cells (p<0.001) was significantly higher in perilesional compared with control skin [Table/Fig-7].

Relationship between LXR-α % and Clinical Data of Studied Cases

Higher LXR-α% in epidermis and pilosebaceous units was significantly associated with papulopustular acne (p=0.01) and higher acne score (p=0.03) [Table/Fig-6].

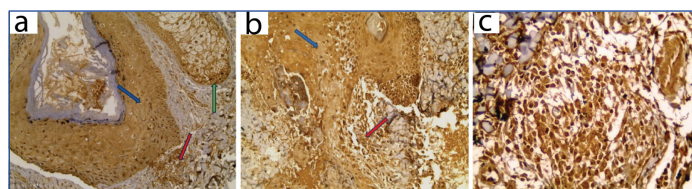| Variables	Cases No= 45	
Age (years)		
Mean ± SD	20.91±3.456	
Range	16-30	
Median	20	
Duration of disease (months)		
Mean ± SD	3.8±2.5	
Range	1-10	
Median	5.5	
Variables	**%**	**No (n)**
Sex:		
Male	40.0	18
Female	60.0	27
Site of Acne:		
Face	40.0	18
Back	15.6	7
Both	44.4	20
Biopsied lesion		
Comedonal	17.8	8
Papulpustular	66.6	30
Nodular	15.6	7
Exacerbating Factors:		
No	57.8	26
Food (chocolate, dairy products)	24.6	11
Menstruation	11.1	5
Sun exposure	6.5	3
Severity:		
Mild	42.2	19
Moderate	51.1	23
Severe	6.7	3
Acne scoring system:		
1	17.8	8
2	24.4	11
3	51.1	23
4	6.7	3
Family History:		
Negative	22.2	10
Positive	77.8	35

[Table/Fig-1]: Clinical data of studied cases.
SD: Standard deviation

Variables	Lesional No= 45	Perilesional No= 45	Control Nos=20	p-value
COX2 expression in epidermis				
COX2 % Mean ± SD Median Range	90.22 ± 10.97 90.0 70-100	84.00±13.55 80.0 50-100	70.50 ± 11.459 70.0 50-90	p1=0.001* p2=0.001* p3=0.029*
COX2 H-Score Mean ± SD Median Range	145.33 ± 76.325 100.0 70-300	108.89±45.23 90.0 50-200	70.50 ± 11.459 70.0 50-90	p1=0.001* p2=0.001* p3=0.021*
	No (%)	No (%)	No (%)	
COX2 Distribution Diffuse Patchy	39 (86.7) 6 (13.3)	39 (86.7) 6 (13.3)	9 (45.0) 11 (55.0)	p1=0.001* p2=0.001* p3=1.00
COX2 Distribution Partial thickness Whole thickness	18 (40.0) 27 (60.0)	29 (64.4) 16 (35.6)	15 (75.0) 5 (25.0)	p1=0.001* p2=0.04** p3=0.02*
COX2 expression in pilosebaceous unit				
COX2 % Mean± SD Median Range	86.89 ± 11.643 90.0 70-100	77.33±17.109 70.0 40-100	66.00 ± 11.425 65.0 40-90	p1=0.001* p2=0.009* p3=0.004*
COX2 H-score Mean± SD Median Range	139.78 ± 70.630 100.0 70-300	100.67±44.69 80.0 40-200	66.00 ± 11.425 65.0 40-90	p1=0.001* p2=0.001* p3=0.005*
	No (%)	No (%)	No (%)	
COX2 Distribution Diffuse Patchy	37 (82.2) 8 (17.8)	39 (86.7) 6 (13.3)	9 (45.0) 11 (55.0)	p1=0.002* p2=0.001* p3=0.561
COX2 Distribution Partial thickness Whole thickness	22 (48.9) 23 (51.1)	30 (66.7) 15 (33.3)	15 (75) 5 (20)	p1=0.091 p2=0.502 p3=0.088
COX2 expression in dermis				
Inflammatory cells Negative Positive	0 45 (100.0)	1 (2.2) 44 (97.8)	20 (100.0) 0	p1=0.001* p2=0.001* p3=1.000

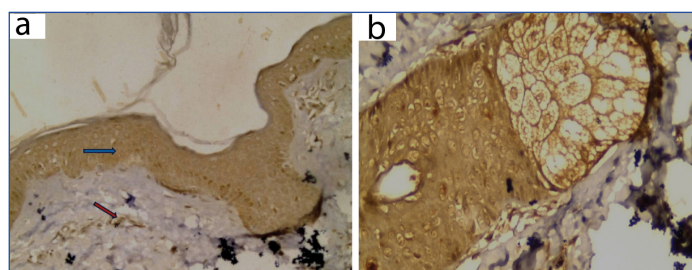**[Table/Fig-2]:** COX2 expression in studied groups.
SD: Standard deviation, p1: lesion vs control. p2: perilesion vs control. p3: lesion vs perilesion, *: Significant.

[Table/Fig-3]: COX2 expression in lesional skin: a) Positive expression in hair follicle (blue arrow), sebaceous gland (green arrow) and dermal inflammatory cells (red arrow) in comedone; b) Positive expression in hair follicle and epidermal keratinocytes (blue arrow) and derma inflammatory cells (red arrow) in papule; c) Positive expression in dermal inflammatory cells in pustule (IHC X200 for a, b and X 400 for c).

[Table/Fig-4]: COX2 expression in perilesional skin: a) Positive expression in epidermis (green arrow) and inflammatory cells (blue arrows); b,c) Positive expression in hair follicle (green arrows); d) Positive expression in hair follicle (green arrow) and inflammatory cells (blue arrow) (IHC X200 for a, X400 for b, c, d).

[Table/Fig-5]: COX2 expression in control skin: a) Positive epidermal expression; b) Positive expression in sebaceous gland (IHC X200 for a, X400 for b).

Significant positive correlation was detected between COX2% and LXR-α% in epidermis (p=0.001, r=0.87) and pilosebaceous units (p=0.001, r=0.65) [Table/Fig-6].
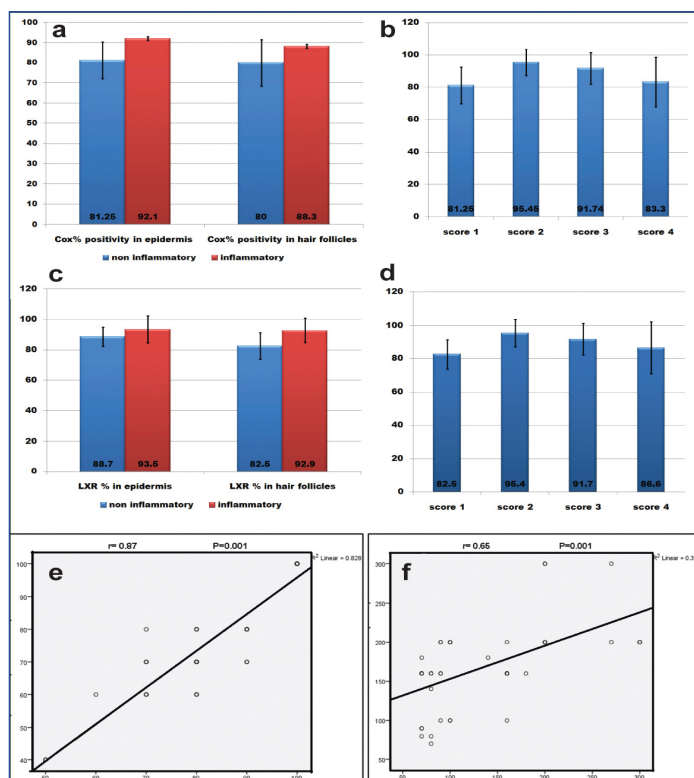
DISCUSSION

In the present work, LXR-α expression was positive in epidermis of all control group with whole thickness staining in 50% of sections. This was in agreement with Hanley K et al., who reported that LXR-α is expressed in cultured human keratinocytes throughout all layers of the human epidermis [16].

LXRs may play an important role within the skin. In vivo and in vitro studies showed that LXR ligands share in stratum corneum formation and induce down-regulation of cell proliferation. These ligands stimulate cornified envelope formation through increased transcription of transglutaminase 1, involucrin, loricrin and filaggrin [17].

In the present work, LXR-α expression was positive in hair follicles and sebaceous glands of all control group. This was in agreement with Komuves LG et al., and Hong I et al., [17,18].
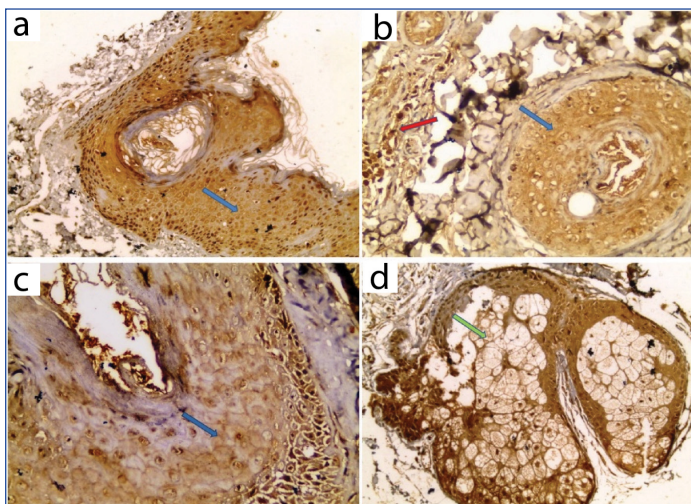
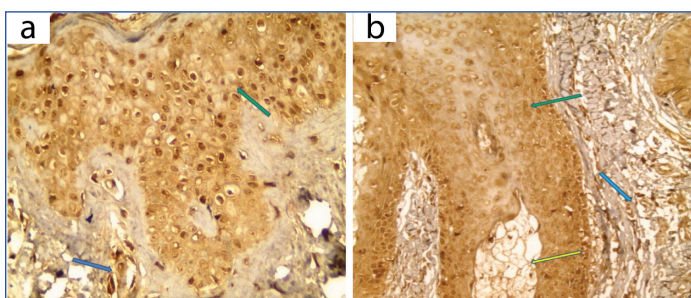It was found that activation of LXR-α induces lipid synthesis in

[Table/Fig-6]: a) association between COX2% and AV lesions; b) association between COX2% and acne score; c) association between LXR-α % and AV lesions; d) association between LXR-α % and acne score; e) correlation between COX2 and LXR-% in epidermis; f) correlation between COX2 and LXR- % in pilosebaceous units.

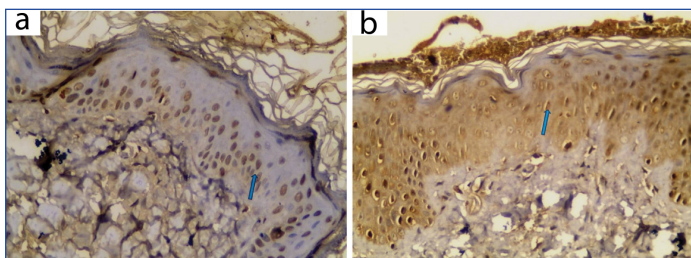| Variable	Lesional No= 45	Perilesional No= 45	Control No=20	p-value
LXR-α expression in epidermis				
LXR α % Mean± SD Median Range	92.67±8.634 100.0 70-100	77.78±16.772 70.0 40-100	70.50 ± 9.98 70.0 50-90	p1=0.001* p2=0.033 p3=0.001*
LXR α H-Score Mean± SD Median Range	190.89±60.784 180.0 80-300	98.44±41.284 80.0 40-200	70.50 ± 9.98 70.0 50-90	p1=0.001* p2=0.002* p3=0.001*
	No (%)	No (%)	No (%)	
LXR α distribution Diffuse Patchy	39 (86.7) 6 (13.3)	39 (86.7) 6 (13.3)	10 (50) 10 (50.0)	p1=0.004* p2=0.004* p3=1.00
LXR α distribution Partial Whole thickness	18 (40.0) 27 (60.0)	30 (66.7) 15 (33.3)	18 (90.0) 2 (10.0)	p1=0.011* p2=0.048* p3=0.011*
LXR-α expression in pilosebaceous unit				
LXR α % Mean± SD Median Range	90.67±10.313 100.0 70-100	86.44±89.700 70.0 30-660	64.00±10.46 70.0 40-80	p1=0.001* p2=0.099 p3=0.001*
LXR α H-score Mean± SD Median Range	170.00 ± 53.55 160.0 70-300	89.78±37.749 80.0 30-200	64.00±10.46 70.0 40-80	P1=0.001* P2=0.002* P3=0.001*
	No (%)	No (%)	No (%)	
LXR α distribution Diffuse Patchy	39 (86.7) 6 (13.3)	39 (86.7) 6 (13.3)	10 (50) 10 (50.0)	p1=1.00 p2=0.004* p3=1.00
LXR α distribution Partial Whole thickness	18 (38.6) 27 (61.4)	30 (66.7) 15 (33.3)	18 (90) 2 (10.0)	p1=0.008* p2=0.048* p3=0.008*
LXR-α expression in dermis				
inflammatory cells Negative Positive	8 (7.8) 37 (82.2)	11 (24.4) 34 (75.6)	20 (100. 0) 0	p1=0.001* p2=0.001* p3=0.438

[Table/Fig-7]: LXR-α expression in studied groups.
SD: Standard deviation, p1: lesion vs control. p2: perilesion vs control. p3: lesion vsperilesion, *: Significant.

[Table/Fig-8]: LXR-α expression in lesional skin: a) Positive patchy whole thickness expression in hair follicle (blue arrow) in comedone; b) Positive diffuse whole thickness expression in hair follicle (blue arrow) and inflammatory cells (red arrow) in papule; c) Positive patchy expression in hair follicle in comedone; d) Positive expression in hyperplastic sebaceous gland (IHC X 100 for a, X 200 for b, X400 for c,d).

[Table/Fig-9]: LXR-α expression in perilesional skin: a) Positive patchy expression in epidermis (green arrow) and inflammatory cells (blue arrow); b) Positive patchy expression in hair follicle (green arrow), sebaceous gland (yellow arrow) and inflammatory cells (blue arrow) (IHC X400 for a,b).

[Table/Fig-10]: LXR-α expression in control skin: a,b) Positive patchy epidermal expression (IHC X400 for a,b).

sebocytes. Additionally, it induces Sterol Regulatory Element-Binding Protein 1 (SREBP-1) that regulates genes required for fatty acid and lipid metabolism and production [18].

In hair follicles, LXR-α has been shown to be expressed not only in the outer root sheath but also in the dermal papilla, connective tissue sheath, and hair bulb. This pattern of expression suggests an important role in mediating hair follicle differentiation programs [19].

The current study showed that LXR-α was upregulated in lesional AV skin compared with perilesional skin and control group providing evidence of its role in disease pathogenesis.

As mentioned earlier, LXR-α plays vital roles in lipid metabolism and cholesterol homeostasis. In the human epidermis, cholesterol contributes in the formation of the permeability barrier via lamellar body formation [20].

It has been reported that male and female acne patients have significantly elevated total cholesterol and low-density lipoprotein cholesterol [21,22].

LXR-α acts as cholesterol sensor; when cellular oxysterol accumulates as a result of increasing concentrations of cholesterol, LXR-α induces the transcription of genes such as the ATP-Binding

Cassette (ABCA1), leading to cholesterol efflux thus lowering intracellular cholesterol [23] protecting cells from cholesterol overload [24,25].

It has been reported that lipogenic enzymes such as fatty acid synthase are regulated by the sterol regulatory binding protein-1 which is regulated by liver LXR-α [26]. LXR-α agonists were shown to increase triglyceride accumulation and to promote lipogenesis [27].

Acne patients have increased sebum production and there is positive correlation between acne severity and rate of sebum production [28]. Lipoperoxides and monounsaturated fatty acids affect keratinocyte proliferation and keratinization. Peroxides are proinflammatory and stimulate the release of inflammatory cytokines [29].

Therefore, it is assumed that the effects of LXR-α on the induction of acne are related to control of cellular cholesterol metabolism inducing excess lipogenesis, which promotes the progress of the disease [30].

The current work showed that, LXR-α was upregulated in perilesional skin compared with control skin. It was reported that inflammatory events begin in perilesional skin in the early stage of the disease even before hyperproliferation or abnormal cornification of follicular keratinocytes. Inflammatory micro-environment is present in perilesional area before evident clinical lesions appear [31].

LXR-α is an important regulator of inflammatory gene expression and innate immunity [32]. Topical treatment with LXR-α endogenous and synthetic agonists have potent anti-inflammatory activity in cutaneous inflammation by inhibition of cytokine production [33]. In addition, LXR-α inhibits the expression of macrophage inflammatory genes, including inducible Nitric Oxide Synthase (iNOS), COX-2, interleukin (IL-6), IL-1β, Monocyte Chemo-attractant Protein-1 (MCP-1) and MCP-3, in response to bacterial, Tumour Necrosis Factor-α (TNF-α) or lipopolysaccharide stimulation [34,35]. This role may explain the positive LXR-α expression by inflammatory cells in lesional and perilesional skin, noted in the present study.

The current study demonstrated significantly higher LXR-α% in papulopustular AV than comedonal lesions.

This was in agreement with Bosseila M et al., and may indicate that LXR-α may play a role in the progression of disease from comedonal to papulopustular lesions [36,37] through its role in cholesterol metabolism and lipogenesis, as mentioned earlier [30].

In the present work, positive diffuse COX2 expression was present in epidermis of all control specimens. This was in agreement with Ikai K [38].

Others detected restricted COX2 expression to keratinocytes of the granular and spinous layers [39,40]. However, An KP et al., reported complete absence of COX2 expression in normal skin [41].

COX2 expression and its role in normal epidermis are controversial issues. Scholz K et al., postulated that COX2 expression is part of normal human keratinocyte differentiation [42]. An increasing intensity of expression is observed as one moves from the suprabasilar stratum spinosum of the epidermis to the stratum granulosum where the signal is most intense [43]. However, Ikai K concluded that, the positive expression in basal and suprabasal cell layers may provide evidence about its role in normal keratinocyte proliferation as well as differentiation [38]. Findings of the present work underscore the possible role of COX2 in cell proliferation and differentiation.

This role can be explained through the effects of mediators produced by COX2 activation. Cutaneous Prostaglandin E2 (PGE2) is produced by keratinocytes and fibroblasts. It has proinflammatory, proproliferative and immunomodulatory effects [44].

PGE2 trigger the generation of inflammatory mediators, and the latter enhance the local leukotriene and PG production. Even if PGE2 would not only be mainly formed by sebocytes in acne, but

also from the participating inflammatory cells, the final result would be identical [45].

In addition, in the mouse, transgenic overexpression of COX2 in the basal compartment of the epidermis correlating with increased PGE2 levels caused sebaceous gland hyperplasia and overshooting sebum production, pointing to a possible role of COX2-mediated PGE2 synthesis in sebum production [46]. However, the exact sequence of events regarding the interaction of human sebaceous gland cells with inflammatory cells or other agents leading to the induction of PG pathways in acne-involved sebaceous glands is not known [45].

The current study showed positive COX2 expression in sebaceous glands in control skin. This was in agreement with Alestas T et al., and Zhang Q et al., [45,47].

The positive hair follicle expression of COX2, demonstrated in the current work, was previously reported by Müller-Decker K et al., who stated that COX2 expression is hair cycling-dependent, becoming apparent in elongated hair germs, later on in the Outer Root Sheath (ORS) of the distal and proximal hair follicles, declining in catagen and telogen, and then being re-induced in the ORS of anagen hair follicles [48].

The current study showed positive COX2 expression in epidermis and pilosebaceous follicles in all cases of acne vulgaris. Furthermore, COX2 was up regulated in acne lesions compared with perilesional and normal skin. This supports the fact that increased COX2 expression may have a role in acne pathogenesis. This was in agreement of Aletas T et al., and Neufang G et al., [45,46].

Ottaviani M et al., reported increased expression of COX2 and PGE2 in acne involved skin associated with enhanced release of pro-inflammatory cytokines and a higher degree of lipoperoxidation. Authors stated that their findings support the interplay between lipoinflammation and lipid signaling in AV development [28].

All cases of acne vulgaris examined in the current study showed positive COX2 expression by dermal inflammatory cells. There was significant association between COX2 % in (lesional epidermis and pilosebaceous units) and papulopustular acne.

This role can be explained through the effects of mediators produced by COX2 activation. In response to an inflammatory insult, the release of PGs, and more importantly PGE2, constitutes a key event in the development of inflammation [49].

The presence of inflammation is the critical link between acne and eicosanoids. Sebaceous gland seems to be the key tissue in this relationship [50,51].

The current study showed that there was higher COX2% in epidermis pilosebaceous units in perilesional skin more than control. There was also higher COX2 positivity in inflammatory cells in perilesional skin more than control. This could be explained by the presence of inflammatory microenvironment in perilesional skin even before the apparent clinical disease [31].

In the current study, there was significant positive correlation between COX2% and LXR-α % and between COX2 H-score and LXR-α H-score in lesional epidermis and pilosebaceous units.

Contrary to our results, Fowler AJ et al., stated that activation of LXR-α inhibited the expression of COX2 in the SZ95 sebocytes [33]. It was reported that LXR-α pathway antagonizes inflammatory gene expression [52,53].

Now, a question arises; what is the therapeutic value of LXR-α and COX2 antagonists in AV treatment?

Hong I et al., documented that LXR-α antagonists could be clinically implicated for the treatment of seborrhea and acne [18]. Viennois E et al., stated that selective LXR-α agonists that are isoform and tissue-specific could provide localized and specific cutaneous effects that do not influence other LXR receptor pools [54]. Clinical trials using COX2 inhibitors in management of AV are needed.

LIMITATION
The study was carried out on a small number of cases who were of the same ethnic background. Future large-scaled research on population of different ethnicities including other AV variants and acneform eruptions is needed.

CONCLUSION
Both LXR-α and COX-2 play a role in the pathogenesis of acne vulgaris through their effects on cellular proliferation, inflammation and lipid synthesis. Research for new therapeutic modalities for acne vulgaris based on their inhibition is needed. More understanding of the interaction between LXR-α, COX2 and acne lesions may lead to effective therapies, possibly directed toward specific cell types or steps within the inflammatory pathways.

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