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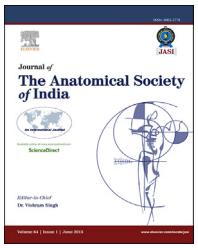

Original Article

Epidermal androgen receptors in acne vulgaris patients before and following oral isotretinoin

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ARTICLE INFO

Article history:
Received 11 February 2015
Accepted 1 April 2015
Available online 20 April 2015

Keywords:
Androgens
Androgen receptor
Androgen receptor index
Isotretinoin
Acne

ABSTRACT

Introduction: The role of androgens and androgen receptors (AR) in the pathophysiology of acne vulgaris appears to be a complex phenomenon. It has been suggested earlier that oral administration of isotretinoin, the drug of choice for severe cases of acne, exerts its action through ARs, which is quite debatable. The aim is to study the response of androgen receptor (AR) in the skin of acne vulgaris patients by administrating isotretinoin orally.

Methods: Skin biopsy was procured from untreated patients of severe cases of acne vulgaris. Out of these, twenty histopathologically confirmed patients were included in the study. They were treated with oral isotretinoin in the dose of 0.5 mg/kg/day for 12 weeks, following which their skin biopsies were repeated. Immunostaining for androgen receptor was performed using mouse monoclonal antibodies. Androgen receptor index (AR index) was calculated for the acne patients before and following treatment with oral isotretinoin. Statistical analysis was done using paired t-test.

Result: The AR indices in skin of untreated acne patients were higher in male patients (26.62 ± 20.74) as compared to the female patients (7.5 ± 10.61). AR indices after 12 weeks of oral isotretinoin treatment showed a reduction in both males and female patients (20.55 ± 16 and 4.71 ± 6.75 respectively). However, the post treatment reduction in AR index was statistically significant in male patients only.

Discussion: Determination of AR status can be helpful in planning the treatment methodology of severe cases of acne. Our study also implicates the effectiveness of oral isotretinoin on acne patients through its interaction with androgen receptor.

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http://dx.doi.org/10.1016/j.jasi.2015.04.002

1. Introduction

Acne vulgaris is a multifactorial disease seen primarily in adolescents, involving the pilosebaceous unit. It is characterized by hypersecretion of androgens, abnormal cornification of the pilosebaceous duct and secondary bacterial invasion of the blocked glands. Although, superficial and not life threatening, acne is a disease that, if untreated, can have serious physical and psychological consequences. Severe acne can result in permanent physical scarring, that has been implicated as a risk factor for suicide, particularly in men. Other psychological stigmas associated with acne include, lowered self esteem and professional expectations, social inhibition, depression and anxiety.[1] Acne vulgaris is the single most common skin disease, which affects 85% of teenage boys and 80% of teenage girls.[1] The condition usually starts in adolescence and frequently resolves by the mid twenties.[2] However, there are differences in the presentation of acne in different gender, race and ethnic groups.[3]

The primary site of acne is the face and to a lesser extent, the back, chest, and shoulders. The lesions may be either non inflammatory or inflammatory.[4] Severe cases of inflammatory acne with large nodule are termed as Nodulocystic or severe nodular acne. Patients may have hypertrophic scars especially on trunk.[5]

Androgens play a role in the keratinocyte proliferation and follicular hyperkeratinisation of sebaceous follicles that is seen in acne vulgaris[6]. Androgens exert their effect through androgen receptors. Androgen receptors (AR) have been localized in the pilosebaceous unit and it has been observed clinically that antiandrogens may reduce follicular casts in these regions.[7] Androgen receptors are present in normal skin, being localized in the basal and suprabasal layer of epidermis and differentiating cells of the sebaceous glands.[8,9] Studies suggest significant association between testosterone and acne vulgaris.[10] The most potent androgen is Dihydrotestosterone (DHT), which is formed in the sebaceous glands, by a 5α reduction of testosterone. Local tissue conversion of testosterone to the more androgenic Dihydrotestosterone, by the enzyme 5α-reductase, has been shown to be increased in the affected skin of acne patients.[11,12]

At the cellular level, the androgens act by intracellular conversion of testosterone to Dihydrotestosterone (DHT) by the enzyme 5α-reductase and subsequently bind to its androgen cytosol and nuclear receptor. The binding of the nuclear hormone receptor complex to the nuclear chromatin promotes gene expression as a direct response to hormone stimuli. The extent of hormone stimulation correlates with the number of androgen receptor binding sites.[13] Androgen receptors on keratinocytes and sebocytes mediate hyperkeratinisation, sebaceous gland development and the production of sebum.[14] It is also suggested that the whole skin of acne patients, have higher target organ sensitivity than the skin of normal controls of the same age.[15]

Based on the earlier studies, it has been suggested that AR and Androgens play distinct roles in the skin pathogenesis and AR seems to be a better target than androgens for the treatment of acne vulgaris and other skin diseases.[16] Androgen receptors have affinities for a wide variety of steroid and non-steroid drugs, therefore androgen receptor expression has been used to predict clinical response to antiandrogenic treatment.[17]

Isotretinoin is becoming the drug of choice for severe recalcitrant nodulocystic acne.[18,19] A study indicates that the skin androgen receptors are sensitive to oral isotretinoin administration in acne patients.[20] It has been observed that isotretinoin causes a significant decrease in androgen receptor binding capacity without an alteration in its affinity.[20]

Extensive review of literature indicates the complexity of the role of androgens and androgen receptors in the pathophysiology of acne vulgaris. The mechanism by which isotretinoin exerts its response on acneic skin is equally debatable. Additionally, ethnic and racial differences have been seen in the presentation of acne vulgaris. Most of the published work on androgen receptor in patients with acne refers to western population. To the best of our knowledge no literature is available on AR expression in the skin of acne patients in Indian population. To the best of our knowledge, there is hardly any data on the effect of isotretinoin on androgen receptors in acne patients in Indian population. Therefore, the present study was an attempt to determine the status of epidermal androgen receptors in the skin of acne vulgaris patients before and following treatment with oral isotretinoin in Indian population.

2. Materials and methods

2.1. *Sample collection*

The study was conducted on patients between 16 and 25 years of age who presented in dermatology OPD with acne vulgaris in LN Hospital, New Delhi, India. A detailed history of patients presenting with acne was taken. Their lesions were examined thoroughly.

Their lesions were graded as follows:

- Grade I – < 25 comedones without pustules.
- Grade II – 25 – 50 comedones with pustules.
- Grade III – > 50 comedones with pustules and truncal involvement.
- Grade IV – nodular and cystic lesions truncal involvement.

We included twenty histopathologically confirmed patients (twelve males and eight females) with grade III and IV acne vulgaris in our study. A written and informed consent was taken from the patients for the investigations, biopsy and subsequent oral isotretinoin treatment. Before starting the treatment, each patient underwent the following investigations: Complete haemogram, Liver function tests, Kidney function tests, Lipid profile and Serum calcium levels. Patients having normal blood investigations were included in the study. Pregnant patients and patients with past history of hepatitis were excluded from the study.

After taking due approval from Institutional ethical committee, the patients were subjected to punch biopsy from the affected skin before starting the treatment. Oral isotretinoin in the dose of 0.5 mg/kg/day was administered to the patients

for 12 weeks. Punch biopsy was obtained from the patients two weeks after stopping isotretinoin treatment.

3. Study design

We divided our study into following groups:

- Group I A – 20 patients of grade III & IV acne vulgaris, before oral isotretinoin treatment.
- Group I B – 20 patients of Group IA after 12 weeks of oral isotretinoin treatment.

Specimens were fixed in 10% formalin for 24 h. Paraffin blocks were made and 5 μm thick sections were cut by rotary microtome. The sections were stained with haematoxylin and eosin.

3.1. *Immunohistochemical staining*

Thin sections of paraffin block were cut using microtome and taken on poly-L-Lysine coated slides. The sections were deparaffinised followed by three changes of acetone. The slides were then washed and kept in P.B.S. buffer. The slides were then kept in 0.03% Hydrogen Peroxide – methanol block for 30 min on shaker. The peroxide block was discarded and three washes of P.B.S. buffer were given. Antigen retrieval was done. The slides were dipped into pre-warmed citrate buffer (pH 6.0) and were micro waved for 15–30 min and then cooled. After antigen retrieval, three washes of P.B.S. buffer were given for 5 min each on shaker. Fresh 5% milk block was prepared in working P.B.S. buffer and slides were kept in it for 30 min on shaker.

The slides were then cleaned and the sections were covered with primary antibody and kept in the moist chamber for overnight at 4 °C followed by three washes of P.B.S. The sections were covered with secondary antibody and kept in the moist chamber at room temperature for 20 min. The sections were recovered with tertiary antibody and kept in the moist chamber at room temperature for 20 min. The area surrounding the tissue section was cleaned & covered with working DAB solution and observed under the microscope to see appearance of brown colour. The colour reaction was stopped by dipping the slide into the distilled water. The slides were then washed in running tap water. The sections were counter stained with Harri's Haematoxylin for 10 s to 2 min. The slides were then washed in running tap water. The sections were dehydrated using three changes of acetone for 5 min each. The sections were cleared using three changes of Xylene for 5 min each. The sections were then mounted in D.P.X and slides were viewed under the microscope.

3.2. *Calculation of AR index*

Single block per biopsied material was cut and ten slides per case were made. Digital images of 5 randomly selected high-power fields were obtained per case using a Nikon microscope (Nikon Corp, Tokyo, Japan). Positive cells showing nuclear staining were counted in the basal and suprabasal layers of epidermis. AR index per case was calculated by the following formula-number of positive cells/total number of cells counted × 100. The mean AR index was calculated for each group and data was tabulated separately for males and females.

4. Statistical analysis

Pre and post treatment data (mean ± SD) was tabulated separately for males and female patients. Statistical analysis was done using Wilcoxon Signed-Rank Test for non-parametric data. W-value was used to evaluate our hypothesis. However, Wilcoxon test was not successful in female patients as sample size was very less. Therefore, Levene's test was used to compare AR index in female patients. The p-value <0.05 was considered to be statistically significant.

5. Results

AR positivity was seen in 11 out of 20 untreated acne patients (8 males and 3 females). The AR index (mean ± SD) in these patients was higher in males (26.62 ± 20.74) as compared to females (7.5 ± 10.61) (Table 1). The AR index (mean ± SD) in patients of acne after 12 weeks of treatment showed a reduction in both males and females (20.55 ± 16 and 4.71 ± 6.75 respectively) as compared to acne patients before treatment (26.62 ± 20.74 in males and 7.5 ± 10.61 in females). Statistically significant post-treatment reduction in AR index was seen in male patients only ($W = 0$, critical value of W, at $p \leq 0.05$ was 3, Tables 1 and 2 Figs. 1 and 2).

An unusual finding in our study was presence of few basal and suprabasal epidermal keratinocytes showing AR positivity expressed in cytoplasm only and few showing both in nucleus and cytoplasm (Figs. 1 and 2). The cytoplasmic positivity was observed in both treated and untreated acne patients. However, we did not count cytoplasmic AR positive cells for calculation of AR index for reasons discussed below.

6. Discussion

Acne vulgaris is a very challenging dermatological disorder seen in all age groups but predominantly in adolescents.

Table 1 – Wilcoxon Signed-rank test for Group IA (male acne patients before treatment) & Group IB (male acne patients after isotretinoin treatment) showing statistically significant post treatment reduction in AR index.

W value	Z value	Mean difference	Sum of positive ranks	Sum of negative ranks	Critical value of W at $p \leq 0.05$
0	−2.5205	−14.36	36	0	3 (statistically significant)

Table 2 – Levene's Test showing statistically significant post-treatment reduction in AR index in males having acne but not in female patients.

Cases	n	AR index (mean ± SD)		Paired differences	p-value
		Group IA	Group IB		
Males	12	26.62 ± 20.74	20.55 ± 16.78	6.075 ± 5.37	0.002
Females	8	7.5 ± 10.71	4.7 ± 6.75	2.78 ± 8.8	0.402

n – no. of cases.
S.D – Standard deviation.
AR Index – Androgen receptor index.
Group IA – Acne patients before treatment.
Group IB – Acne patients after isotretinoin treatment.
$p < 0.05$, statistically significant.

Androgens are known to play an important role in pathogenesis of acne, having a role in follicular hyperkeratinisation of sebaceous follicle, a feature seen in acne. Androgens cause enlargement of the sebaceous glands with an increase in sebum production. It has been suggested that the entire integument of acne patients is dependent and influenced by androgens.[21] The action of androgens is mediated by a high affinity intracellular receptor – androgen receptor (AR) which is located in the basal and suprabasal layers of epidermis and sebaceous glands.

An extensive review of literature has revealed the localization of AR in skin in the nuclear compartment of the cell.[8,22] Few researchers, although, report both cytoplasmic and nuclear AR positivity.[23,24] The cytoplasmic androgen receptors are the unbound AR which are internalized or translocated in the nucleus only in presence of its ligand.[25]However, according to some authors, it is thought that bound steroid receptors like oestrogen receptors, progesterone receptors and androgen receptors, are associated tightly with the nuclear chromatin, whereas unbound receptors are more readily displaced from the cell nucleus.[25] Thus, abundant steroid receptors measured in preparations of cell cytosol have been considered as an artifact of cell fractionation. The cytoplasmic staining seen in our study may represent artifactual staining due to tissue processing or post mortem translocation of nuclear receptors as suggested by few authors. Therefore, we only considered nuclear AR positivity, which is the bound form of AR for calculation of AR index. Studies suggest that there exists a parallelism between cytoplasmic AR positivity and the levels of androgens.[26] However, in our study, we did not measure the serum androgen levels in the acne patients.

Androgen receptors are present in normal skin in 62–75% of normal skin specimen.[27] Previous studies also observed more androgen receptor positive males (27%) as compared to females (23%).[28] On the contrary, a study reports no difference between concentrations of AR in male and female[11] indicating that the androgen receptor concentration is not solely regulated by androgens.

AR positivity was observed in 11 out of 20 (55%) acne patients in our study. A previous study reported AR positivity in 78.8% acne patients.[29] In our study, 66.6% (8 out of 12) male acne patients showed AR positivity and only 37.5% female patients (3 out of 8 female patients) were AR positive. A previous study found 27% of female acne patients and 50% of male acne patients to be AR positive[27] which corroborated with the present study. These findings reinforce the importance of androgens on the androgen receptor concentration

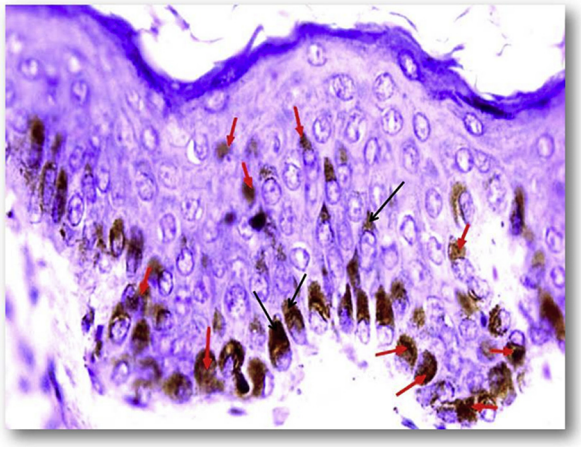

Fig. 1 – Epidermis from Group IA (acne patients) showing an increase in AR positive cells. Nuclear AR positivity can be seen in basal and suprabasal layers, marked as red arrows. Cytoplasmic AR positivity is marked as black arrows, seen under 400× magnification.

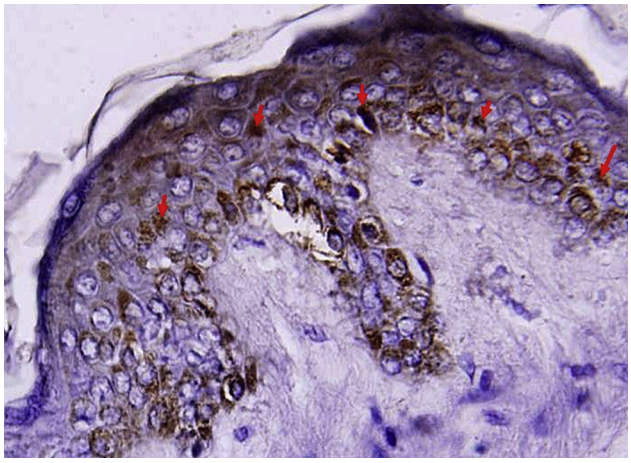

Fig. 2 – Epidermis from Group I B (acne patients after isotretinoin treatment) showing a decrease in AR positivity, marked as red arrows (400×).

particularly in males who have higher levels of androgens than females.

Isotretinoin is widely used as a drug of choice in severe cases of acne. Previous study suggests that AR is sensitive to oral isotretinoin administration in acne patients.[20] The present study showed a decrease in the androgen receptor positivity in acne patients after 12 weeks of isotretinoin treatment in both males and females. The results of the present study is in agreement with a study which demonstrated 2.6 fold decrease in the skin AR levels in acne patients after isotretinoin treatment.[30] However, in our study, the decrease in AR index was statistically significant in male patients but not in female patients indicating that androgens are important but not mandatory in the pathogenesis of acne vulgaris.

7. Conclusion

Our study indicates that determination of AR status in acne patients can be used as a marker of response to oral isotretinoin particularly in males. These findings suggest that the determination of androgen receptor may be taken as a baseline marker to plan the treatment of severe cases of acne vulgaris. Based on our immunohistochemical studies of androgen receptors it is also suggested that isotretinoin is effective on severe cases of acne vulgaris through a mechanism involving androgen receptor.

8. Limitations of our study

The study was a part of PG training program with a limited availability of time. Moreover, owing to certain ethical issues with respect to taking biopsies twice in a patient who was already seeking medical advice for a clinical condition associated with scarring and social stigma, we limited the sample size to 20 patients. Additionally, only clinical grade III & IV patients were histopathologically confirmed and included in our study. However, clinical Grade II patients could exhibit features of moderate acne histopathologically, but these patients are administered only topical and not oral isotretinoin, hence they were not included in our study.

Conflicts of interest

All authors have none to declare.

Acknowledgements

Our study was funded by Maulana Azad Medical College, New Delhi for the purpose of research work and we acknowledge our Institution for the support. There were no other financial grants and there were no conflict of interests. We also want to thank the support given by our colleagues and the technical staff who assisted in our research work.

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