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Efficacy of Adapalene 0.1% and Benzoyl Peroxide 2.5% Gel for the Treatment of Acne Vulgaris Using FTIR-ATR Spectroscopic Technique

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Abstract: ***Acne Vulgaris is the most common skin disease affecting about 85% of adolescents. Acne, is driven by androgenic hormones, which typically become active during the teenage years. Excess secretion of hormones, combined with bacteria on the skin, and fatty acids within oil glands, cause acne. The aim of the present study is to evaluate the safety and efficacy of the Adapalene 0.1% & Benzoyl Peroxide 2.5% (ADP+BPO) fixed-dose combination gel, in a few human (both male and female) for the treatment of Acne vulgaris. Instead of analyzing blood to diagnose Acne Vulgaris, hair could be used to detect acne vulgaris using FTIR-ATR spectroscopic technique. FTIR spectra of the Acne, hair samples, Pre and Post-treatment along with healthy persons were recorded in the mid-infrared region. The biomarkers for the acne vulgaris samples with specific peaks during the before treatment were analyzed and compared with, hair samples of healthy subjects. The absorption values of some of the specific bands of the biomolecules present in the hair samples viz., Protein, lipids, and Squalene both the pre- and post-treatment subjects are noted. It was observed that these biomarkers are significantly different between pre- and post-treatment hair samples of acne patients and hence the efficacy of Adapalene 0.1% and Benzoyl Peroxide 2.5% is estimated. The results are further validated with statistical analysis by applying the dependent t-test, which indicated that the spectral variations are statistically significant.***

Keywords: FTIR-ATR, Acne Vulgaris, Propionibacterium, ADP, BPO, Sebum, Lipid, Squalene

1. Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit (comprising the hair follicle and sebaceous gland) and is among the most common dermatological conditions worldwide, with an estimated 650 million people affected most people experience acne during adolescence, with >90% of teenage boys and 85% of teenage girls affected [1,2]. Acne vulgaris is an exceptionally common, recurring disease involving multiple etiological factors, including follicular hyper keratinization, increased sebum production, Propionibacterium acnes shown in **Fig. 2**proliferation and inflammation. It typically starts around the age of 15 to 20 years, but tends to manifest earlier in female patients. The mixture of oil and cells provide a favorable environment for bacteria Propionibacterium acnes (P acnes) that normally live on the skin to grow in the plugged follicles which causes acne. The increased sebum production and alteration regarding lipid profile, cause LDL level significantly higher in patients than control ones and the quality of sebum lipids play a major role in acne pathogenesis. The scientist believes that an increase in male hormones that are present in both males and females lead to an over production of sebum. Scientists hypothesize that one important factor is the way skin interacts with androgens in the bloodstream. The fatty acid content of sebum may play an important role in pore clogging, that fatty acids trigger the body's production of a specific inflammatory substance called Interleukin-1 (IL-1), which is known to trigger acne. ILs are groups of cytokines (secreted protein's and signal molecules) first seen by the white blood cells (leukocytes).It is also caused by inflammation of the hair follicles and oil-producing (sebaceous) glands of the skin called pilosebaceous unit. Pilosebaceous density is greatest on the face, upper neck, and chest and is roughly nine times the concentration found elsewhere on the body. The sebaceous gland is attached to the upper third of the hair follicle as shown in **Fig.1,** in the dermis layer.

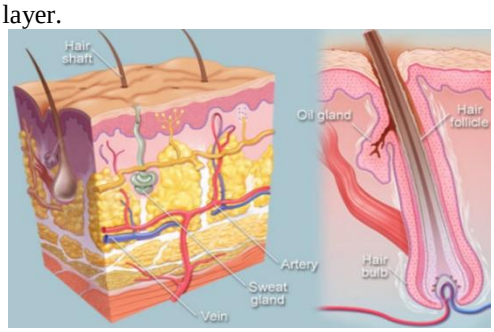

Figure 1: Hair Follicle

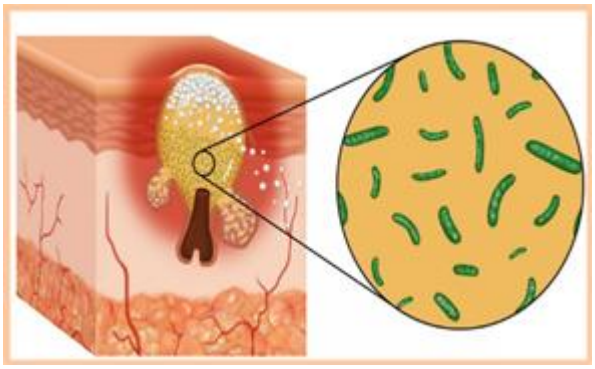

Figure 2: Propionibacterium acnes

Sebum and keratinocytes that fill the narrow follicle may produce a plug, an early sign of acne. The follicular plugging (comedones) prevents sebum from reaching the skin surface through a pore. Human sebum consists of Squalene, esters of glycerol, wax, and cholesterol, as well as free cholesterol and fatty acid. The most characteristic products of sebaceous secretion are wax esters (26%) and Squalene (12%), they are unique to sebum. In acne patients, oxidative modification of proteins, lipids, and nucleic acids have been implicated in the mechanism of various diseases. Investigators reported that components of sebum, particularly Squalene, show enhanced comedogenicity when oxidized [3]. Indeed, squalene was reported to be highly sensitive to oxidation and researchers reported that both squalene and its oxidized metabolites are found at much higher levels in the acne vs. Healthy controls [4]. Squalene is a triterpene of the general formula is C_{30} H_{30} that comprises 6 non-conjugated double bonds, making this compound one of the most unsaturated lipids [5].

Upon oxidative challenge, Squalene is readily oxidized giving rise to different Squalene peroxidation by-products exerting harmful activities in skin cell cultures and in vivo, including keratinocytes cytotoxicity. It is the biochemical precursor to the whole family of steroids [6] and also which is a precursor of cholesterol. A possible explanation for the Squalene build-up in the sebaceous gland may be linked to over expression or an increase in the activity of squalene-synthesis in the cells, or it may be related to decreased levels or activity of the enzymes involved in the conversion to cholesterol [7]. Specifically, sebum of patients with acne contains lipoperoxides resulting from the peroxidation of the lipid Squalene [8]. In addition to activating the immune system, studies show that P. acnes can also activate groups of proteins called inflammasome complexes in the skin, which stimulate the release of inflammatory molecules called cytokines and enzymes that lead to the accumulation of neutrophils at the site of acne comedones [9]. Due to the phagocytosis, these accumulated neutrophils generate Reactive oxygen species (ROS). ROS refers to a diverse group of reactive, short lived, oxygen containing species, such as superoxide radicals (O^-), hydrogen peroxide (H_2O_2), hydroxyl radical (^-OH), singlet oxygen ($^1O_2$), peroyl radical (LOO^-). These species cause inflammation and tissue injury [10].

Based on expert consensus on relative effectiveness, the American Academy of Dermatology recommends doxycycline and minocycline (Minocin) rather than Tetracycline [11]. Kotori in his investigation, he found three months of treatment with low-dose isotretinoin (20 mg/d) was found to be effective in the treatment of moderate acne. SeongdaeKim evaluate antibacterial activity against P.acnes, and in the vitro antioxidant activities of Sanguisorb officinalis L [12]. Sebacia microparticles are now FDA-cleared for use in the treatment of acne .The clearance comes on the heels of a pivotal study demonstrating the clinical safety and efficacy of the microparticles.These sebacia microparticles selectively target the sebaceous glands and are indicated for use as an accessory to 1064 nm lasers to facilitate photothermal heating of sebaceous glands for the treatment of mild to moderate inflammatory acne vulgaris [13]. Moore A et al. 2018 evaluate the efficacy and safety of once-daily sarecycline, a novel, narrow-spectrum tetracycline- class antibiotic, in moderate to severe-acne [14]. In multiple mouse models triafarotene exhibited superior comedolytic, anti-inflammatory and depigmenting activity compared with other topical retinoid. Samples of patients with acne treated with trifarotine 0.005% cream was helpful to establish further clinical relevance [15].

Topical retinoids are indicated as monotherapy for non-inflammatory acne and as combination therapy with antibiotics to treat inflammatory acne. Adapalene (Differin) is the best tolerated topical retinoid [4]. Several studies have been conducted to study the safety and efficacy of low-dose isotretinoin in the treatment of moderate to severe acne vulgaris [16, 11, 19, 17, 18] Doxycycline is a tetracycline antibiotic that fights bacteria in the body are used to treat many different bacterial infections, such as acne, urinary tract infections, intestinal infections, eye infections, gonorrhea, chlamydia, Periodontitis (gum disease), and others. These combinations can also improve patient compliance, increase the effectiveness of the treatment and decrease the development of bacterial resistance [20,21,22] have compared the efficacy and safety of 5% benzoyl peroxide, 0.1% adapalene, and their combinations. Topical retinoids, derivatives of vitamin A have been used to treat acne for almost three decades. Sebum of patients with acne contains lipoperoxide resulting from the peroxidation of the lipid Squalene [23], lipoperoxides and MUFAS (Monounsaturated fatty acids) influence keratinocyte proliferation and differentiation, contributing to follicular hyperkeratinization [23, 24]. Topical retinoids, derivatives of vitamin A are the most effective comedolytic agents for the treatment of acne vulgaris, decreasing the formation and the number of microcomedones. They also promote the clearing of preexisting comedones [25] and decrease in papulopustular lesions [26, 27, 28]. Benzoyl peroxide and adapalene are among the most effective topical agents used in the treatment of acne vulgaris [29]**.** **C**ompared the efficacy and safety of benzoyl peroxide 4% gel used twice daily with adapalene 0.1% gel used once daily, they found benzoyl peroxide more effective than adapalene on noninflammatory and inflammatory lesions at weeks 2 and 5, and they found both drugs safe.

Efficacy is the ability of a drug to produce the desired therapeutic effect after taking the drug or applying the gel or ointment. There are many methods to find out efficacy, but FTIR Spectroscopy is one of the recent scientific and unique tool to characterize the inorganic and organic compounds by

chemists. Its application in biology for studying the structure and conformation of proteins, nucleic acids and lipids have been well documented in the literature [30, 31]. The aim of the present study is to evaluate the efficacy of the adapalene 0.1% and benzoyl peroxide 2.5% (ADP+BPO) gel in the treatment of acne vulgaris using, a single human scalp hair in the FTIR-ATR spectroscopic method. Treatment goals in patients with acne include the prevention of scars, the reduction of psychological morbidity, and the resolution of noninflammatory and inflammatory lesions. Therapy should be continued for a minimum of eight weeks maximum of 3 months. The human hair is composed of Biochemical mposition such as alpha keratin, proteins, lipids, nucleus acids, glycogen (carbohydrates) and a small amount of water [32, 33, 34, 35, and 36]. The Hair units possess complex and abundant vascular network to ensure adequate blood supply [37]. Today a growing number of proofs-of-principle assays have been established using Hair as a case study of diabetes [38], thyroid [39] and breast cancer [40]. The actual biomolecular changes of the acne vulgaris skin disease in the sebum and blood, human hair one of the tissues will also reflect the same biomolecular changes which are present in the sebum. Diagnosing acne vulgaris using human tissue, hair is quite challenging in the clinical laboratory. Hence science gives an opportunity to use hair samples as a tool for diagnosing any disease. In the hair, biomolecular changes could be well diagnosed using FTIR-ATR spectroscopic technique.

2. Materials and Methods

Twenty Acne vulgaris patients were introduced in the present investigation. All the 20 patients were applied the combination topical gel adapalene 0.1% and benzoyl peroxide 2.5% (ADP+BPO) daily on the night for one course of 3 months. After the 3 months treatment, single scalp hair samples were collected from the patients (Post-treatment) and samples of single scalp hair samples were obtained from 20 healthy subjects (control group) for FTIR-ATR spectral measurements. The samples before medication of acne vulgaris patients were considered as the Pre-treatment samples. The collected hair samples were subjected for FTIR-ATR spectral measurements were packed in an airtight plastic cover stored away from heat and moisture. In order to eliminate any surface contaminations, specimens were washed by dipping in distilled water for many times and the washed hair samples were admitted into laminar air flow to remove the water thoroughly, as water is a good absorbent of infrared radiation, it affects the actual spectral response of the test material. The hair samples collected were labeled with the respective subjects in a clean polythene bags at room temperature.

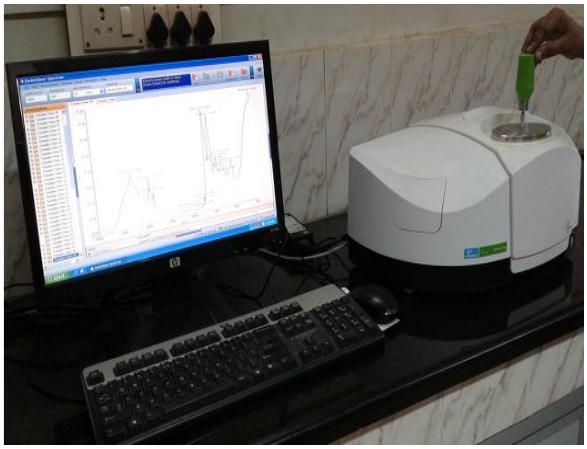

Figure 3: FTIR-ATR Spectral recordings ofHuman Scalp hair tissues

The root end of the hair sample was placed on the internal reflectance crystal, and force was applied by the pressure gauge on the hair sample to provide good optical contact with the crystal. The FTIR-ATR spectra for all the samples were recorded in the mid-infrared region of 4000-450 cm^{-1}. Using Perkin Elmer Spectrum-Two FTIR Spectrophotometer having highly reliable and single bounce diamond as its Internal Reflectance Element (IRE) as shown in the **Fig.3**. The incident IR beam strikes the interface between the IRE and the sample of a lower refractive index. The refractive index of the tissue sample hair in FTIR-ATR must be lower than the IRE employed. Diamond is the IRE which has a refractive index as 2.4, the Hair tissue has a refractive of 1.55, and this enhances the spectra and gives detailed analysis of the tissue samples. This internal reflectance creates an evanescent wave that extends beyond the surface of the crystal into the tissue sample held in contact with the crystal. FTIR-ATR spectra of human scalp hair samples of the 20 acne patients before applying gel (Pre-treatment) adapalene 0.1% and benzoyl peroxide 2.5% were recorded. After applying the gel adapalene 0.1% and benzoyl peroxide 2.5% for a course of 3 months, (Post-treatment), FTIR –ATR spectra hair samples were recorded. The average overlaid spectra of the hair samples of Pretreatment and Post treatment along with the healthy subjects are presented in the **Fig.4.**

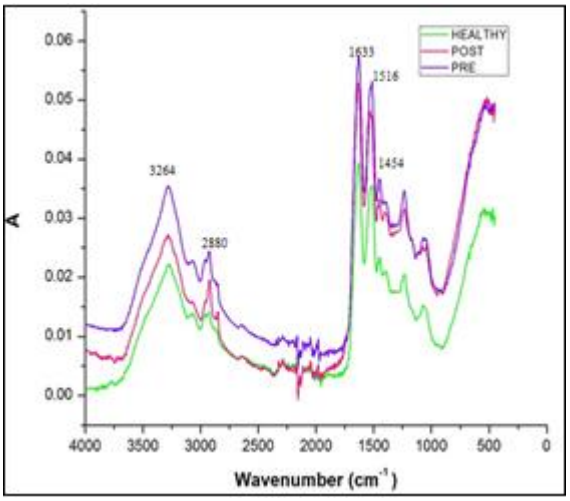

Figure 4: The average overlaid FTIR-ATR spectra of Healthy, Pre and Post- treatment of Acne vulgaris patients Human Scalp Hair

3. Treatment of Acne

Medicines in different forms like oral, ointment and tablets are used as a treatment for acne are often effective alone in the mild to moderate acne and are important adjuncts to oral antibiotics in more severe acne. The treatments like Light and laser therapies can be used for the treatment of acne. Examples include visible light, pulsed-dye laser, and Photodynamic therapies. Overall, adapalene (Differin) is the best tolerated topical retinoid. Limited evidence suggests that tazarotene (Tazorac) is more effective than adapalene and tretinoin (Retin-A). Adapalene 0.1% and & Benzoyl peroxide 2.5 % gel is a topical formulation used for the treatment of different types of acne, blackheads, whiteheads, cysts, nodules, and pustules, which is an antibacterial skin-peeling agent. Patients should also be made aware that it may take 3–6 weeks until an improvement can be observed [41, 42, and 43]. Benzoyl peroxide is an important treatment for mild to moderate acne and although it can be used as monotherapy for a period of 6–8 weeks, is often combined with topical antibiotics in order to reduce the resistance of the P. acnes species and to increase the efficacy of treatment [42, 44]. The comedones are treated with topical tretinoin and Mild inflammatory acne is treated by topical retinoid like adapalene 0.1% + benzoyl peroxide 2.5 % (ADP+BPO) as a combination therapy. Moderate acne is treated with oral antibiotic plus topical therapy like tetracycline, minocycline, erythromycin, and doxycycline. Oral drug isotretinoin applies to severe acne, an oral treatment that needs to be taken for 16 to 20 weeks. Cystic acne may be treated with a corticosteroid injection called triamcinolone. This injection into the lesion aims to reduce scarring caused by the inflammation. The purpose of treatment for acne is for reducing sebum production, comedone formation, inflammation, and bacterial counts and normalizing keratinization. Today's therapeutic modalities for acne are aimed at one or more of its pathogenic precipitants, which include androgenic hormonal stimulation, hyper secretion of sebum, faulty occlusion of the follicular orifice, P. acnes colonization, and inflammation.

3.1 Adapalene

Adapalene shown in **Fig.5**is a synthetic naphthoic acid derivative with retinoid activity. The effect of ADP+BPO was sustained for 4 months, and it was also safe as a long-term treatment for up to 12 months [45]. The chemical name of adapalene is 6-[3-(1-adamantyl) - 4- methoxyphenyl] -2-naphthoic acid. Adapalene is a white to off-white powder which is soluble in tetrahydrofuran, sparingly soluble in ethanol, and practically insoluble in water. The molecular formula is $C_{28}H_{28}O_3$ and molecular weight is 412.52. Some of its biological activities are the same with tretinoin, however, it is chemically more stable and lipophilic. By this way, it can reach higher concentrations in the pilosebaceous unit. Its mechanism of action is twofold: first, it inhibits comedo formation through its ability to bind to retinoic acid receptors and modulate cell differentiation; and second, it possesses direct anti-inflammatory activity. This anti-inflammatory effect is due to inhibition of the lipoxygenase activity and also to the oxidative metabolism of arachidonic acid. These mechanisms may be the reason for the decreased risk of irritation with adapalene. Adapalene has a very low percutaneous absorption once the drug has penetrated the stratum corneum so that it becomes entrapped in the epidermis and hair follicle, which are targeted areas.

3.2Benzoyl Peroxide

The chemical formula of benzoyl peroxide as shown in **Fig.6** is $C_{14}H_{10}O_4$ and its molecular mass is 242.23 g/Mol and poorly soluble in water. Benzoyl peroxide is effective for reducing the number and severity of acne lesions. It has a bactericidal effect on propionibacterium acnes associated with acne and does not induce antibiotic resistance. In the past, dermatologists and researchers believed that P. acnes bacteria were the cause of acne, so the treatment for acne was focused on finding ways to kill the bacteria by oxidizing its proteins through the formation of oxygen free radicals and benzoic acid. These free radicals are thought to interfere with the bacterium's metabolism and ability to make proteins [46, 47]. One of the ways that benzoyl peroxide fight acne infection is by irritating acne bacteria and forcing them to spend energy in maintaining the integrity of their outer membranes. In the treatment of all but the most severe recalcitrant acne vulgaris, the combination of a topical retinoid and benz benzoyl peroxide (BPO) with an oral antibiotic has been recommended as first-line therapy [48,49].

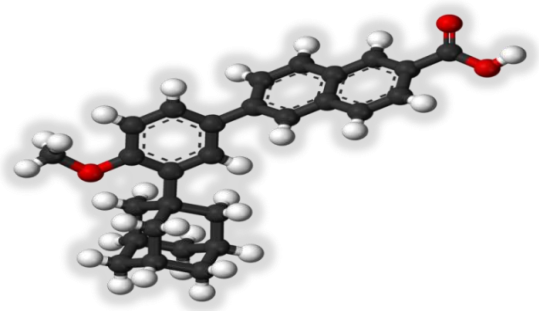

Figure 5: Adapalene

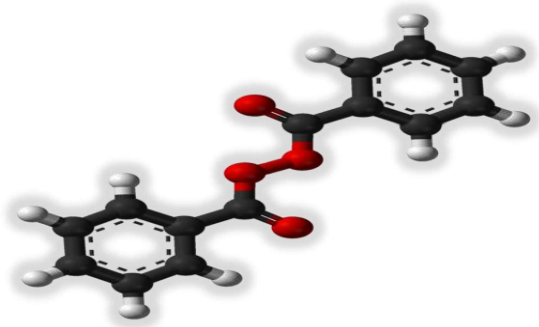

Figure 6: Benzoyl Peroxide

4. Results and Discussion

Using the FTIR-ATR technique the spectral deviations are also identified very accurately for the Pretreatment and Post-treatment of the Acne vulgaris individuals. From the overlaid spectra as shown in **Fig.4,** the absorption peaks of proteins (3264 cm^{-1}), Amide I (1633 cm^{-1}), Amide II (1516 cm^{-1}), and Squalene (1454 cm^{-1}) are severe for the acne vulgaris patients when compared to healthy subjects because of the proteins, lipids, and Squalene (LDL). From the pretreatment spectrum of the acne patients, it's viewed that,

protein band which occurs at 3264 cm^{-1} is due to N-H stretching mode of (Amide A) of protein and the main absorption bands are those associated with aliphatic C-H stretches of the saturated and unsaturated long chain fatty acids, alcohols and esters. The asymmetric and symmetric stretching C-H vibrations of methane and methylene groups belong to free fatty acids are found to be present around 2930-2873 cm^{-1}. The bands occur at 2880 cm^{-1} are belong to –CH3 groups are due lipids and 1446 cm^{-1} is due to $–CH_2$ scissoring is due to Squalene (LDL) [50]. The strong absorption band at 1633 cm^{-1} at corresponds to C=O stretching, vibration (Amide I) whereas the amide II band centered around 1516 cm^{-1} due to C=O is stretching coupled with C-N stretching and bending deformation of N-H in the protein backbone. The absorption in the keratin spectrum is attributed to the deformation and bending modes of the C-H/CH_2/CH_3 groups originating from the various amino acid (SC) side chains.

From the **Fig.4** the prominent absorption peak at 3264 cm^{-1} is due to protein band after post treatment with gel adapalene 0.1% and benzoyl peroxide 2.5% shows that absorption level is very well decreased.Amide I band 1633 cm^{-1}, Amide II band 1516 cm^{-1} and squalene at 1454 cm^{-1} absorption values also show differences in absorption levels after the post treatment. The sensitivity exhibited by the FTIR spectral bands of proteins, lipids due to the IR absorption of acne vulgaris tissue samples indicates that these were the key biomarkers in the investigation of acne vulgaris. Topical application of ADP + BPO, a lipid-soluble antioxidant, reduces the severity of acne. Squalene (LDL), which is in the region 1454 cm^{-1} is specific to human sebum, protects the skin surface from lipid peroxidation. The main aim is to control the level of protein, lipids, and Squalene (LDL) present in the sebum.

5. Statistical Analysis

In order to find the efficacy of the Gel ADP+BPO in the treatment of acne vulgaris, the absorption values of the vibrational bands at 3264, 1633, 1515 cm^{-1} and 1454 cm^{-1} corresponding to protein, lipids, amide I, amide II, Squalene (LDL) peaks respectively on (After 3 month course) spectra were noted. Bio-markers for acne vulgaris samples with specific peaks (Proteins/ lipids), (Amide I/lipids), (Amide II/lipids), (Squalene /lipids) and the intensity ratio parameters were calculated. The significance of the intensity ratio results was estimated using dependent t-test statistic method. For the statistical interpretation, lowest p-value ($p<0.05$) is taken as statistically significant.

The efficacy is found out from the formula,
% of Efficacy = $(A_{Pre}-A_{Post}/ A_{Pre}) X100$

And the results were tabulated in **Table 1.** The FTIR spectral data were statistically analyzed by paired sample t-test for pre and post-treatment of the tissue samples. The mean and the standard deviation for pre and post treatment of the acne vulgaris individual tissue samples were found from the above said four intensity ratio parameters Viz., R1 = $I_{(3264/2864)}$, R2 = $I_{(1633/2864)}$, R3 = $I_{(1516/2864)}$ and R4 =$I_{(1454/2864)}$ have been introduced and calculated for Pre and Post- Treatment.

Acne vulgaris hair tissue samples of pre and post –treatment patient's tissue samples were found from the above said four intensity ratio parameters are given in **Table.2** and similarly the mean and the standard deviation for healthy persons and post-treatment acne vulgaris patient's intensity ratio parameters are presented in the **Table.3** respectively. The results obtained from the tissue samples for the statistical analysis by t-test shows that the pre and post-treatment mean values for the ratios R1= $I_{(3264/2864)}$ and R2= $I_{(1633/2864)}$ mean value have changed from 2.0460 to 0.7190, and 4.2167 to 1.7859, R3=$I_{(1516/2864)}$ and R4 =$I_{(1454/2864)}$ mean value changes from 3.8105 to 1.7609 and 2.6935 to 1.0057 respectively. The histogram with a comparison of the mean intensity Ratio Parameters of Healthy, Pre, and Post-treatment of Acne vulgaris individual's hair samples are shown in **Fig. 7**. There is a statistically significant difference in levels of proteins, lipids, and squalene, for each of the internal ratio parameters R1-R4.

Table 1: Intensity ratio parameters of pre and post treatment and Efficacy

Samples	Stages	$R_1 = I_{3264}$ (Proteins)/I_{2864} (Lipids)	$R_2 = I_{1633}$ (AmideI) / I_{2864} (Lipids)	$R_3 = I_{1516}$ (Amide II) / I_{2864} (Lipids)	$R_4 = I_{1454}$ (Squalene) / I_{2864} (Lipids)
1	Pre	2.8254	7.2222	6.6508	4.3968
	Post	0.7761	2.449	3.8878	1.6429
	% of Efficacy	72.53	66.09	41.54	62.63
2	Pre	1.9606	3.6535	3.3386	2.1732
	Post	0.3893	1.1409	1.5711	0.2013
	% of Efficacy	80.14	68.77	52.94	90.73
3	Pre	2.9211	6.9605	6.3158	4.3026
	Post	2.0703	4.1094	3.6953	1.5859
	% of Efficacy	41.09	40.96	41.49	63.14
4	Pre	2.0758	4.2424	3.7273	2.6136
	Post	0.0094	0.8447	1.5031	0.441
	% of Efficacy	99.54	80.08	59.67	83.12
5	Pre	2.2661	5.211	4.6881	3.3028
	Post	1.0816	1.3776	2.1224	1.0306
	% of Efficacy	52.27	73.56	54.72	68.79
6	Pre	2.2427	5.3786	4.8155	3.2816
	Post	0.9857	2.5571	2.3143	0.4071
	% of Efficacy	56.04	52.45	51.94	87.59
7	Pre	1.7591	3.4234	3.0949	2.1825

	Post	0.4634	1.8976	0.7707	0.9024
	% of Efficacy	73.65	44.56	75.09	58.65
8	Pre	2.0918	5.2449	4.7347	3.2347
	Post	0.5071	2.0071	2.0500	0.1214
	% of Efficacy	75.75	61.73	56.79	96.24
9	Pre	1.8293	3.0732	2.6768	1.8293
	Post	0.7459	1.2486	1.1271	0.4530
	% of Efficacy	59.22	59.37	57.89	75.23
10	Pre	1.7349	2.9628	2.7372	1.7930
	Post	0.3390	1.3631	1.0446	1.0210
	% of Efficacy	80.45	53.99	61.83	43.05
11	Pre	1.7667	2.9528	2.7444	1.7922
	Post	0.1077	1.3385	1.0692	1.0077
	% of Efficacy	93.90	54.67	61.04	43.77
12	Pre	1.8122	2.9771	2.7863	1.9542
	Post	1.0000	1.3681	1.0123	1.0552
	% of Efficacy	44.81	54.04	63.66	46.00
13	Pre	2.1529	3.9529	3.6000	2.4118
	Post	0.8854	1.9167	1.6823	0.8750
	% of Efficacy	58.87	51.51	53.26	64.72
14	Pre	1.7725	2.5059	2.2797	1.5536
	Post	0.4583	1.4722	1.0000	0.6944
	% of Efficacy	74.14	41.25	56.13	55.30
15	Pre	1.7035	3.6627	2.7209	2.5930
	Post	0.1412	1.2040	1.5028	1.4181
	% of Efficacy	91.71	67.12	44.76	45.31
16	Pre	1.6901	3.7777	3.4327	2.6783
	Post	1.0014	1.0408	1.7041	1.5408
	% of Efficacy	40.74	72.44	50.35	42.47
17	Pre	2.0000	4.1066	3.8361	2.5000
	Post	1.1418	2.1560	1.7518	1.4052
	% of Efficacy	42.91	47.49	54.33	43.79
18	Pre	2.0544	3.9116	3.4966	3.2176
	Post	1.0048	1.7762	1.3762	1.3714
	% of Efficacy	51.09	54.59	60.64	57.37
19	Pre	2.4944	5.4607	5.0899	3.3371
	Post	1.0163	3.0976	2.6748	1.6260
	% of Efficacy	59.25	43.27	47.44	51.27
20	Pre	1.7666	3.6527	3.4444	2.7222
	Post	0.2562	1.3518	1.3577	1.3139
	% of Efficacy	85.49	62.99	60.58	51.73

From the **Table.2,** lowest p-value ($p<0.05$) indicates that there is a significant difference between the means of the internal ratio parameters calculated for pre and post-treatment hair tissue samples, and hence there is discrimination between Pre and Post-treatment have been thus proved from this statistical method. One study reported that there was not any significant difference of total cholesterol and triglyceride between acne vulgaris and control subjects, but there was significant decrease of HDL and increase of LDL levels in severe acne vulgaris subjects [51,52]. Also some report showed a significant difference in lipid profile levels between acne vulgaris and control subjects ($p<0.001$) [53]. From the **Table.3**, the p-value ($p>0.05$) indicates that there is no difference between Healthy subjects and post-treatment, respectively, therefore almost a better results occur between the means of the internal ratio parameters calculated for post-treatment human scalp hair tissue and healthy subjects as evidence.

Table 2: Mean Intensity Ratio Parameters Pre and Post Treatment Acne Vulgaris Patients using t-test

IRP Ratios	Pre Treatment			Post Treatment			t	p
	N	Mean	SD	N	Mean	SD		
Protein/Lipid $I_{3264/2864}$	20	2.0460	0.3584	20	0.7190	0.4826	15.7230	0.0000
AmideI/Lipid $I_{1633/2864}$	20	4.2167	1.3056	20	1.7859	0.7901	12.1790	0.0000
Amide II/Lipid $I_{1516/2864}$	20	3.8105	1.2124	20	1.7609	0.8468	19.8950	0.0000
Squalene/Lipid $I_{1454/2864}$	20	2.6935	0.7971	20	1.0057	0.4873	10.1230	0.0000

Table 3: Mean Intensity Ratio Parameters of Healthy and Post Treatment Acne Vulgaris Patients using t-test

IRP Ratios	Healthy			Post Treatment			t	p
	N	Mean	SD	N	Mean	SD		
Protein/Lipid $I_{3264/2864}$	20	0.6004	0.4567	20	0.7190	0.4826	0.7990	0.4290
AmideI/Lipid $I_{1633/2864}$	20	1.6548	0.7943	20	1.7859	0.7901	0.5230	0.6040
Amide II/Lipid $I_{1516/2864}$	20	1.6308	0.7774	20	1.7609	0.8468	0.5060	0.6160
Squalene/Lipid $I_{1454/2864}$	20	0.9030	0.4831	20	1.0057	0.4873	0.6690	0.5070

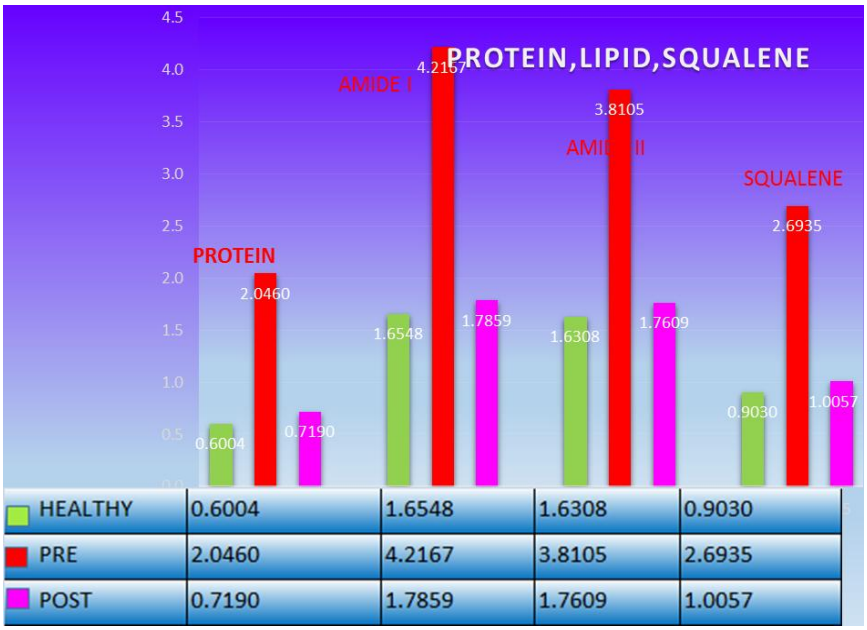

Figure 7: Histogram indicating the mean intensity ratio parameter of healthy subjects, Pre and Post Treatment of Acne vulgaris patient's Human scalp hair tissue

6. Conclusion

Thus the FTIR-ATR is most powerful, quick, simple and fastest technique using hair tissue samples to detect any diseases within a little span of the time period. Based on the spectral variations in the hair tissue samples, intensity ratio parameters viz., R1=$I_{(3264/2864)}$, R2=$I_{(1633/2864)}$, R3=$I_{(1516/2864)}$, R4 =$I_{(1454/2864)}$ was used for the discrimination between the pre and post-treatment of the acne vulgaris disease. The biomarkers for the acne vulgaris samples with specific peaks protein/lipid, amide I/lipid, amide II/ lipid, and Squalene/lipid significantly differed after the treatment. The efficacy of the gel adapalene 0.1% and benzoyl peroxide 2.5% shows satisfactory results. And also statistical analysis $p<0.05$ by dependent t-test has provided significant differences in the pre and post-treatment of Acne vulgaris. FTIR-ATR proved a rapid reduction in Propionibacterium acnes in the combination therapy of ADP/BPO particularly within the first 4 weeks. These findings provide evidence for the efficacy of ADP+BPO 0.1 & 2.5 % in the treatment of mild to severe acne vulgaris.

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