Original Research Article

A Randomized Control Study To Evaluate The Influence Of Autogenic Training And To Establish A Non-Invasive Biomarker Pannel For Psoariasis

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ABSTRACT
Psoriasis is one of the main dermatological disorders caused by stress. It is important to consider a psychological treatment in parallel to the dermatological treatment Hence we aim to evaluate whether autogenic training is helpful in treating psoriasis as assessed by perceived stress score (PSS),establish a non-invasive panel of biomarkers (salivary versus blood) which may be useful in predicting the disease severity and effectiveness of the therapy in psoriasis. Hundred patients of moderate and severe chronic plaque psoriasis defined as PASI >6 or DLQI >6 , more than 15 years of age will be recruited in this randomized controlled study. Daily autogenic training sessions will be conducted for a week followed by a psycho-education session. Blood samples will be collected to measure the biomarkers using ELISA Technique. Saliva will be collected for the quantification of genetic markers using RT-PCR, miRNA22 BY Real Time PCR-qPCR and whole genome sequencing using Next generation sequencing. Statistical analysis will be carried out using SPSS version 23.

Expected Outcome- Genetic markers, especially miRNA 22 may be both could be used as diagnostic and prognostic marker for psoriasis. Our study may show that the benefits of the study extend beyond the immediate duration of the intervention. In India, where single dermatologist caters for a large number of patients, a group autogenic training followed by daily autosuggestion by the individuals may be more feasible.

Keywords: psoarisis, autogenic training, genetics

Introduction
Psoriasis is one of the main dermatological disorders associated with psychological distress [1]. Stress is one of the best-known triggers for psoriasis. It has been associated with disease onset, flare-ups, and psychological distress [2]. Stress is likely to impact the therapeutic efficacy of treatments. It is important to consider a psychological treatment in parallel to the dermatological treatment. Autogenic training is a method of autosuggestion which teaches a clearly structured process where the patient concentrates on relaxing.

When assessing the severity of the disorder and choosing the treatment, the physician must also consider the patients perception of the disease [3]. Several tools for quality-of-life measurement are nowadays available. The Dermatology Life Quality Index (DLQI) is a self-reported questionnaire of

ten items which evaluates the impact of the disease on daily activities, feelings, work, studies, personal relationships, and treatment [4]. The Psoriasis Life Stress Inventory (PLSI) is a 15-item questionnaire which measures the psychosocial stress associated with coping with everyday events [5,6]. The Psoriasis Disability Index (PDI) consists of 15 psoriasis-specific questions which address the disability in daily activities, employment, personal relationships, leisure, and treatment [5,7]. Psoriasis Index of Quality of Life (PSORIQoL) is a 25-dichotomous item instrument designed for clinical practice and trials which assesses the capability of individuals to satisfy their needs [5,8,9]. Diagnosis and monitoring often require painful invasive procedures such as biopsies and repeated blood draws, adding undue stress to an already unpleasant experience. The discovery of saliva-based biomarkers may offer unique opportunities to bypass these measures by utilizing oral fluids to evaluate the condition of both healthy and patients.

Objectives of the study are to
1.Assess whether autogenic training is useful in improving stress levels in psoriasis as assessed by perceived stress score
2.Assess whether autogenic training reduces the severity of the disease and improves response to therapy in psoriasis
3.Establish a non-invasive panel of biomarkers (salivary versus blood) which may be useful in predicting the disease severity and effectiveness of the therapy in psoriasis
4.Establish a panel of non-invasive genetic markers in the saliva which may be useful in predicting the disease progression, prognosis and effectiveness to therapy
Methodology

Study setting:
Collaboration between Depts of Dermatology. Biochemistry and Psychiatry

Study design
A randomized controlled design will be adopted. Standardized assessment tools will be used at the beginning and end of the intervention by the dermatologist and the self-rated instruments by the participants . Wherever the second assessment is not possible, the result of the first assessment will be carried forward in an intention-to-treat (ITT) analysis[10]

Recruitment of subjects
Participants will be approached and recruited by the dermatologist and randomized using random number tables into an intervention arm (group 1) and a control arm (group 2).
Sample size calculation : Given the exploratory nature of this pilot study and the need to be able to detect small differences, a standardized effect size of 0.2 and a sample size of 40 in each arm will be calculated [11] Assuming an attrition rate of 20%, a total of 100 participants will be recruited.
Inclusion criteria - patients of moderate and severe chronic plaque psoriasis defined as PASI >6 or DLQI >6 , more than 15 years.
Exclusion criteria - Patients of pustular psoriasis and erythrodermic psoriasis, pregnant patients, patients with concomitant psoriatic arthritis and other medical comorbidities such as coronary artery disease, stroke, hepaticorenal dysfunction, and patients with current suicidal ideation will be excluded from the study.

Assessment tools
Sociodemographic and clinical data will be obtained from all patients at baseline. Psoriasis severity will be assessed using PASI,[12] and dermatological QOL will be assessed using DLQI[13].
Subjective psychological well-being will be assessed using the WHO-5which is a Likert scale[14].

This scale generates raw scores from 0 to 25 which is then transformed to 0–100 by multiplying by 4 for ease of understanding and use. Higher percentage scores indicate more well-being. The psychiatric diagnoses at baseline will be generated using the PHQ which is a self-rated questionnaire-based instrument. The PHQ contains five modules that generate five common psychiatric diagnoses, namely, depressive disorder, somatoform disorder, anxiety disorder, alcohol use disorder, and eating disorder. Depression, anxiety, and somatic symptom severity were assessed using the PHQ-9, GAD-7, and PHQ-15 subscales of the PHQ, respectively. AH in diversion of the PHQ will be used.[15,16]

Their perception of stress will be analyzed before and after autogenic training using a validated questionnaire, Perceived stress scale (PSS)[17].This questionnaire consists of ten 4-choice questions. To determine each individual's total stress score, following rules are followed; Reverse scores on four positive items (4,5,7,8) as follows- 0=4,1=3,2=2,1=4.

Assessment time points

Participant assessments will be done twice. In the intervention arm, the first assessment will be done before the first autogenic training session. Psychiatric diagnoses will be assessed using PHQ at baseline. The second assessment will be done 6 months after the end of the study intervention. At both time points, PASI, DLQI, WHO-5, and PHQ subscales,PSS scores will be assessed.

In the control arm, the same assessments will be done at recruitment and at follow-up at similar time points as the intervention arm. The patients in this group will receive treatment as usual.

Intervention

Psoriasis patients will be recruited in batches of 10–15 and randomized by nursing staff using computer- generated random number sequences. Allocation concealment will be done using sequentially numbered opaque-sealed envelopes. The intervention consists of daily autogenic training sessions for a week, which will be conducted in the Psychiatry outpatient department.

The sessions will follow a semi-structured format lasting 45 minutes. Patients in group 1 will be demonstrated the autogenic relaxation technique[18]. As the name suggests, this relaxation technique uses self-visualization and self-instruction to produce feelings of warmth and heaviness in the body, thus producing a state of relaxation. The autogenic training session will be followed by a psychoeducation session including the association, recognition, and management of depression and anxiety in psoriasis. This will be followed by an interactive session with a clinical psychologist. The areas covered will be the alleviation of self-stigma using cognitive behavior techniques, distraction techniques, imagery training, and thought stopping. In addition, This will be followed by a feedback and interactive session.

Followed by this one-week sessions, patients in group 1 will be instructed to practice autosuggestion for 15 minutes twice daily for 6 months. Patients' adherence to autosuggestion will be noted on daily basis with a checklist and total score will be noted as practice scores.

Clinical Assessment

The Psoriasis Area Severity Index (PASI), Total Sign Score (TSS), and Laser Doppler Skin Blood Flow (LDBF) of a selected reference plaque will be measured in a blinded fashion at baseline (week 0) and at the end of 6 months.

Laboratory investigations

Five ml of blood will be collected in plain tubes with aseptic precautions ,sample will be centrifuged, and serum will be stored at -80 degrees. 5 ml saliva samples will be collected from the patients in both the groups ,at baseline (before the autogenic training) and at the end of 6 months.

Saliva Collection and Sample Preparation

Before saliva collection, a skilled dentist and dental hygienist will check the oral hygiene of all participants. Remarkable dental plaque and calculus deposits will be removed using a toothbrush without dentifrice and ultrasonic scaling at ≥ 3 h before saliva collection.

All participants will be asked to refrain from eating and drinking for ≥ 1.5 h before saliva collection. The participants will be asked to rinse their mouths with water before sample collection and split their saliva into 50 cc Falcon tubes in a paper cup filled with crushed ice. Subsequently, approximately 3 mL of unstimulated whole saliva will be collected for approximately 5 min. Finally, the samples will be aliquoted into smaller volumes and stored at room temperature.

Precautions taken

However, salivary metabolic profiles are sensitive to various factors irrelevant to the disease, and the biomarkers should be robust to withstand these. Pre-conditioning of sample collection (such as fasting duration), sampling methods (stimulated or unstimulated), sampling timing, and storage conditions also affect the salivary metabolite profiles. Therefore, saliva donors will be instructed to strictly follow the SOPs at the marker discovery stage to minimize unexpected bias.

Following biomarkers will be assayed using ELISA technique.
- Biomarkers for both psychotherapy and psoariasis - CRP, soluble P-selectin, TNF-α, IL-6
- Soluble biomarkers - Haptoglobin, C3,C4 compliment proteins, fibrinogen VEGF,TGF-β, TIMP-1, MMP-1, S100 proteins like S100A8/A9, S100A12, IFN -γ,IL-8,IL-12,IL-18
- Tissue associated biomarkers - Keratins ,K6, K16, K1,K10,Connexins- 30,26, BCL-2,BCL-X,
- Antimicrobial peptides - Human beta defensin 2 (HBD-2) and cathelicidin (LL-37)
- Analysis of genetic markers in the saliva :
- RUNX3, TAGAP, STAT3 ,DDX58, ZC3H12C, CARD14 and CARM1, IL-23 receptor(IL-23R) and IL-12B, zinc-finger protein 313 (ZNF313), TNFAIP3 interacting protein 1 (TNIP1), and TNF-α-induced protein 3 (TNFAIP3)
- Salivary DNA will be isolated and whole gene sequencing will be done by Next generation technique
- Salivary micro-RNA 22 will be isolated and quantified as follows

Gene (mRNA) Expression by Real time PCR (qPCR) Analysis
a. RNA extraction
Five millilitres of saliva will be collected and will be stored at −80°C until RNA isolation. RNA will be isolated from saliva by using commercially available kits, following the manufacturer's instructions. RNA purification and extraction will be performed using the columns. The extracted RNA will be stored at −80˚C until further analysis.

b. cDNA Synthesis
Purity and RNA concentration will be assessed by measuring the absorbance at 260 and 280nm using Nano drop 2000. 1 ug of RNA will be converted into cDNA by using High-Capacity cDNA Reverse Transcription Kit. The gene-specific suitable oligonucleotide primers will be used.

c. Real time PCR (qPCR)
CFX96 Real-Time PCR Detection System will be used for evaluating the gene expression levels by using SYBR green and probe master mix (Roche, Indianapolis, IN).In order to confirm the presence

of a single PCR product in PCR reaction, melting curve analysis will be performed. Relative fold change will be calculated by using 2–ΔCt method (Livak & amp; amp; Schmittgen, 2001).

Statistical analysis

Statistical analysis will be carried out using SPSS version 23. Comparison of biomarkers and stress perception scores before and after autosuggestion will be done using Wilcoxon rank signed test. Association of stress perception scores and bio marker levels (both serum and saliva) and genetic markers will be done using Chi square test .Correlations of the stress perception, disease severity scores and biomarkers will be studied using Spearman correlation test**.** Mann Whitney U test will be done for comparison of stress scores, disease severity scores and biomarkers between the intervention and control groups. Chi square test will be used for the association of effectiveness of autogenic training and disease severity and responsiveness to therapy, as well as stress scores.

Outcome measures: Before and after autogenic training at base line and at 6 months

- Serum biomarkers
- Salivary biomarkers
- Salivary genetic markers
- PSS
- Disease severity assessment scores

Expected Outcome and Significance of the study

Genetic markers ,especially miRNA 22 may be both diagnostic and prognostic marker for psoariasis. This may be of great value as it is a non-invasive marker throwing light on genetic aspect of psoariasis. This pilot randomized controlled study to assess the effect of a simple, pragmatic, multi‑ professional, autogenic training delivered to patients with psoriasis in addition to standard treatment. Our autogenic training session is designed to be semi‑ structured and brief, encompassing the physical as well as psychological aspects of psoriasis, free of excessive technicality and jargon, and easily delivered. The study overcomes most methodological issues confronting the existing literature in this area. Rather than focusing on the immediate effects of the intervention, the outcome measures in our study will be assessed at 6 months and thus show the sustained effect of intervention at this point of time. The autosuggestion intervention may increase treatment compliance and adherence by educating the patient on the natural disease course, treatment options, and possible adverse effects. This suggests that such interventions make lasting changes. In developing countries like India, where single dermatologist caters for a large number of patients, time constraints may hamper the delivery of one‑ to‑ one autogenic training. Thus, a group autogenic training followed by daily autosuggestion by the individuals may be more feasible.

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