DOI: 10.7860/JCDR/2022/60281.17204

Research Protocol

Dermatology Section

Assessment of Cell-mediated Immunity to *Trichophyton* Antigen in Patients with Dermatophytosis: A Case-control Study

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ABSTRACT

Introduction: Dermatophytosis are presenting for longer durations and at atypical sites and are quite persistent. If changes in the Cell-Mediated Immunity (CMI) will be studied then, it will help to set treatment guidelines for the management of dermatophytosis to certain extent, considering the current changes in the resistance to dermatophytosis. There is paucity in literature regarding the CMI to *Trichophyton* antigen in dermatophytosis patients in the geographical region of Vidarbha, Maharashtra, India.

Aim: To assess the CMI to intradermal *Trichophyton* antigen in dermatophytosis patients and to study its adverse reaction in these patients.

Materials and Methods: The present prospective cross-sectional case-control study will be conducted on patients of dermatophytosis attending Skin Outpatient Department (OPD) in Acharya Vinoba Bhave Rural Hospital (AVBRH), Sawangi, Wardha, Maharashtra, India, during January 2022 to January 2023 (13 months). Patients will be enrolled following fulfillment of study's eligibility criteria. Clearance has been acquired from the Institutional Ethical Committee (IEC). Participants will be required to sign a written informed consent form in their native language. A detailed history will be collected and cutaneous examination will be done before conducting the study. The area for intradermal injection will be marked. For the study 0.1 mL, Intradermal *Trichophyton* antigen will be injected in the patient's forearm. Patient will be called after 48 hours to check for the CMI response (delayed response).

Keywords: Filamentous fungi, Hypersensitivity, Intradermal skin test, *Trichophyton* cell

INTRODUCTION

Dermatophytes are an assemblage of filamentous fungi which invades the keratinised tissues of human being or natural world to produce a contagion termed "Dermatophytosis" commonly referred as ringworm. Owing to their filamentous structure they cannot penetrate deeper tissues and hence the infection is limited to the immunocompromised host's non living cornified skin layers [1,2]. The cutaneous reactions could be varied from mild to severe depending upon the host's reactions to the inherent products of fungus, its hostility, host's infection site and local factors. *Trichophyton rubrum* being commonest causative variant responsible for preponderance of fungal infections which are superficial [3-5]. A distinctive feature of *T. rubrum* immunity is its competency to reduce hypersensitivity response (immediate or delayed). This scrupulous immune response is host dependent and the preceding exposure to the antigen [1]. A preliminary defence mechanism of host includes Toll Like Receptor (TLR) 2, 4 and 6 and Human Beta Defensin 1, -2, -1 B and Interleukin-8. Annotations suggest that "T Lymphocyte Infection" is critical in recuperation from a dermatophytic infection. Induration is the symptomatic trademark event of a delayed type hypersensitivity response [6,7].

In a study by Kaaman T, importance of CMI in the eradication of cutaneous infections was highlighted. The authors suggested that, the CMI in humans could be assessed using intradermal skin test causing delayed-type skin reactions are to dermatophyte antigen which are indicative of previous or actual dermatophytosis [8].

Over the course of dermatophyte contaminations, the delayed-type skin reactivity is positive, at peak and at last decreases as a sign of clinical insusceptibility. (Th1) CD4-T lymphocyte is seen in classical postponed sort extreme hypersensitivity [6,7]. In current scenario, the need for a dermatologist is to form an evidence based treatment protocol for better patient management. Till date, literature regarding the CMI to *Trichophyton* antigen in dermatophytosis patients in Vidarbha (Maharashtra) as, its soil is known to be a significant reservoirs of dermatophytes [9,10]. Hence, the present study aimed to assess the CMI to *Trichophyton* antigen in persistent dermatophytosis patients.

Research Question

What is the CMI response (delayed response) in a patient with persistent dermatophyte infection to intradermal *Trichophyton* antigen?

Objectives

1. To study the CMI to intradermal *Trichophyton* antigen in patients with dermatophytosis.
2. To study the adverse effect of intradermal *Trichophyton* antigen in patients with persistent dermatophyte infection.

MATERIALS AND METHODS

The present prospective case-control study will be carried out in the Skin OPD of AVBRH, Sawangi (M), Wardha, Maharashtra, India, during January 2022-January 2023 (13 months). Approval was obtained from IEC with approval number Ref No. DMIMS(DU) IEC/2022/854 (5/4/22).

Study Population

a) Eighty gender-matched participants who are clinical diagnosed cases of dermatophytosis, willing to give written informed consent, from rural population of Sawangi, Wardha, Maharashtra, India.

b) Eighty gender-matched participants who are clinically fit i.e. control and do not have dermatophyte infection, willing to give written informed consent, from rural population of Sawangi, Wardha, Maharashtra, India.

c) The sample size was estimated using the formula, $N=(Z_{\alpha/2})^2 P(1-P)*1/E^2$

Where, P is the prevalence or proportion of event of interest for the study, E is the Precision (or margin of error). $(1.96)^2*0.276*(1-0.276)/(0.07)^2=156.66$. The prevalence of 27.6% dermatophytosis as obtained from the study of Lakshmanan A et al., [9].

Inclusion criteria:

1. Patients of both genders.
2. Patients willing to give informed consent and participate in the study.
3. Clinically-diagnosed cases of persistent dermatophytosis of age above 18 years.

Exclusion criteria:

1. Patients who refuse to take part in the research.
2. Clinically suspected cases of dermatophytosis.

Study Procedure

Dermatophytosis patients attending Skin OPD in AVBRH, Sawangi, Wardha, India will be included when the study's eligibility criteria have been considered. Clearance has been acquired from the Institutional Ethical Committee (IEC). Participants will be required to sign a written informed consent form in their native language. A detailed history will be collected and cutaneous examination will be done before conducting the study. The area where the intradermal injection will be given will be marked. For the study, 0.1 mL Intradermal *Trichophyton* antigen will be injected in the patient's forearm. Patient will be called after 48 hours, to check for the CMI response (delayed response) using wheal test, which involve formation of wheal at the sites of injection, the size and degree of erythema, and degree of induration at 48 hours. A wheal greater than 10 mm in diameter, with or without a flare will be considered a positive immediate reaction. The CMI response will be intercepted as positive, if any degree of erythema, oedema observed [11].

Outcome Measures

Primary outcome: Delayed response CMI to *Trichophyton* antigen in a patient, who has been suffering from persistent dermatophyte infection (case group) and those, who were suffering from non dermatophyte skin conditions (control group).

Secondary outcome: Any adverse event post injection will be recorded in both the groups.

STATISTICAL ANALYSIS

Standard statistical methods will be used, to evaluate both categorical like demographic characteristics and non categorical data like positive or negative wheal test or any reported adverse event, using paired 't'-test and Chi-square test respectively. Statistical Package for the Social Sciences (SPSS) software version 26.0 will be used for analysing these data. A p-value <0.05 will be deemed significant.

DISCUSSION

Jones HE, 1994 [1], conducted an in human experiment with injected dermatophytes and on the basis of their cellular resistive response, participants were divided into two groups: (1) Those who mount conclusive delayed-type excessive responsiveness as a result of contamination clearance, (2) Those who lack or have a fault in their cellular insusceptibility, which prevents them from building a powerful reaction to their presence in the body and hence, predisposes them to infection. The authors discovered that, the severe provoking illness was linked to T-cell mediated delayed type of sensitivity to a "*Trichophyton* intradermal test" and the ability of those impacted to recognise mycologic involvement. Constant contamination was related to a tall (anti-*Trichophyton* IgE-mediated) responsiveness and a nil or vanishing T-cell mediated delayed type of sensitivity response to *Trichophyton* in differentiated mice [1].

Khosravi AR et al., demonstrated cell-mediated immunity in 98 patients with acute dermatophytosis (group I) and 131 chronic dermatophytosis patients (group 2). They found 96 members of group I (98%) had positive delayed-type hypersensitivity responses to *Trichophyton*, whereas only 43 subjects (32.8%) of group 2 had positive delayed-type hypersensitivity responses [11].

Begum J et al., studied about specificity over the ordinary strategies of culture and microscopy for dermatophytes distinguishing competency. The symptomatic methods within the study, which give the objective and reproducible species distinguishing proof for the particular treatment, observing and control of dermatophytosis. All the strategies talked about within this audit, have a critical benefit in terms of affectability, the time required, financial matters, complexity and species range [2].

Jain S et al., observed *Tinea corporis* to be the most prevalent clinical variant in 1,200 cases from eastern Odisha in 2020. Culture positive was 61.75% and coordinate potassium hydroxide (KOH) positivity was 89.4%. *T. mentagrophyte* was the most frequent dermatophyte (77.5%), followed by *T. rubrum* (13.3%) [3].

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 19, 2022
- Manual Googling: Oct 13, 2022
- iThenticate Software: Oct 15, 2022 (3%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 16, 2022**
Date of Peer Review: **Oct 01, 2022**
Date of Acceptance: **Oct 17, 2022**
Date of Publishing: **Dec 01, 2022**