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Pathophysiology of psoriasis and the roles of nucleolar phosphoprotein-1 (NPM1) and glucose-dependent insulinotropic polypeptide 3 (G1P-3)

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ABSTRACT
Background: Psoriasis is a prevalent chronic systemic inflammatory disease affecting over 2% of the global population, characterized by skin and systemic manifestations. Recent research has highlighted the role of non-coding RNAs, such as PRINS and the gene Interferon Alpha Inducible Protein 6 (IFI6 or G1P-3), in the disease's pathogenesis. These molecules, particularly in response to stress, may influence psoriasis susceptibility and severity. This study investigates the levels of G1P-3 and nucleolar phosphoprotein (NPM) in serum and tissue samples from psoriasis patients compared to healthy controls to assess their roles in psoriasis development**.**
Methods:A total of 20 individuals, including 10 psoriasis patients and 10 healthy controls matched for age and sex, were recruited from the Department of Dermatology at NMCH, Patna between January 2023 and January 2024. Patients had plaque psoriasis of varying duration (8 to 360 months). Exclusion criteria included chronic inflammatory or autoimmune conditions, cancer, pregnancy, and lactation. Written consent was obtained from all participants. Serum and 4mm skin punch biopsy samples were collected. Levels of G1P-3 and NPM were quantified using molecular biology techniques, including the miRNeasy mini kit for RNA purification, cDNA synthesis with the miScript II RT kit, and real-time PCR using the Rotor Gene Q System. Statistical analysis was performed using SPSS 17, with significance determined by p-values <0.05.
Results: Significantly higher levels of both G1P-3 and NPM were observed in serum and tissue samples from psoriasis patients compared to controls. Median values for serum G1P-3 and NPM were 3.330 and 2.030, respectively, versus 1.085 and 1.040 in controls ($p<0.001$). Similarly, tissue levels of G1P-3 and NPM were significantly elevated in psoriasis patients (6.495 and 5.425) compared to controls (1.040 for both) ($p<0.001$). No significant correlation was found between these biomarkers and clinical data such as disease onset, course, or family history.
Conclusion: Elevated levels of G1P-3 and nucleolar phosphoprotein in psoriasis patients suggest their critical role in the disease's pathogenesis. These biomarkers may serve as non-invasive prognostic tools and novel therapeutic targets for psoriasis.

****Keywords**:** Psoriasis, G1P-3, Nucleolar Phosphoprotein, Non-Coding RNA, Biomarkers, Molecular Biology, Real-Time PCR.

INTRODUCTION
Psoriasis is known to be one of the most common chronic systemic inflammatory diseases, characterised by the presence of both Manifestations that occur on the skin and throughout the body. The disease affects over 2% of the global population.[1,2]The occurrence and progression of psoriasis are influenced by various factors, and inheritance is polygenic. Non-coding RNAs have a crucial impact on an individual's susceptibility to psoriasis. Research has demonstrated that there is an imbalance in long-non-coding RNAs in both the peripheral blood and skin lesions of individuals with psoriasis.[3,4] Stress is a frequent catalyst for the occurrence of psoriasis flare-ups.PRINS is a non-coding RNA that controls G1P3, a gene that has anti-apoptotic effects in keratinocytes. The official designation for PRINS is "psoriasis associated non-protein coding RNA induced by stress." The

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deregulation of the PRINS non-coding RNA may play a role in the development of psoriasis.[5]Computational structural and homology analyses indicate that PRINS likely operates as a noncoding RNA.The gene consists of two distinct elements, and it is transcribed by RNA polymerase II. It is expressed at varying levels in different human tissues.[6]

PRINS is known to be up regulated in both affected and unaffected psoriatic epidermis in response to stress. The expression of PRINS was elevated in the uninvolved epidermis of psoriatic patients when compared to both psoriatic lesional and healthy epidermis. This indicates that PRINS may play a role in the susceptibility to psoriasis.[6] The nucleolar phosphoprotein may function as a cell proliferation regulator. PRINS is known to interact physically with it. Immunohistochemical experiments have demonstrated a significant upregulation of nucleolar phosphoprotein expression in the cells located in the basal layers of psoriatic skin lesions, in contrast to both healthy skin and non-lesional psoriatic skin.[7]The nucleolar phosphoprotein is translocated into the nucleus in cells treated with UVB.UV-B irradiation of keratinocytes results in the retention of nucleolar phosphoprotein within the nucleoli when the PRINS gene is eliminated.[8] Nucleolar phosphoprotein may play a significant role in chronic inflammatory skin conditions, such as atopic dermatitis and malignant cutaneous melanoma.[9,10]

In this study, we evaluated the presence of the gene Interferon Alpha Inducible Protein 6 (IFI6) or G1P-3, as well as nucleolar phosphoprotein, in the serum and tissue of Egyptian patients with psoriasis. We compared these findings with those of healthy individuals to better understand their involvement in the development of psoriasis. This research aims to contribute to the development of innovative treatment approaches for psoriasis.

Methods

The present study was carried out on a sample of 10 individuals diagnosed with psoriasis and 10 individuals without the condition who were selected as controls. The participants were matched in terms of age and sex. The study was conducted between January 2023 and January 2024. Participants were enlisted from the Department of Dermatology in NMCH,Patna. The disease duration exhibited considerable variation, spanning from 8 to 360 months, with an average ± standard deviation of 122.55 ± 106.91 months.

Inclusion criteria

All patients included in the study exhibited plaque psoriasis.

Exclusion criteria

Patients with any additional chronic inflammatory or autoimmune conditions, patients with metabolic disorders, individuals with any form of cancer, pregnant women, and lactating females were excluded.All patients were treatment-naïve and had not received any prior treatment for psoriasis.

Prior to enrollment in the study, written consent was obtained from each participant. A comprehensive medical history of psoriasis was obtained, including specific details regarding the onset, progression, and duration of the condition. The clinical evaluation of the type and severity of psoriasis, as well as the calculation of the Psoriasis Area and Severity Index (PASI), were performed.11 Serum and a 4mm skin punch biopsy were obtained from each patient and control.

Molecular biology techniques were used to measure the levels of long noncoding G1P-3 and nucleolar phosphoprotein in both serum and skin biopsy samples. The miRNeasy mini kit, manufactured by Qiagen in Valencia, CA, USA, was employed for the purification of long noncoding RNA. The quantification and purity of RNA were evaluated using a NanoDrop®(ND)—1000 spectrophotometer. The RNA was converted into complementary DNA (cDNA) using the miScript II RT kit (Qiagen, Valencia, CA, USA) according to the following procedure:

1. The components used are Hiflex buffer, Nucleics mix, miScript Reverse transcriptase mix, and RNA (60 ug).

2. The gentle mixing was achieved through centrifugation. The conventional PCR was carried out by incubating the sample at 37ºC for 60 minutes.A 5-minute incubation at 95ºC was performed to deactivate Reverse Transcriptase using conventional PCR.

Detection of both biomarkers

The fold change for both long noncoding RNAs was determined using pre-made primers and a customised GAPDH gene as an internal control housekeeping gene for both the cases and healthy controls. The forward primer sequence is 5'-CCCTTCATTGACCTCAACTA-3', and the reverse primer sequence is 5'-TGGAAGATGGTGATGGGATTThe Rotor Gene Q System (Qiagen) was utilised to perform real-time PCR.The fold change was calculated using the 2^(-ΔΔCt) method relative to the control samples.
Statistical analysis of data the data analysis was conducted using SPSS 17 on Windows 8.1. Parametric numerical data was analysed using the mean ± standard deviation for descriptive statistics. The median was employed for non-parametric numerical data.The t-test was utilised to assess the significance of differences in parametric data obtained from the two groups, while the U-test (Mann-Whitney) was employed in a similar manner to measure the non-parametric variables. The chi-square test was employed to demonstrate the association between categorical variables. The Pearson correlation coefficient was used to test the association between groups, specifically serum and tissue samples from controls and psoriatic patients. The test of significance was conducted using a two-tailed approach. The sensitivity and specificity of measuring G1P-3 and nucleolar.

Results

Table1:Biomarkerslevelforthestudiedgroups(serumandtissuelesional)

Biomarkers (Median)	Psoriasis	Control	Mann-Whitney Statistic *U*	*P*-value
NPM(serum)	2.030	1.040	31.500	<0.001[a]
G1P-3(serum)	3.330	1.085	38.000	<0.001[a]
NPM(tissue)	5.425	1.040	610.000	<0.001[a]
G1P-3(tissue)	6.495	1.040	610.000	<0.001[a]

***P*valuesinboldarestatisticallysignificant(*P*<0.05).aOneway,NPM:Nucleolarphosphoprotein,G1P-3:Glucose-1phosphate-3**

The phosphoprotein was analysed using a Receiver Operating Characteristic (ROC) Curve. Significance was attributed to P-values less than 0.05 for all statistical analyses.

Table2:**Level of NPM and G1P3 in relation to clinical data of psoriasis patients (serum and tissueelesional)**

Clinical data	NPM(serum)	NPM(tissue)	G1P-3(serum)	G1P-3(tissue)
Disease onset				
Gradual Sudden	2.87±0.34	5.87±2.33	3.63±1.90	**6.54±2.16**
	2.66±0.80	3.92±1.02	3.30±1.41	**6.72±1.45**
Disease course				
Remissions & exacerbations progressive	2.83±0.42	5.29±2.49	3.07±1.92	**6.25±1.91**
	2.82±0.45	5.84±2.24	3.88±1.52	**7.18±2.24**
Family history				
Positive Negative	2.91±0.79	3.29±0.69	6.58±2.54	**6.00±1.37**
	2.44±0.25	3.37±0.99	5.20±2.04	**6.72±2.12**

NPM:Nucleolarphosphoprotein,G1P-3:Glucose-1phosphate-3

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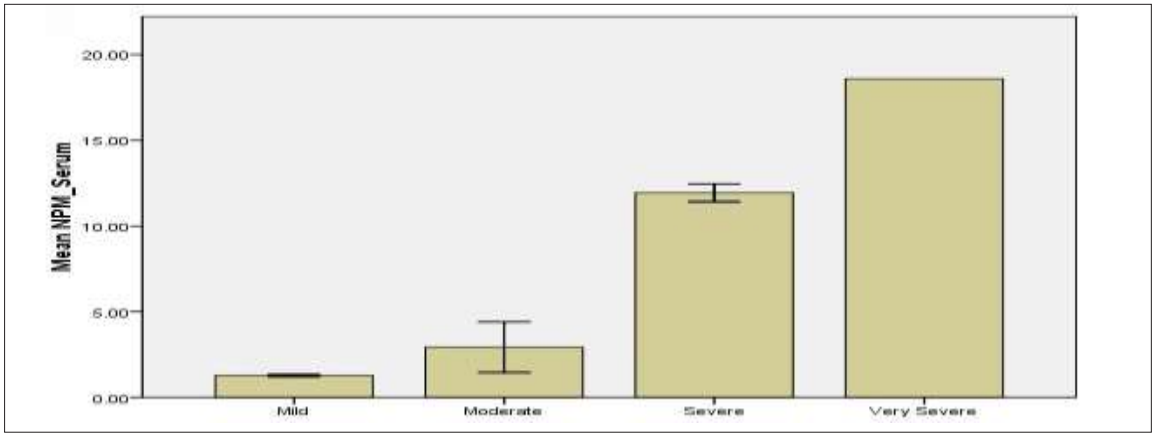

Figure1a: Relation ship of NPM and G1P-3 levels of psoriatic patients with regard to disease severity (a) NPM level in serum

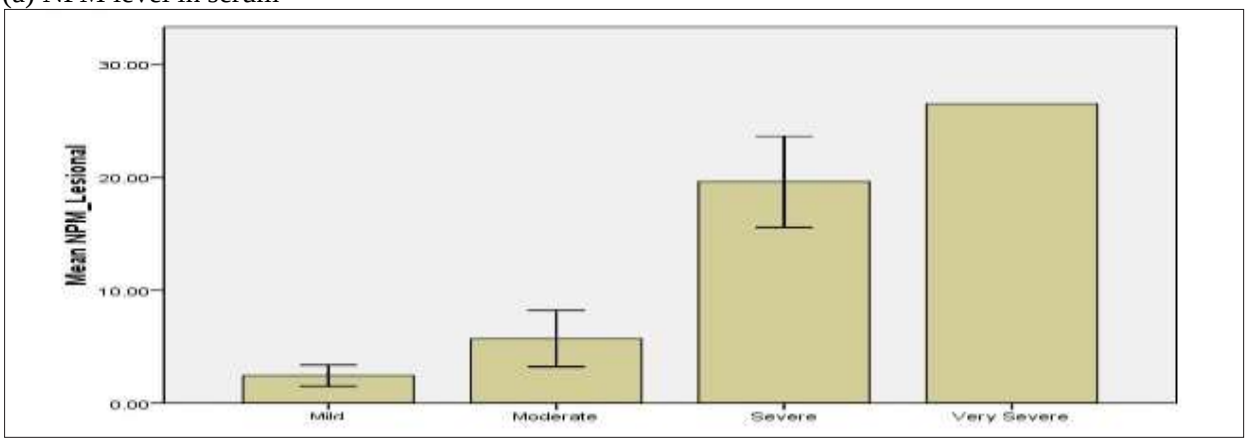

Figure 1b: Relationship of NPM andG1P-3 levels of psoriatic patients with regard to disease severity (b) NPM level in lesion skin

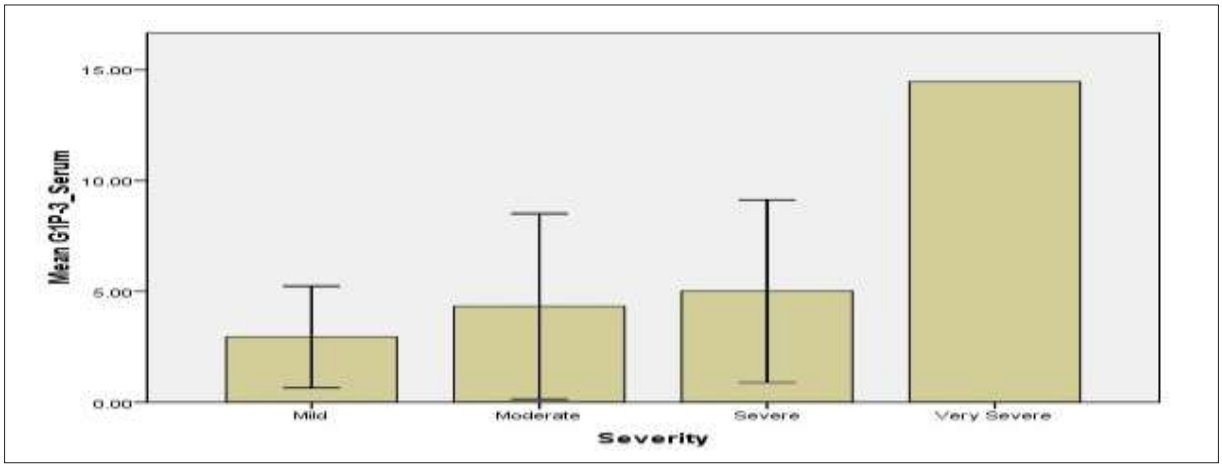

Figure1c: Relationship of NPM andG1P-3 levels of psoriatic patients with regard to disease severity (c) G1P-3 level in serum

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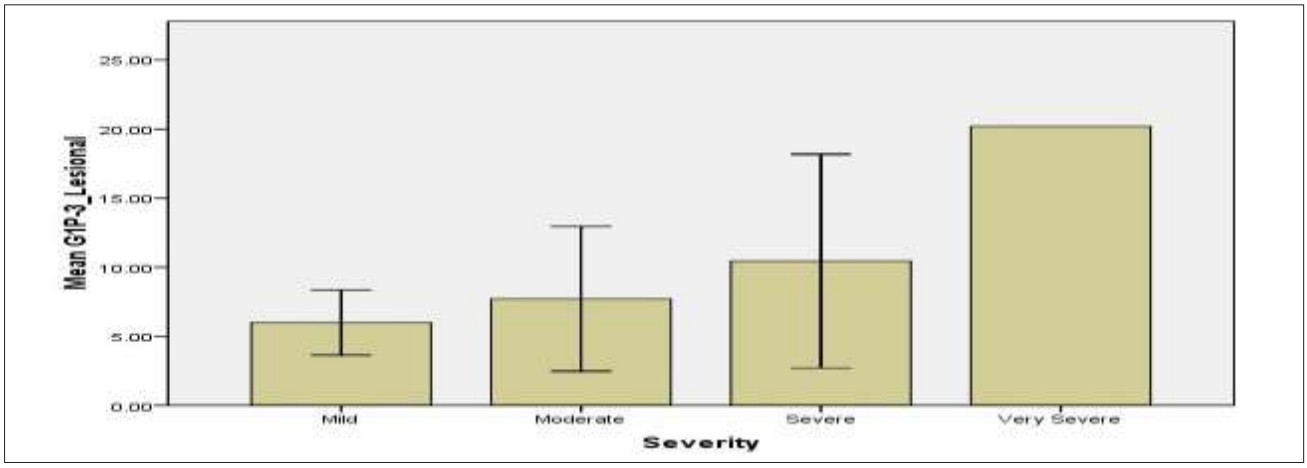

Figure1d: RelationshipofNPMandG1P-3levelsofpsoriaticpatients with regard to disease severity (d) G1P-3 level inlesional skin

Discussion

The PRINS gene is the primary regulator of both the G1P-3 activity and the nucleolar phosphoprotein activity. There is a possibility that G1P3 plays a part in the pathogenesis of psoriasis.[7]Desegregation of the PRINS systems Through the regulation of G1P-3, non-coding RNA is responsible for the development of psoriasis and leads to a reduction in the sensitivity of keratinocytes to spontaneous apoptosis. In keratinocytes, PRINS regulates one of the genes that has anti-apoptotic effects.Additionally, nucleolar phosphoprotein is a potential physical actor with PRINS.[7] The objective of this study was to examine the levels of nucleolar phosphoprotein and G1P-3 expression in both serum and lesional skin among our different groups. Szegedi et al. established that PRINS induces alterations in both cellular morphology and gene expression profile.[5] G1P-3 plays a crucial role in the development of psoriasis by controlling the anti-apoptotic gene G1P-3 in keratinocytes.PRINS controls the activity of G1P-3, which has been observed to be increased in both hyperproliferative lesions and non-lesional epidermis compared to healthy controls.[10,12]

Our investigation revealed that G1P-3 exhibited higher levels of expression in the tissue of individuals diagnosed with psoriasis. Our findings indicate that G1P-3 plays a crucial role in determining susceptibility to psoriasis and in the cellular response to stress. According to the experimental findings in this study, it is hypothesised that G1P-3 may function as a regulatory RNA that alters the expression of genes that code for proteins. This hypothesis is corroborated by other studies that have demonstrated that the up-regulation of G1P-3 stimulates cell proliferation and inhibits apoptosis.[12–14]Prior studies have demonstrated the significance of the PRINS non-coding RNA gene in individuals with psoriasis.[14–16]

We subsequently examined the expression of nucleolar phosphoprotein in the tissue of patients with psoriasis. The study revealed a significant increase in the expression of nucleolar phosphoprotein in the epidermis of individuals with psoriasis.

Additional research supports our findings[17,18] indicating that nucleolar phosphoprotein is highly expressed in the epidermis affected by psoriasis. Previous observations indicate that it

Plays a crucial role in the normal growth of keratinocytes and its increased expression is observed in certain skin diseases characterised by excessive cell growth and malignancy, which aligns with our research findings.[7]In the past, TGF-β1 or VEGF-A have been suggested as effective therapeutic targets for atopic dermatitis. Multiple reports have highlighted the significant role and connection of nucleolar phosphor protein in the TGF-β1 pathway for skin diseases. A group of authors concluded that nucleolar phosphor protein offers a new understanding of diseases related to TGF-β1 and VEGF-A.9 A separate study discovered elevated levels of TGF-β1 in the tissue and serum of individuals with

psoriasis.2 VEGF has been demonstrated to play a crucial role in the process of angiogenesis in psoriasis.[19]
No previous reports were found to establish the correlation between serum and tissue expression of G1P-3 and nucleolar phosphoprotein in psoriatic patients with clinical data. The results of our study indicate that there was no statistically significant distinction observed between G1P-3 and nucleolar phosphoprotein in both serum and tissue samples, in terms of age, sex, family history, disease onset, or disease progression.
We additionally investigated the correlation between the PASI score and the biomarkers. The results indicated notable disparities in the levels of nucleolar phosphor protein and G1P-3, both in serum and tissue, when comparing patients with varying degrees of psoriasis severity. Several of these differences were extremely statistically significant (elaborated upon in greater depth in the results section).We observed a consistent increase in the expression of all four biomarkers as the PASI scores increased.

Upon further analysis, we discovered a positive correlation between the levels of serum and tissue nucleolar phosphoprotein, as well as between serum and tissue G1P-3 levels. Additionally, we observed a correlation between both biomarkers in tissue.

Conclusion

Psoriatic epidermis exhibited elevated levels of both G1P-3 and high nucleolar phosphoprotein expression. This indicates that they play a crucial role in the development of psoriasis. In the future, these parameters could be utilised as non-invasive prognostic biomarkers and novel therapeutic targets for patients with psoriasis.

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