

Assessment of macrophage migration inhibitory factor in patients with verruca vulgaris

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Background: Common warts are caused by human papillomaviruses (HPVs), they are among the most common cutaneous viral infections. Macrophage migration inhibitory factor (MIF) is an essential contributor in many inflammatory and immune skin diseases. Yet, its role in the pathology of common warts is unclear.

Objective: To assess MIF levels in lesional and perilesional skin in patients with common warts in comparison to apparently healthy control group with matching age and sex.

Subjects and methods: A case-control study performed on 60 patients with common warts (group A) and 30 age and sex matching healthy controls (group B). Two biopsies were taken from each patient in group A; one from the lesion (lesional) and the other one from the skin around the wart (perilesional), while biopsies of controls were taken from matched sites to patients. Measurement of MIF in all groups was done by quantitative ELISA kits.

Results: Significant high MIF levels were detected in lesional and perilesional skin biopsies compared to controls ($P < 0.001$). Yet, the difference in MIF levels between lesional and perilesional skin biopsy was non-significant. No significant relations were found between lesional and perilesional MIF levels and clinical characteristics of the studied patients while both lesional and perilesional MIF levels were significantly correlated ($r = 0.269$, $P = 0.021$).

Conclusion: The significantly elevated MIF levels in lesional and perilesional skin biopsies compared to controls point to its role in wart progression from HPV infected cells.

Keywords: macrophage migration inhibitory factor, common warts, human papillomavirus

Introduction

Cutaneous warts are benign skin proliferations caused by the human papillomavirus (HPV).¹ Since papillomaviruses can cause chronic infections, without any apparent systemic manifestations, that rarely kill the host, they are considered very infectious agents.²

Patients suffering from active warts demonstrate both antibody and cellular immune reactions with the latter being proved by a histopathological assessment of spontaneously regressing genital warts.³

Macrophage migration inhibitory factor (MIF) is the first discovered lymphokine⁴ that holds characteristic structure and biological activities, combining the features of growth factors, chemokines, and cytokines.^{5,6} Cutaneous MIF is produced in the basal keratinocyte layer.⁷ MIF is a ligand of cell surface receptor complexes which consists of CD74 and CD44, CXCR2, CXCR4, or CXCR7.⁸ It is responsible for some pro-inflammatory functions, including chemo-attraction of T cells, neutrophils, and monocytes and activation of T cells and macrophage as well.^{5,9}

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Macrophage MIF participates in triggering the innate immune system during infections via stimulating the production of IL-2, IL-6, INF γ , and TNF- α .¹⁰ It also exerts major functions in cutaneous wound healing, inflammatory responses, immune reactions, and other skin disorders such as psoriasis vulgaris and atopic dermatitis.¹¹ Yet, data about its role in the pathology of common warts are scanty. So, the aim of this work was to assess MIF in lesional and perilesional skin in patients with common warts (verruca vulgaris) compared with age- and sex-matched control subjects.

Subjects and methods

This case-control study included 60 verruca vulgaris cases who were gathered from the Outpatient Clinic of the Dermatology and Andrology Department of Benha University Hospital between October 2017 and June 2018. The approval of the Research Ethical Committee involving human subjects of Benha Faculty of Medicine was granted before commencing the study which also followed the terms of the Declaration of Helsinki. Each participant signed an informed written consent before joining the study.

Both males and females who were 18 years or older and suffering from verruca vulgaris, yet, did not receive systemic (immunotherapy) or topical treatment for at least a month before collection of the skin biopsies were enrolled in the study. While cases who were pregnant, breast feeding, suffering from other skin disorders which affect the level of MIF like atopic dermatitis and psoriasis vulgaris, or had a history of any systemic disorders like autoimmune disorders, diabetes mellitus, malignancies, or hypertension were excluded from the study.

A purposely designed sheet was filled for each participant demonstrating age, gender, onset, duration of the warts, and medical history. Meticulous general and local examination (focusing on number, and site of warts) was performed for each participant.

Participants were grouped into two groups: group A (patients group including 60 patients with verruca vulgaris) and group B (control group including 30 age- and sex-matched apparently healthy controls).

Two biopsies were taken from each patient in group A; one was the lesional skin biopsy from the verruca vulgaris lesion and the other was the perilesional biopsy taken from skin surrounding the wart (1.5 cm from the edge of the lesion), while biopsies from controls were taken from matched sites to patients.

Skin biopsies were homogenized in 1.5 mL extraction buffer (containing 10 mM Tris pH 7.4, 150 mM NaCl, 1%

Triton X-100) per gram of tissue using rotor-stator homogenizer (Art-Micra D-8 Germany) for 20 s. The resulting homogenate transferred to 1.5 mL Eppendorf tube to be centrifuged at 13,000 \times g for 10 mins in 4°C and the resulting supernatant was kept in -80°C until analyzed.

MIF levels were measured by the quantitative sandwich ELISA technique using Quantikine[®] ELISA (DMF00B; R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis

Data collection and tabulation were performed using by the 16th version of SPSS software (SPSS Inc., Chicago, ILL Company). Chi-square test (χ^2) was adopted for analyzing categorical data which were presented as numbers and percentages. While mean \pm standard deviation, median, and range were used to present continuous data. Shapiro–Wilks test was used to test the normality of data for which $P>0.05$ was the normality reference. Variables with normal distribution among two independent groups were evaluated using the Student's *t*-test, while nonparametric variables were evaluated using Man–Whitney *U*-test. Kruskal–Wallis test was adopted to analyze differences among three independent means of nonparametric variables. Differences in nonparametric data among matched groups were evaluated using Wilcoxon test. Correlation between nonparametric variables was assessed using Spearman's correlation coefficient (ρ). $P\leq 0.05$ was considered significant.

Results

The current study included 60 patients; 23 males (38.33%) and 37 females (61.67%). Their ages ranged between 18 and 60 years with a mean \pm SD for the age of 27.1 years \pm 8.7. Thirty clinically free individuals served as a control group; 16 males (53.33%) and 14 females (46.67%). Their ages ranged between 19 and 38 years with a mean \pm SD for the age of 25.4 \pm 5.2 years. Both patients and controls were age and sex-matched with no statistically significant difference ($P=0.33$ and 0.17, respectively).

Macrophage migration inhibition factor was significantly higher in lesional and perilesional skin biopsies than controls ($P=0.001$ for both); however, the difference in MIF levels between lesional and perilesional skin biopsies was non-significant ($P=0.31$) (Table 1).

No significant relations were found between lesional and perilesional MIF levels and clinical characteristics of the studied patients (Tables 2 and 3) while both lesional and perilesional MIF levels were significantly correlated ($\rho=0.269$, $P=0.021$) (Table 4).

Table 1 Comparison between studied groups regarding MIF levels

Variable	Patients (N=60)				Controls (N=30)				Wilcoxon test & P	Z _{MWU1} test & P ₁	Z _{MWU2} test & P ₂		
	Lesional		Perilesional		Mean	±SD	Median (range)	Mean				±SD	Median (range)
	Mean	±SD	Mean	±SD									
MIF level (µg/L)	51.4	16.1	50.3 (23–82.3)	48.2	18.8	48.1 (22.5–93.2)	3.6	2.3	3.35 (0.45–8.5)	1.02 & 0.31 (NS)	7.7 & <0.001*	7.4 & <0.001*	

Notes: Wilcoxon test & P₁ → for comparing lesional and perilesional. Z_{MWU1} test & P₁ → for comparing controls with lesional. Z_{MWU2} test & P₂ → for comparing controls with perilesional.*Significant. Abbreviation: NS, non-significant.

Discussion

The current study was designed to evaluate the level of MIF in lesional and perilesional skin in patients with verruca vulgaris in comparison to age- and sex-matched controls.

As far as we know, this is the first study to assess lesional and perilesional MIF levels in cases suffering from verruca vulgaris compared to their controls.

The increased level of MIF in lesional biopsies could be attributed to its production by many immune and non-immune cells, including fibroblasts, macrophages, and lymphocytes, as well as, cells of endocrine, nervous, and reproductive systems.⁵ Additionally, histological examination of warts demonstrated an inflammatory cellular infiltration mostly by lymphocytes and mononuclear phagocytes at the destructed epidermal layers which could be the source of MIF.¹²

The relationship between MIF secretion and HPV infection was studied by Kindt et al.¹³ They found for the first time that the secretion (not expression) of MIF measured by quantitative analysis to MIF-mRNA was higher in HPV-positive human head and neck squamous cell carcinomas cell lines than in HPV-negative cell lines, an observation which was confirmed by resistance to an inhibitor of MIF (4-iodo-6-phenylpyrimidine). They hypothesized that the acidic environment created by HPV (evidenced by increased lactate production) explained the increase in MIF secretion through the activation of hypoxia-inducible factor 1. Consequently, the MIF-mRNA level increases in human HPV-positive cell lines, thus leading to elevated protein synthesis and the secretion of MIF.

At the cellular level, MIF signaling mainly depends on interaction with the cytokine receptor CD74/CD44. Binding to its cognate receptor triggers the mitogen-activated protein kinase /extracellular signal-regulated kinase signaling cascade, leading to an activation of cyclin D1 transcription and cyclin-dependent kinase-mediated phosphorylation of the Retinoblastomaprotein, resulting in stimulation of cell proliferation.¹⁴

The postulated role of MIF in progression of warts could be explained by the fact that high MIF levels may act as a trigger for proliferation of keratinocytes as MIF is a potential enhancer of cellular growth¹⁵ and is a pro-inflammatory agent as well, since it stimulates the production of cytokines including IL-1 IL-2, IL-6, IL-8, TNF, interferon IFN- γ , the secretion of nitric oxide, and the induction of the cyclooxygenase-2 pathway.¹⁶ IL-8 also stimulates cellular growth and proliferation,¹⁷ and

Table 2 Level of lesional MIF according to the clinical data of patients group

Variable		n.	Lesional MIF level			Z _{MWU}	P
			Mean	±SD	Range		
Sex	Male	23	50.5	15.07	23–82.3	0.35	0.72 (NS)
	Female	37	52.0	16.93	23–82.3		
Site	Head	4	48.7	18.8	24.4–70.6	0.36	0.72 (NS)
	Extremities	56	51.6	16.1	23–82.3		
Number	Single	32	52.8	16.7	23–82.3	KW=0.79	0.67 (NS)
	Two	3	47.2	23.7	28.2–73.9		
	Multiple	25	50.1	14.9	23–80		

Notes: Z_{MWU}→Z value of Mann–Whitney U-test. KW→Kruskal–Wallis test.

Abbreviations: MIF, migratory inhibitory factor; NS, non-significant.

Table 3 Level of perilesional MIF according to the clinical data

Variable		n.	Perilesional MIF level			Z _{MWU}	P
			Mean	±SD	Range		
Sex	Male	23	50.7	21.22	22.5–93.2	0.88	0.37 (NS)
	Female	37	46.6	17.36	27.6–93.2		
Site	Head	4	37.7	11.2	29.8–54.5	0.83	0.41 (NS)
	Extremities	56	48.9	19.1	22.5–93.2		
Number	Single	32	47.4	18.4	22.5–93.2	KW=2.72	0.25 (NS)
	Two	3	35.7	13.6	27.6–51.5		
	Multiple	25	50.6	19.8	22.5–93.2		

Notes: Z_{MWU}→Z value of Mann–Whitney U-test. KW→Kruskal–Wallis test.

Abbreviations: MIF, migratory inhibitory factor; NS, non-significant.

Table 4 Correlation between MIF levels with age and duration of warts

		Lesional MIF Level (µg/L)	Perilesional MIF Level (µg/L)
Perilesional MIF Level (µg/L)	rho	0.296	–
	P	0.021*	–
	N	60	
Age	rho	–0.079	–0.069
	P	0.55 (NS)	0.60 (NS)
	N	60	60
Duration	rho	0.013	0.249
	P	0.92 (NS)	0.055 (NS)
	N	60	60

Note: *Significant.

Abbreviations: NS, non-significant; MIF, migratory inhibitory factor.

angiogenesis.¹⁸ MIF induces expression of matrix metalloproteinase 9 which helps in tumor cell invasiveness.¹⁹

Clues for the role of MIF in wart progression come from the observation that it has been investigated in many clinical and experimental studies in both inflammatory diseases and cancer²⁰ specifically its role is in stimulating proliferation of keratinocytes in psoriasis animal models.²¹

Moreover, it plays many roles in the pathogenesis of human cancer stimulating cellular proliferation, growth, progression and escape from the immune system, promoting angiogenesis and cellular migration and inhibiting apoptosis as well as autophagy in tumor cells.²²

Results obtained from this study showed that lesional MIF levels were higher than perilesional levels with no statistically significant difference, despite they were correlated which could be explained as a stepwise rise in MIF levels, an observation which needs further studies.

On the other hand, El Hamd et al²³ found that serum levels of MIF were significantly lower in patients with cutaneous warts compared to their healthy controls which could be explained by shift of MIF to the wart sites, a result which needs further research.

Limitations of the study

The limitations of this study were the small number of patients and the study of only one type of cutaneous warts which was verruca vulgaris.

Recommendations

We recommend further studies to evaluate MIF in other types of warts, eg, plane, planter, and genital types and correlate it with the type of HPV. Further studies are needed to investigate the genetic polymorphisms.

Conclusion

The significantly elevated MIF levels in lesional and perilesional biopsies compared to controls point to its role in the progression of warts from HPV infected cells through postulated increase in keratinocytes proliferation, enhanced angiogenesis, and cellular invasion.

Disclosure

All authors declare no conflicts of interest in this work.

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