Measurement of serum macrophage migration inhibitory factor level in patients with external ano-genital warts treated with intra-lesional purified protein derivative injection: an interventional study

Original Article

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ABSTRACT

Background: The most prevalent sexually transmitted disease is anogenital warts (AGW), which are produced by different genotypes of the human papilloma virus (HPV). Immunotherapy promotes a delayed type hypersensitive reaction to a variety of antigens as well as to wart tissue.

Aim: To evaluate the serum level of macrophage migration inhibitory factor (MIF) in patients with external ano-genital warts before and after intra-lesional purified protein derivative (PPD) injection.

Patients and Methods: This study was carried out as pre–post interventional study on 40 patients with external anogenital warts. The study was conducted at the dermatology clinic and the andrology, infertility and sexually transmitted diseases clinic, Suez Canal University Hospital in Ismailia. Using an insulin syringe, each patient was injected 0.2 ml of tuberculin PPD (containing 10 TU of tuberculin PPD) intra-lesionally in the largest wart at regular interval of 2 weeks for a maximum of six injections or less in cases of complete clearance of wart. Serum levels of MIF were measured using ELISA before and after intra-lesional PPD injection.

Results: 22 (55%) patients achieved complete response while 8 (20%) patients achieved partial response. No response occurred in 10 (25%) patients. Serum level of MIF after PPD injection was significantly higher than serum level of MIF before PPD injection. There was statistically significant relation between serum MIF level after PPD injection and clinical response to PPD injection.

Conclusion: Immunotherapy with intralesional PPD injection provides an efficient, financially viable method of treating genital warts. Serum levels of MIF in patients with anogenital warts after intralesional PPD injection were significantly higher than serum levels of MIF before intralesional PPD injection and have statistically significant relation to clinical response to intralesional PPD injection.

Key Words: Genital warts, migration inhibitory factor, tuberculin purified protein derivative

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INTRODUCTION

The most prevalent sexually transmitted disease is anogenital warts (AGWs)[1]. Several human papillomavirus (HPV) genotypes are responsible for AGWs. Low-risk types 6 and 11 of the HPV account for more than 90% of all incidences of genital warts[2]. Through microscopic abrasions in the skin or mucosa,the HPV gets into cells in the basal layer of the epidermis[3].

One of the most important immunoregulatory cytokines is called macrophage migration inhibitory factor (MIF). Itsupports macrophage phagocytosis, motility, as well as transendothelial migration processes, in addition to immunological and inflammatory responses[4]. Innate immunity and adaptive immunity are controlled by MIF. It is released by granulocytes, tumor cells, endocrine, endothelium, and B and T cells as well as macrophages[5].

AGWs are treated topically or by immunotherapy or by damaging methods. The site, size, and number of warts, as well as the efficacy of previous therapies, coexisting circumstances (pregnancy and immunosuppression), patient preferences, adherence to therapy, expectations,

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and cost of treatment all go into the decision of how to treat AGWs. Each patient must receive specialized treatment[6].

Immunotherapy induces delayed hypersensitivity response to various antigens and warts. Immunotherapy causes T helper 1 (Th1) cytokines to be released. This leads to activation of natural killer cells in addition to cytotoxic cells to eliminate HPVat the location of the injected lesions and at surrounding and distant warts[7].

A protein known as purified protein derivative (PPD) is extracted from mycobacterium tuberculosis. It serves as a diagnostic tool for determining whether or not an individual has been exposed to the tuberculin protein[8]. PPD induces a delayed hypersensitivity reaction that is T cell-mediated[8].

Intralesional immunotherapy with PPD causes the synthesis of a number of cytokines, including interleukin 2 (IL-2), IL-4, IL-5, IL-8,and IL-12. It also causes synthesisof interferon gamma(IFN-γ)and tumor necrosis factor alpha (TNF-α), which in turn induces a powerful immune response against HPV[9]. Cell-mediated immunity (also known as CMI) is essential in the process of removing warts[10].

According to El-Hamd *et al.*[11], patients with cutaneous warts had significantly lower MIF serum levels than their healthy controls, which contribute to the risk of HPV infections and the growth of various cutaneous warts.

The current study was designed to find out the influence of PPD on serum MIFin the treatment of genital warts. This was accomplished by comparing the serum levels of MIF before and after receiving an intralesional injection of PPD in patients having external AGWs.

PATIENTS AND METHODS

This research was conducted both before and after an intervention.The investigation was conducted in Ismailia's Suez Canal University Hospitalfrom August 2019 to December 2020 at the dermatology clinic as well as the andrology, infertility, and sexually transmitted diseases clinic.

Patient selection

A random sample of 40 patients who had previously consented to take part in the study was selected in accordance with the inclusion criteria and the exclusion criteria. Patients who were male, above the age of 18 years, and had external AGWs were considered to meet the inclusion criteria. Patients who had a history of tuberculosis infection, patients with chronic or debilitating illnesses, patients with immune-suppressive conditions, and patients taking immunosuppressive medications were all excluded from the study. Other patients with external AGWs who had recently received treatment with another modality at the last 3 months were also excluded. Patients who were known to be hypersensitive to PPD met the exclusion criteria.

Medical history and examination

A full history was taken of every patient. To exclude systemic disorders, a general examination was conducted. To rule out other skin conditions, a full body skin examination was performed. A local dermatological examination was performed to identify the location, size, quantity, and distribution of AGWs as well as to rule out any other sexually transmitted diseases.

Intralesional tuberculin purified protein derivative injection

To first identify PPD sensitivity, a tuberculin test was conducted. To complete the test, 0.1ml of tuberculin PPD containing five tuberculin units was injected intradermally into the patient's left forearm. At the injection site, a white bleb that is well defined and measures 10ml in diameter should form. Sensitization was detected by the presence of induration and/or erythema 48–72h after the injection[12].

After the patients had been sensitized, 0.2ml of PPD (containing 10 international units of tuberculin PPD) was injected intralesionally into the biggest wart on each patient's body using an insulin syringe at frequent basis of 2 weeks, up to an a total of six sessionsor less in circumstances of full elimination of the wart. At follow-up appointments, the treatment was stopped if the patient showed complete clearance. However, intralesional PPD therapy was repeated at a predetermined interval if there was no improvement or only partial clearance[13].

A photographic examination served as the basis for the clinical assessment at the start of therapy, before to each session, and again after 2 weeks had passed.

Therapy response was determined by the percentage of wart reduction from the initial assessment and by comparing before-and-after pictures taken at the beginning of treatment and at the end. Adverse effects were evaluated both immediately and many days after each therapeutic session.

The findings were classified as follows: complete response when warts completely disappeared and the skin marks returned to normal, partial response when the warts decreased by 25–99%, and no response when the warts decreased by 0–25%[14]. After the warts had been entirely removed, the patients went in for frequent follow-up appointments once a month for a period of 3 months to check for any signs of a return of AGWs.

The researchers carefully reviewed and reevaluated partial responders and nonresponders. Patients were switched to alternative management strategies until full recovery.

Assessment of serum migration inhibitory factor

Before the first session and again 2 weeks after the final session,enzyme-linked immunosorbent assay was used to measure serum MIF. The immunoassay was performed according to the manufacturer's guidelines (cat#: 201-

12-0142; Sun Red Biotechnology Company, Shanghai, China)

Ethical considerations

The Research Ethics Committee of the Faculty of Medicine at Suez Canal University gave its permission to proceed with the investigation. The number of the Ethical Committee's permission was 3813, and the date was April 22, 2019. Each participant started the study by filling out an informed consent document that outlined the goal of the research, the benefits, and any potential adverse effects that may be caused by the therapy that was used.

Statistical analysis

The statistical study was carried out with IBM SPSS Statistics, version 22, IBM Corp.,Armonk, New York, USA. Quantitative information was presented in the form of numbers and percentages. The range of values, including minimum and maximum, as well as mean, SD, median, and interquartile range, were used to express the numerical data. The significance of the obtained results was evaluated at the 5% level.

Table 1: Sociodemographic characteristics of the studied patients with anogenital warts (N=40)

Parameters	n(%)
Age (years)	
Minimum–maximum	22.0–52.0
Mean±SD	35.05±8.64
Site of warts	
Pubic area	22 (55.0)
Shaft of penis	36 (90.0)
Frenulum of penis	10 (25.0)
Scrotum	22 (55.0)
Perianal area	6 (15.0)
Number of warts	
Minimum–maximum	1.0–24.0
Mean±SD	13.40±9.89
Duration (months)	
Minimum–maximum	3.0–18.0
Mean±SD	8.40±4.57
Number of sessions	
Minimum–maximum	4.0–6.0
Mean±SD	5.65±0.59

IQR, interquartile range.

A total of 12 (30%) patients had no adverse effects to PPD injection,whereas 28 (70%) patients had adverse effects to PPD injection. Flu-like symptoms were the most common adverse effects and occurred in 18 (45%) patients. Pain at the injection site occurred in 16 (40%) patients, and transient edema at the injection site occurred in six (15%) patients. Overall, four (10%) patients developed mild erythema and two (5%) patients showed transient postinflammatory hypopigmentation. Of 40 patients, 22 (55%) patients achieved complete response,whereaseight (20%) patients achieved partial response. No response occurred in 10 (25%) patients. A total of four (18.2%) patients showed recurrence of warts after complete response of warts after follow-up of 3 months and 18 (81.8%) patients showed no recurrence of warts after complete response of warts after follow-up of 3 months (Table 2).

Serum levels of MIF before intralesional PPD injection ranged from 0.19 to 3.46ng/ml, with a mean of 0.75±0.91ng/ml,whereas serum levels of MIF after intralesional PPD injection ranged from 0.13 to 5.54ng/ml, with a mean of 1.41±1.41ng/ml. Serum level of MIF after PPD injection was significantly higher than serum level of MIF before PPD injection (P=0.025) (Table 3).

Table 2: Frequency distribution of the studied patients with anogenital warts according to adverse effects to purified protein derivative, clinical response to purified protein derivative, and recurrence after complete response (N=40)

Parameters	n(%)
Adverse effects to PPD	
No	12(30.0)
Yes	28 (70.0)
Flu-like symptoms	18 (45.0)
Pain	16 (40.0)
Erythema	4 (10.00)
Hypopigmentation	2 (5.0)
Edema	6 (15.0)
Clinical response	
No response	10 (25.0)
Partial response	8 (20.0)
Complete response	22 (55.0)
Recurrence after complete response	
No	18 (81.8)
Yes	4 (18.2)

PPD, purified protein derivative.

Table 3: Comparison between before and after purified protein derivative injection according to serum migration inhibitory factor (N=40)

Serum MIF (ng/ml)	Before injection	After injection	*Z*	*P*
Minimum–maximum	0.19–3.46	0.13–5.54		
Mean±SD	0.75±0.91	1.41±1.41	2.240*	0.025*
Median (IQR)	0.40(0.22–0.68)	1.17(0.38–1.73)		

IQR, interquartile range; MIF, migration inhibitory factor.
Z: Wilcoxon signed-rank test
P: *P* value for comparing between before and after
*Statistically significant at *P* value less than or equal to 0.05.

No significant difference was found between serum level of MIF before PPD injection and clinical response for PPD injection. There was a statistically significant relation between serum MIF level after PPD injection and clinical response of PPD injection. Mean serum level of MIF after PPD injection was higher in patients with complete response to PPD injection than patients with partial response and no response to PPD injection (Table 4). A statistically significant relationwas found between clinical response to PPD injection and age of patients ($P<0.001$), as the younger the age, the better the response. There was a statistically significant relation between clinical response to PPD injection and number of warts ($P=0.001$), as the fewer the number of warts, the better the response. There was no a statistically significant relation between clinical response to PPD injection and site of warts. There was a statistically significant relation between clinical response to PPD injection and duration of warts ($P=0.001$) as greater frequency of full wart removal was found in warts that had shorter duration (Table 5 and Figs 1–3).

Table 4: Relation between clinical response and serum migration inhibitory factor (ng/ml) (N=40)

Serum MIF (ng/ml)	Clinical response			*H*	*P*
	No response (n = 10)	Partial response (n = 8)	Complete response (n = 22)		
Before injection					
Minimum – Maximum.	0.20 – 0.63	0.19 – 3.0	0.22 – 3.46	2.619	0.270
Mean ± SD.	0.32 ± 0.18	1.10 ± 1.29	0.82 ± 0.96		
Median	0.21	0.60	0.46		
After injection					
Minimum – Maximum.	0.13 – 0.45	0.14 – 2.50	1.01 – 5.54	11.343*	0.003*
Mean ± SD.	0.25 ± 0.13	0.92 ± 1.07	2.12 ± 1.46		
Median	0.18	0.53	1.38		

H, Kruskal–Wallis test; MIF, migration inhibitory factor; *P, P* value for comparing between different parameters.
*Statistically significant at P value less than or equal to 0.05.

Table 5: Relation between clinical response and different parameters (N = 40)

Serum MIF (ng/ml)	Clinical response						Test of significance	*P*
	No response (n = 10)		Partial response (n = 8)		Complete response (n = 22)			
	N	%	N	%	N	%		
Age (years)								
Min. – Max.	39.0 – 52.0		36.0 – 47.0		7.0 – 35.0		F= 15.548*	<0.001*
Mean ± SD.	44.60 ± 4.88		41.25 ± 4.57		26.64 ± 7.71			
Median	44.0		41.0		28.0			
Site of warts								
Pubic area	8	80.0	6	75.0	8	36.4	γ2=3.192	^{MC}p=0.272
Shaft of penis	10	100.0	6	75.0	20	90.9	γ2=1.668	^{MC}p=0.415
Frenulum of penis	0	0.0	4	50.0	6	27.3	γ2=2.777	^{MC}p=0.231
Scrotum	8	80.0	4	50.0	10	45.5	γ2=1.708	^{MC}p=0.491
Perianal area	2	20.0	2	25.0	2	9.1	γ2=1.336	^{MC}p=0.749
Number of warts								
Min. – Max.	16.0 – 34.0		11.0 – 24.0		1.0 – 14.0		H= 13.973	0.001*
Mean ± SD.	25.60 ± 7.77		18.0 ± 6.48		6.18 ± 2.96			
Median	28.0		18.50		6.0			
Duration (months)								
Min. – Max.	12.0 – 18.0		9.0 – 12.0		3.0 – 8.0		H= 15.407*	0.001*
Mean ± SD.	14.60 ± 2.41		10.25 ± 1.26		4.91 ± 1.70			
Median	14.0		10.0		5.0			

γ2, γ2 test; F, F for analysis of varinacetest; H, H for Kruskal–Wallis test; MC, Monte-Carlo; P, P value for comparing between different parameters.
*Statistically significant at *P* value less than or equal to 0.05.

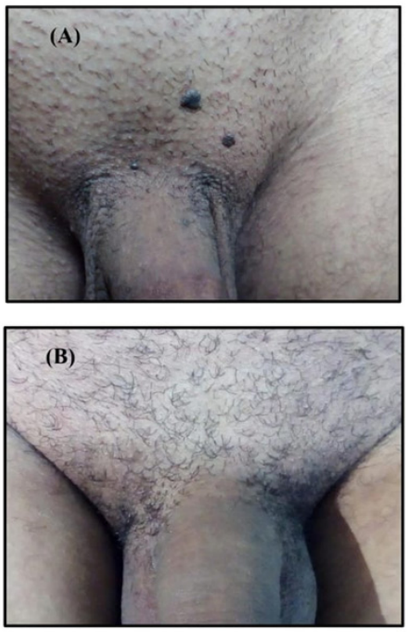

Fig. 1: (a) A case of a 35-year-old male patient with four genital warts in the pubic area before intralesional PPD injection. Serum MIF level was 0.222ng/ml before PPD injection. (b) The same patient showed complete response after six sessions of intralesional injections of 0.2ml of PPD every 2 weeks. Serum MIF level was 1.11 ng/ml after PPD injection.MIF, migration inhibitory factor; PPD, purified protein derivative.

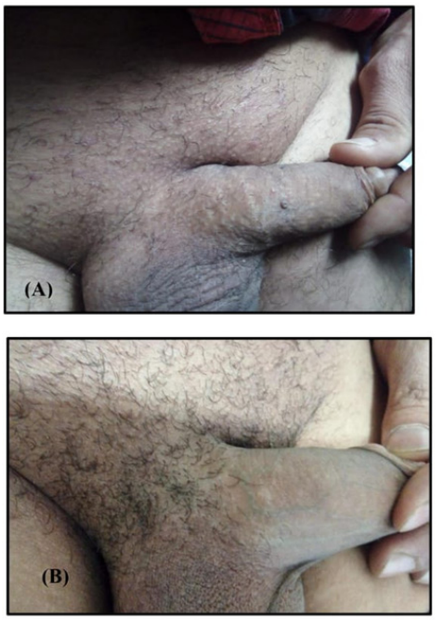

Fig. 2: (a) A case of a 31-year-old male patient with one genital wart on the shaft of penis before intralesional PPD injection. Serum MIF level was 0.589ng/ml before PPD injection. (b) The same patient showed complete response after five sessions of intralesional injection of 0.2ml of PPD every 2 weeks. Serum MIF level was 1.76ng/ml after PPD injection.MIF, migration inhibitory factor; PPD, purified protein derivative.

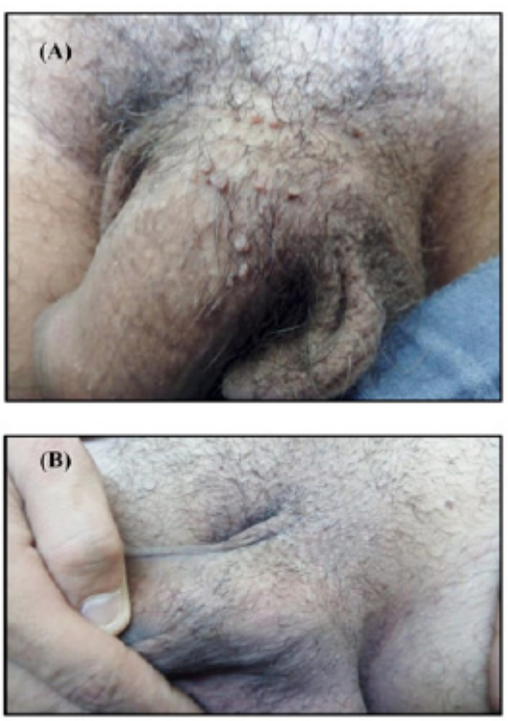

Fig. 3: (a) A case of a 26-year-old male patient with multiple genital warts on the pubic area and shaft of penis before intralesional PPD injection. Serum MIF level was 0.216ng/ml before PPD injection. (b) The same patients showed complete response after four sessions of intralesional injections of 0.2ml of PPD every 2 weeks. Serum MIF level was 1.384ng/ml after PPD injection.MIF, migration inhibitory factor; PPD, purified protein derivative.

PATIENTS AND METHODS

The most frequently identified viral sexually transmitted infection is AGWs. The HPV, which affects both men's and women's anogenital regions, has many genotypes that are responsible for AGW.In excess of 90% of incidence of AGW can be attributed to low-risk HPV strains 6 and 11[2].

Immunotherapy for warts induces a hypersensitive reaction of the delayed type to numerous antigens in wart tissue.Through the activation of cytotoxic cells and natural killer cells, which are stimulated by the production of Th1 cytokines, the HPV infection can be eradicated. When compared with conventional treatments, this eliminates both nearby and distant warts[15]. Intralesional PPD injection causes the synthesis of a variety of cytokines, such asIL-2, IL-4, IL-5,IL-8, and IL-12. It also causes synthesis ofIFN-γ and TNF-α, which in turn stimulates a powerful immune response against HPV[9]. Intralesional PPD injection is a successful, well-tolerated, and low-risk form of therapy[16].

In the present study, we injected tuberculin PPD intralesionally as a kind of immunotherapy for the treating of AGWs. Of 40 patients, 22 (55%) had a complete response, whereaseight (20%) had a partial response, and 10 (25%) patients showed no sign of improvement.

These findings are consistent with those of Fawzy *et al.*[17], who reported that PPD-treated patients exhibited complete removal of warts in 22 (55%) patients, partial response in six (18%) patients, and no response in 12 (30%) patients. Chandra *et al.*[15] discovered that 16 (50%) of 32 patients experienced full elimination of their warts.Gupta *et al.*[18] found that 35 (68.6%) patients had total wart removal, six (11.7%) had partial clearance, and 10 (19.6%) had no response.

Singh *et al.*[16] observed better outcomes and found that 80% had their warts completely disappear after intralesional PPD injection, 15% patients had a partial clearance, and 5% patients had no change. Raveendra *et al.*[19] showed higher results than our results and reported that 38 (76%) of 50 patients exhibited complete wart elimination, whereas 12 (24%) exhibited only partial clearance.

On the contrary, Kusand colleagues gave patients with resistant warts an intralesional tuberculin PPD injection, and they found that five (29%) of the patients experienced a complete cure, 10 (59%) of the patients experienced a partial response, and two (12%) of the patients saw no reaction at all. This lower success rate in recovery can be attributed to the fact that the study was conducted with a smaller sample size than ours, fewer sessions, and a longer period between injections (3 weeks as opposed to 2 weeks)[20].

MIF is implicated in innate and adaptive immunity as well as inflammatory and autoimmune skin disorders[21]. Numerous disorders, including pemphigus vulgaris, alopecia areata, vitiligo, psoriasis, allergic and irritant contact dermatitis, and bullous pemphigoid, are affected by MIF[11].

The current study revealed that serum MIF levels were found to be significantly increased after PPD injection compared with levels before PPD administration (P=0.025).

According to El-Hamd *et al.*[11], patients with cutaneous warts had significantly lower MIF serum levels than their healthy controls, which contribute to the risk of HPV infections and the growth of various cutaneous warts. Sorourand colleagues demonstrated that the MIF level was much higher in lesional and perilesional skin biopsies compared with the levels found in controls; however, no significant difference between lesional MIF level and perilesional MIF level was found. Numerous immune and nonimmune cells, such as fibroblasts, macrophages, lymphocytes, and cells of the endocrine system, produce MIF, which is why its concentration was elevated in lesional biopsies[22].

Nassarand colleagues examined the serum levels of MIF in people who had several common warts after administering intralesional injections of candida antigen into the biggest wart at intervals of 2 weeks until full elimination or for a maximum of five therapy session. They observed that the change in serum MIF levels between before and after injection was statistically significant[4].

There was no statistically significant difference found inthis study between serum level of MIF before PPD injection and clinical response for PPD injection. There was a statistically significant relation between serum MIF level after PPD injection and clinical response of PPD injection. Mean serum level of MIF after PPD injection was higher in patients with complete response to PPD injection than patients with partial response and no response to PPD injection.

The findings of our study were in agreement with the findings that were reported by Nassar and colleagues. They revealed that theserumMIF levels before treatment in the responders group were statistically insignificant when compared with the levels in the nonresponders group. After the completion of therapy, significant difference in MIF levels was observed between those who responded to the treatment and those who did not[4].

In the skin, MIF recruits antigen-presenting cells, which leads to an increase in delayed-type hypersensitivity responses. These reactions provide the bulk of the protection against intracellular pathogens. It is responsible for the activation of a number of Th1 cytokines, including TNF-α, IL-1, IL-6, and IFN-γ, among others[23].

The current study has some limitations, such as a small sample size that makes it unable to make generalizations andthe follow-up period was too short to detect recurrence. It was quite challenging to get patients to take multiple injections and wait 3 months to end of sessions. Other

restrictions include poor patient compliance, intolerance of adverse effects, and challenges evaluating partners. PPD was extremely vulnerable to heat and light, and if these conditions werenot regulated, it got spoilt. There isnot enough information to compare with our findings about the effect of immunotherapy using PPD and MIF levels.

To get more reliable results, we suggest larger-scale follow-up investigations. We suggest a larger study be conducted to evaluate other ILs in individuals with other forms of warts whether alone or in conjunction with various therapeutic approaches, notably immunotherapy. We recommend using PCR to detect if there is a correlation between clinical response to PPD injection and the HPV subtypes or not. We advise more research into the efficacy of MIF in treating different forms of warts.Long-term patient follow-up is recommended to evaluate the success of PPD in reducing recurrence.

CONCLUSION

With its high removal rate and low recurrence rate, immunotherapy by intralesional PPD is an economically viable technique for treating genital warts .After intralesional PPD injection, MIF levels in individuals with AGWs were significantly greater than before injection and correlated with clinical response.

CONFLICT OF INTEREST

There are no conflicts of interest.

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