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Expression of Matrix Metalloproteinase-2 (MMP-2) and its Inhibitors in Pyogenic Granuloma

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Abstract

Background- Pyogenic granuloma is a common reactive oral lesion characterized by rapid vascular proliferation and tissue remodeling. Matrix metalloproteinase-2 (MMP-2) and its regulators, TIMP-2 and RECK, are key modulators of extracellular matrix turnover and angiogenesis, but their roles in pyogenic granuloma remain unclear. ***Aim-*** This study aimed to assess the expression and possible role of MMP-2, TIMP-2 and RECK in the biologic behaviour of Pyogenic granuloma. ***Methods-*** This was a laboratory based immunohistochemical study of pyogenic granuloma cases seen at the Department of Oral Pathology/Oral Medicine, UCH Ibadan, Nigeria between January 2000 and December 2011. 50 cases of pyogenic granuloma were sectioned and stained with commercial antibodies for MMP-2, TIMP-2 and RECK. Immunohistochemical staining of cells in individual cases was assessed at X100 magnification. Immunohistochemical assessment of MMP2, TIMP2 and RECK were expressed in proportions/percentages. Mean scores for MMP-2, TIMP-2 and RECK as well as MMP-2: TIMP-2 and MMP-2: RECK in all the cases of pyogenic granuloma were compared using the Independent Sample T test. ***Results-*** All of the cases expressed MMP2 and 88% of cases expressed TIMP-2 while RECK is positive in 80%. The mean MMP2: RECK ratio in pyogenic granuloma is 2:1. TIMP -2 is significantly higher in males than females ($p = 0.005$) while mean MMP2:TIMP2 is significantly higher in females than males ($p = 0.000$). Pearson correlation and regression analyses were performed to explore the relationships between MMP-2 and its inhibitors, TIMP-2 and RECK, in pyogenic granuloma. A weak negative correlation was observed between MMP-2 and TIMP-2 expression ($r = -0.093$), which was not statistically significant ($p = 0.521$). Similarly, simple linear regression analysis showed that MMP-2 expression was not a significant predictor of RECK expression ($\beta = 0.02$, $p = 0.882$), with the model explaining virtually none of the variance ($R^2 = 0.00$). ***Conclusion-*** MMP-2 is expressed in all pyogenic granuloma cases and exceeds the levels of its inhibitors, TIMP-2 and RECK, consistent with its role in driving angiogenesis. Its expression appears largely independent of TIMP-2 and RECK, suggesting additional regulatory factors influence MMP-2 activity in these lesions.

Keywords: Pyogenic Granuloma; Matrix Metalloproteinase-2; Tissue Inhibitor of Metalloproteinases-2; RECK Protein; Angiogenesis.

INTRODUCTION

Pyogenic granuloma (PG) is considered a reactive tumour-like lesion, an inflammatory hyperplasia which arises in response to various stimuli such as low-grade chronic irritation or traumatic injury, as well as hormonal factors and may be drug-related. There is no known local invasive property associated with this lesion as it is non-neoplastic. [1-3] PG presents clinically as an elevated dusky red or purplish lesion which may be soft or firm and may be ulcerated. It bleeds easily, due to its high vascularity and as it matures with more connective tissue formation, may become firmer and more pinkish in colour. On histology, it shows an eroded or ulcerated surface epithelium overlying a connective tissue stroma, containing numerous, thin, endothelial-lined vascular channels engorged with red blood cells, as well as, an admixture of inflammatory cells infiltrate,

predominantly lymphocytes, plasma cells and neutrophils. It also shows proliferating spindle to plump shaped fibroblasts within the stroma. [4, 5] Recurrences are said to characterize oral pyogenic granuloma in only a small percentage of cases and are usually attributed to inadequate excision; a study in Ibadan by Lawoyin *et al* [3] showed no recurrence of the lesion on follow-up of treated cases.

In view of their clinicopathological presentation, it has been affirmed by several authors that pregnancy epulis is the PG that occurs in pregnancy; though some authors believe that it has a unique nature from PG, due to the influence of progesterone and estrogen and should thus, be considered a separate entity.[6] It has however been shown that, in pregnancy tumor or epulis, as well as in PG, gingival tissues are rendered more susceptible to chronic irritation (usually by plaque and calculus) via the effect of serum estrogen and progesterone concentrations. [6, 7] Macrophages and fibroblasts are proven to be induced by estrogen to produce vascular endothelial growth factor (VEGF) and thus, granulation tissue formation. Release of growth factors such as basic fibroblast growth factor (b FGF) and transforming growth factor (TGF- 1) also occurs. [6, 7]

Matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent proteolytic enzymes that play a central role in the regulation of extracellular matrix during embryonic development and tissue remodelling. They also participate in tumour invasion and metastasis in which case, their pathological activity is detected in tumour cells and surrounding stroma.[8-10] Moreover, their roles, not only in tumour initiation and invasion but also in angiogenesis, metastasis, in releasing other tumour-promoting factors and in tumour recurrence have been established.[11, 12] These enzymes are classified according to the substrate they degrade (collagenase, gelatinase, stromelysin). MMP-2, a gelatinase, has substrates including: gelatin types I to III and collagen types IV, V, VII, and X. [13-15]

Endogenous MMP inhibitors comprise a family of proteins and glycoproteins known as tissue inhibitor of matrix metalloproteinases (TIMPs). Four of these inhibitors have been cloned: TIMP-1, TIMP-2. TIMP-3 and TIMP-4.[16] The role played by TIMPs in regulating matrix degradation has been observed in physiological tissue remodelling such as wound healing and trophoblastic implantation as well as in the pathologic process of tumour invasion. [17, 18] TIMPs inhibit active and latent forms of MMPs by forming non-covalent bimolecular complexes with MMPs. They inhibit the activity of fully activated MMPs in a 1:1 stoichiometric ratio, in addition, they appear to block or retard the activation of MMP precursors. [19, 20] However, TIMP-2 is unique because it may function both as a MMP inhibitor and activator.[21]

Reversion-inducing cysteine rich protein with kazal motifs (RECK) was initially discovered due to its ability to induce reversion in ras-activated fibroblasts. RECK gene is therefore a tumour suppressor gene [22, 23]. Reversion-inducing cysteine-rich protein with kazal motifs (RECK), a membrane bound protein, can also exert inhibitory effects on the transcription, synthesis, activation and activity of MMPs [24-26]. A prominent function of RECK is that of inhibition of MMPs, especially MMP-2 and MMP-9 [20, 21]. RECK functions as an endogenous inhibitor of MMP-2 in physiological and pathological processes [26, 27].

Various inflammatory oral diseases have been associated with matrix metalloproteinases (largely as a host related factor).[28] Collagenase activity particularly human collagenase-2, MMP-8 and MMP-9 have been found in inflamed gingivae and crevicular fluid in periodontal disease. [29] Moreover, it was found that the TIMPs in these lesions are not sufficient to down regulate the pathologically elevated MMPs.[30] MMPs are said to be elevated in inflamed pulp and periapical lesions as in other inflammatory conditions.[31] Similarly, granulation tissue - a product of wound healing and tissue repair, which is microscopically similar to oral pyogenic granuloma, expresses MMPs and TIMPs[1, 17]

The aim of this study is to determine the expression and possible role of MMP-2, and its inhibitors, TIMP-2 and RECK, in pyogenic granuloma.

METHODOLOGY

Histopathology registers were first obtained from the Department of Oral Pathology/Oral Medicine, UCH Ibadan, and a list of all histologically diagnosed pyogenic granulomas in the stated period was manually retrieved. The patient's bio data (age and sex) were retrieved alongside. H & E sections and formalin-fixed paraffin-embedded tissues from the listed pyogenic granuloma cases were then retrieved. When the original slides were missing or inadequate for histological evaluation, fresh sections were obtained and stained with H & E. The slides were reviewed by the investigator under the supervision of a trained and experienced Oral Pathologist to confirm the histological diagnosis.

Paraffin blocks of fifty (50) histologically confirmed cases of pyogenic granuloma that met the inclusion criteria were consecutively selected for immunohistochemistry. Test and control slides were stained for MMP-2, TIMP-2 and RECK.

Selected paraffin blocks of pyogenic granuloma were remounted, and representative sections of each cut serially on a manual microtome at 4 microns thickness. Sections were floated on a warm water bath, placed on adhesive-coated slides (pre-treated slides) and then placed on a warmer set at 60°C. Three slide sections were prepared from each block, in preparation for

immunohistochemical staining with antibodies for MMP-2, RECK and TIMP-2, respectively.

Immunohistochemical Staining Procedure:
Deparaffinisation

A total of three slides per paraffin block were produced for immunohistochemical staining for each of the selected pyogenic granuloma cases. They were deparaffinised by passing them through changes of xylene twice for 5 minutes each, then rehydrated in decreasing grades of alcohol as follows: 100% ethanol twice for 3 minutes each, followed by 95% and 70% ethanol for 1 minute each. This was followed by rinsing in phosphate buffered saline (PBS).

Antigen unmasking and retrieval:

After this, the slides were immersed in heat induced epitope retrieval citrate buffer (10 mM sodium citrate buffer at pH 6.0) and pre-treated in a microwave oven set at 90^0C for 1 hour. They were removed and placed in fresh citrate to cool for 20 minutes. This was followed by a 10-minute rinse in phosphate buffered saline (PBS).

Blocking of endogenous peroxidase:

The slides were placed in a humidity chamber, then, the tissue periphery marked with a hydrophobic pen. Blocking of endogenous peroxidase was done with 3% hydrogen peroxide for 10 minutes at room temperature after which the slides were rinsed in PBS.

Immunoperoxidase staining:

The three slide sections prepared from each paraffin block were incubated for an hour with laboratory recommended amount of appropriately characterized and diluted primary antibodies (40 – 130 µl, depending on the surface area of the tissue): anti-RECK (1:50 mouse monoclonal antibody, by Santa Cruz biotechnology), anti-TIMP-2 (1:200 mouse monoclonal antibody, by abcam plc) and anti-MMP-2 (1:200 mouse monoclonal antibody, by abcam plc) respectively. Excess reagent was wiped dry from each slide with laboratory tissue paper.

This was followed by incubation of the slide sections with the appropriately labelled polymer horse radish peroxidase (HRP) conjugated anti-mouse secondary antibody for 30 minutes, after which they were rinsed in PBS.

The areas surrounding the paraffin section were wiped dry with laboratory tissue paper and 1ml of diaminobenzidene (DAB) chromogen was added to cover the specimen. After visualization of the reaction with the diaminobenzidine chromogen, incubation in humidity chamber for 15 minutes was done. The slides were immersed in a bath of aqueous haematoxylin about 10 times for 1 minute each and then rinsed in distilled water for 3 minutes after 10 immersions for counterstaining. The slides were then passed by dipping through a series of graded alcohol to dehydrate the sections (70%, 95% then 100%) and then also rinsed with xylene. A mounting fluid (glycerine gel) was applied and a cover slip placed.

Laboratory positive and negative staining controls for each of the three proteins (antibodies) being tested were used as recommended by the manufacturer to validate the procedure. Breast adenocarcinoma was used as a positive control for MMP-2, human placental tissue was used for TIMP-2 and normal colon specimen was used for RECK.[32-34] Laboratory negative-staining controls were other slide sections produced from each of the three laboratory control paraffin blocks, but incubated with PBS only instead of the indicated primary antibody. Slides of each laboratory positive and negative control followed the same immunohistochemical staining protocol as the other slides in this study to ensure validity.

Evaluation of Immunohistochemical Staining:

Slide sections were evaluated by the investigator and two experienced consultant Oral Pathologists. A consensus opinion on the staining patterns observed under a binocular light microscope (Zeiss Axioscope, Switzerland) was recorded for each case. The indicated positive laboratory control slide was used as a reference guide. The connective tissue cells were evaluated in all cases of pyogenic granuloma. The manufacturer's guide was used to evaluate the staining colour quality for positive cell staining. Cytoplasmic staining was used for positive staining with each antibody, as documented by the manufacturers.

Immunohistochemical staining of cells was assessed using both stain intensity (depth of colour or intensity of staining) and proportion of cells stained. Stain intensity was graded using a semi quantitative 4-point scale: 0 = no staining (-); 1= mild staining; 2= moderate staining; and 3= intense staining. The proportion of cells stained was assessed at X100 magnification (Objective X10) using a semi quantitative 4-point scale: 0 = no cell staining in any microscopic fields; 1= <25% staining; 2= 25-50% staining; and 3= >50% staining. The combined score (proportion plus intensity) was determined. The combined score was categorised as follows: <2, negative staining or low staining (-); 2 and 3, moderate staining (+); and ≥4, strong staining (++). A combined score equalling or exceeding (+) was defined as positive for either MMP-2, TIMP-2 or RECK.[35]

Data was analysed with IBM SPSS version 20.0. Data was presented as tables and graphs using summary statistics such as mean and standard deviation for quantitative data like age, scores of antibody staining (intensity and proportion) for MMP2, TIMP2 and RECK as well as MMP-2: TIMP-2 and MMP-2: RECK.

Qualitative data such as age group, sex, Immunohistochemical staining were expressed as proportions/percentages. Mean scores for MMP-2, TIMP-2 and RECK staining (intensity and proportion) as well as the ratios of MMP-2: TIMP-2 and MMP-2: RECK were compared between genders using the Independent Samples t-test. Correlation analyses were performed to evaluate the relationship between MMP-2 expression and its inhibitors, TIMP-2 and RECK. In addition, Fisher's exact test was applied where appropriate to assess the association between categorical variables, particularly when expected cell counts were small. The level of statistical significance for all analyses was set at $p < 0.05$.

RESULTS

9 (nine) out of Fifty (50) cases of the selected pyogenic granuloma cases had missing records of age-group and gender(sex), hence 41 cases were analysed for age-group and gender distribution.

Table 1: Age Group and Sex Distribution of Pyogenic Granuloma Cases

Age-Group	Male	Female	Total
0-9 YEARS	1	1	2 (4.9%)
10-19 YEARS	3	9	12 (29.3%)
20- 29 YEARS	3	4	7 (17.1%)
30-39 YEARS	1	4	5 (12.2%)
40-49 YEARS	1	3	4(9.8%)
50-59 YEARS	2	4	6 (14.6%)
60-69 YEARS	3	1	4 (9.8%)
70-79 YEARS	1	0	1 (2.4%)
Total	**15 (36.6%)**	**26 (63.4%)**	**41 (100%)**

Though pyogenic granuloma seemed to occur more in females in most of the age groups, especially in the 2nd decade of life, no statistically significant difference was found between genders in the age groups (Fishers Exact = 6.10 df =7 p= 0.528). The 10-19 years age group had the highest proportion of cases (29.3%) followed by the 20-29 years age group (17.1%) (Table 1)

Table 2: Categories of protein expression

Proteins	Staining category	Pyogenic granuloma (50)
MMP2	-	0 (0%)
	+	8 (16%)
	++	42 (84%)
TIMP2	-	6 (12%)
	+	13 (26%)
	++	31 (62%)
RECK	-	10 (20%)
	+	11 (22%)
	++	29 (58%)

KEY- (Negative), + (Positive), ++ (Strongly positive)

Distribution of immunohistochemical staining intensity for MMP-2, TIMP-2, and RECK proteins in 50 cases of pyogenic granuloma. Staining was categorized into three levels: negative (−), positive (+), and strongly positive (++), based on the intensity and proportion of immunoreactive cells observed. All examined lesions demonstrated expression of MMP-2, with the majority showing strong positivity (84%) and the remaining cases showing moderate positivity (16%), while no cases were negative. For TIMP-2, most lesions were positive (88%), including 26% with moderate positivity and 62% with strong positivity, whereas 12% of cases were negative. RECK expression was observed in 80% of cases, with 22% showing moderate positivity and 58% strong positivity, while 20% of lesions showed no detectable staining. (Table 2)

Table 3: Semi quantitative expression of the proteins in pyogenic granuloma

MMP2	TIMP2	RECK	MMP2:TIMP2	MMP2: RECK
4.80 (SD±1.8)	3.54 (SD± 1.42)	3.50 (SD±1.61)	1.8:1 (SD±1.42)	2:1 (SD±1.55)

Table 3 shows that mean MMP2 score was higher than the mean scores of the inhibitors, TIMP2 and RECK. Both inhibitors of MMP-2 (TIMP-2 and RECK) had similar mean scores. It is also notable that the mean ratio of MMP-2 to its inhibitors was 2:1 in the case of RECK and nearing same for TIMP-2.

Table 4: Semi quantitative expression of the proteins in pyogenic granuloma by Gender (Sex)

Protein	Male (15)	Female (28)	Significance
MMP-2	5.40 ± 0.63	4.57± 1.32	0.010
TIMP-2	4.07± 0.88	3.21± 1.62	0.005*
RECK	3.53± 1.55	3.50± 1.69	0.571
MMP-2: TIMP-2	1.41± 0.40	2.19± 1.82	0.000*
MMP-2: RECK	1.96±1.16	2.04± 1.77	0.067

*- statistically significant

NB- 7 out of the 50 cases of pyogenic granuloma used for immuno- histochemical staining had missing records of gender. Hence only 43 cases were analysed for differences in gender expression of these proteins. Using the Independent Sample T test, a statistically significant difference was found between genders in the mean TIMP-2 and MMP-2: TIMP-2 ratio. This ratio was significantly higher in females (F =19.46 t = -1.63 df = 40.00 p= 0.000). While TIMP-2 had a significantly higher value in males than in females (F=8.91 t =1.89 df = 41.00 p= 0.005)-Table 4

Table 5: Pearson correlation analysis between MMP-2 and TIMP-2 expression scores

Variable	MMP-2	TIMP-2
MMP-2	1.000	−0.093
Sig. (2-tailed)	—	0.521
N	50	50
TIMP-2	−0.093	1.000
Sig. (2-tailed)	0.521	—
N	50	50

Pearson correlation analysis was performed to evaluate the relationship between MMP-2 and TIMP-2 expression scores in pyogenic granuloma specimens (Table 5). The analysis revealed a weak negative correlation between MMP-2 and TIMP-2 expression; however, the association was not statistically significant ($r(48) = -0.09$, $p = 0.521$).

Table 6: Simple linear regression analysis assessing the association between MMP-2 and RECK expression Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.02	0.00	−0.02	1.62

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	0.06	1	0.06	0.02	0.882
Residual	126.44	48	2.63		
Total	**126.50**	**49**			

Regression Coefficients

Predictor	B	Std. Error	Beta	t	Sig.
Constant	3.36	0.97	—	3.45	0.001
MMP-2	0.03	0.20	0.02	0.15	0.882

A simple linear regression analysis was further conducted to determine whether MMP-2 expression could predict RECK expression (Table 6). The regression model showed a very weak association between the variables ($R = .02$, $R^2 = .00$) and was not statistically significant ($F(1,48) = 0.02$, $p = .882$). Additionally, MMP-2 was not a significant predictor of RECK expression ($\beta = 0.02$, $p = 0.882$). Overall, these findings indicate that no statistically significant relationship exists between MMP-2 expression and the expression of its inhibitors TIMP-2 and RECK in the studied lesions.

DISCUSSION

In this study, pyogenic granuloma occurred more frequently in females (63.4%) than in males (36.6%), corresponding to a female-to-male ratio of 1.7:1. This female predominance was observed across most age groups, particularly in the 10–19 years group, although the difference did not reach statistical significance. The 10–19 years age group also accounted for the highest proportion of cases overall (29.3%), followed by the 20–29 years group (17.1%). These findings are consistent with prior reports demonstrating a higher incidence of pyogenic granuloma in young females, particularly during adolescence and early

adulthood, periods characterized by significant hormonal fluctuations. [36,37] The predilection for this age group suggests that hormonal status may contribute substantially to lesion development and progression.

The predominance of pyogenic granuloma in females during their second decade of life aligns with the proposed role of sex hormones, particularly estrogen and progesterone, in lesion pathogenesis.[36,37] Elevated hormone levels during puberty and reproductive years are believed to increase vascular permeability and stromal edema, facilitating a mixed inflammatory infiltrate that promotes vascular proliferation.[37] The interplay between hormonal signaling and the local microenvironment may potentiate the rapid growth of capillary networks characteristic of these lesions. Contemporary studies suggest that the dynamic balance between hyperplastic capillary vessels and the fibromyxoid stroma is further regulated by enzymatic activity and local growth factors, which collectively modulate angiogenesis and connective tissue remodeling. [38,39]

Matrix metalloproteinases (MMPs) are key mediators of extracellular matrix turnover and vascular remodeling. Although studies specifically examining MMP-2 and its inhibitors in pyogenic granuloma are limited, their broader role in oral inflammatory conditions is well documented. Collagenase activity, including human collagenase-2 (MMP-8) and MMP-9, is elevated in inflamed gingival tissues and crevicular fluid during periodontal disease, and MMPs are similarly upregulated in inflamed pulp and periapical lesions. [29, 31] In pyogenic granuloma, a lesion with histological features of both reactive and reparative tissue, MMPs are expressed by multiple cellular components, including fibroblasts, basal keratinocytes, and infiltrating inflammatory cells, highlighting their central role in tissue remodeling. [40-42]

In the present study, all pyogenic granuloma lesions stained positive for MMP-2, while the majority expressed TIMP-2 (88%) and RECK (80%). Semi-quantitative analysis revealed that the mean MMP-2 score exceeded those of its inhibitors, TIMP-2 and RECK. The mean MMP-2 to inhibitor ratio was approximately 2:1 for RECK and similar for TIMP-2, suggesting that MMP-2 activity predominates, potentially driving the prominent angiogenesis observed in these lesions. Notably, the mean MMP-2: TIMP-2 ratio was significantly higher in females, which may reflect synergistic effects of hormonal influences and proteolytic activity on vascular proliferation. Conversely, TIMP-2 expression alone was significantly higher in males, possibly representing a compensatory upregulation aimed at counterbalancing excessive MMP-2 activity.

While TIMP-2 is classically an inhibitor of MMP-2, it can paradoxically facilitate MMP-2 activation when expressed at a 1:1 ratio and may also possess independent angiogenic activity.[21, 42, 43] This dual role may explain why angiogenesis persists despite substantial TIMP-2 expression. RECK, a membrane-anchored glycoprotein, exerts inhibitory control over MMP-2, and its expression in pyogenic granuloma may function primarily to restrain excessive proteolytic activity. The limited literature on RECK in these lesions, however, restricts comprehensive understanding of its broader regulatory roles, highlighting a critical area for future investigation. The disproportionate MMP-2 to inhibitor ratio observed in this study provides a mechanistic explanation for the pronounced angiogenic phenotype of pyogenic granuloma, particularly in females. The elevated MMP-2 activity may drive the proliferation of capillaries and stromal remodeling, while the relative insufficiency of inhibitors such as TIMP-2 and RECK allows continued extracellular matrix degradation and vascular expansion. These findings are consistent with previous reports linking MMP-2 overexpression to angiogenesis in other reactive and inflammatory oral lesions. [44,45]

Pearson correlation and regression analyses were performed to explore the relationships between MMP-2 and its inhibitors, TIMP-2 and RECK, in pyogenic granuloma. A weak negative correlation was observed between MMP-2 and TIMP-2 expression ($r = -0.093$), which was not statistically significant. Similarly, simple linear regression analysis showed that MMP-2 expression was not a significant predictor of RECK expression, with the model explaining virtually none of the variance. These results suggest that, within the studied lesions, MMP-2 expression appears largely independent of the levels of its classical inhibitors, TIMP-2 and RECK, highlighting the potential involvement of additional regulatory mechanisms or signaling pathways in controlling MMP-2 activity and angiogenesis in pyogenic granuloma. Beyond the MMP-2/TIMP-2/RECK axis, the pathophysiology of pyogenic granuloma is likely influenced by additional, yet unidentified, signaling pathways and local factors. The interaction between hormonal status, inflammatory mediators, and matrix remodeling enzymes underscores the complex, multifactorial nature of lesion development. Understanding these interactions may inform potential therapeutic interventions, particularly in recurrent or rapidly proliferating lesions.

Strengths and Limitations:

This study has several notable strengths. First, it provides a comprehensive analysis of MMP-2, TIMP-2, and RECK expression in pyogenic granuloma, integrating semi-quantitative protein expression with demographic and clinical patterns. The inclusion of both male and female patients across multiple age groups allowed assessment of gender- and age-related

differences in enzyme expression. Additionally, the study preserved the histopathological context by correlating enzyme expression with the reactive and angiogenic features of the lesion, strengthening the biological relevance of the findings. Several limitations should be acknowledged. The study is cross-sectional and observational in nature, which limits causal inferences regarding the role of MMP-2 and its inhibitors in lesion development. The sample size, while sufficient for semi-quantitative analysis, may be underpowered to detect subtle differences between subgroups, particularly in age-stratified comparisons. Additionally, the study focused on protein expression without assessing functional enzymatic activity or downstream signaling pathways, which may limit mechanistic interpretation. The paucity of comparative studies on RECK in pyogenic granuloma also constrains the broader generalizability of findings related to this inhibitor.

Future Directions:

Future research should aim to expand on these findings using larger, multicenter cohorts to validate the observed demographic and enzymatic patterns. Functional studies assessing MMP-2 activity and its regulation by TIMP-2 and RECK in vitro and in vivo would provide mechanistic insights into angiogenesis and tissue remodeling in pyogenic granuloma. Additionally, exploration of hormonal interactions, inflammatory mediators, and other signaling pathways may clarify the multifactorial drivers of lesion growth and recurrence. Investigating potential therapeutic strategies that target the MMP-2/TIMP-2/RECK axis could offer novel approaches for managing lesions with aggressive angiogenic behavior or high recurrence rates.

CONCLUSION

In conclusion, our findings confirm the pivotal role of MMP-2 in mediating angiogenesis in pyogenic granuloma, with TIMP-2 potentially contributing to MMP-2 activation rather than solely inhibition. RECK appears to serve as a classical inhibitor of MMP-2, though its regulatory complexity may be less than that of TIMP-2. The disproportionate MMP-2 to inhibitor ratio, particularly in females, aligns with the observed demographic and clinical patterns of the lesion. These data reinforce the importance of proteolytic regulation in lesion pathogenesis and highlight the need for further research into additional modulators and signaling pathways that govern MMP-2 activity in pyogenic granuloma.

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