Comparative Analysis of Mast Cell Density in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is one of the most common malignancies of the oral cavity. One of the foremost cells recruited near the tumor front is the mast cells. Mast cells produce angiogenic mediators such as fibroblast growth factor (FGF), transforming growth factor (TGF), tumor necrosis factor, (TNF), and the vascular endothelial growth factor (VEGF) [1.] which are increased in precancerous lesions and found in more abundance in OSCC.

Accumulation of mast cells around the tumor margins and their release of potent pro-angiogenic and angiogenic factors may represent a tumor-host interaction which probably favors tumor progression. The progression of oral lesions from dysplasia to oral squamous cell carcinoma is characterized by an "angiogenic switch" that is associated with an increase in the neovascularization of the subepithelial lamina propria, which may be considered an indicator of malignant transformation. [2]

MCs also represent a rich source of proteases, especially ofmast cell tryptase and chymase, which directly degrade the extracellularmatrix through their proteolytic activity and thus indirectly stimulate angiogenesis and facilitate invasion and metastasis. Theliterature has proven that mast cells can be an indicator of increased angiogenesis and hence can help in the prediction of carcinogenesis, its progression, and also the prognosis of the malignant lesions.

Aim of the Study: To compare the number, morphology, and distribution of mast cells in different grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) and to study their role in tumor growth and pathogenesis

Materials and Methods: A total of 39 cases, which included 13 cases of dysplasia and 26 of OSCC were included in the study. Tissue sections were stained with H&E and 1% toluidine blue was used to evaluate mast cells. Mast cells were counted manually using an ocular grid throughout the tissue sections in 10 representative grid fields in a stepladder fashion. (40x magnification).The mean mast cell density (MCD) of 10 fields was calculated and was expressed as mean (standard deviation) per $mm^{3,4}$. Mast cells were then categorized as typical, atypical, or granular mast cells and statistically analyzed.[5]

Result: In the present study, there is a decrease in the number of mast cells with severe grades of dysplasia and poorer grades of oral squamous cell carcinoma.

Conclusion

Mast cells may induce the tumor progression by providing a mitogenic stimulation or angiogenesis-the hallmark of the tumor growth and metastasis through the release of various mediators.

Keywords: *Mast cell, Mast cell density, Dyspalsia, Oral squamous cell carcinoma,Toludine blue*

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80-90% of all malignant neoplasms of the oral cavity. Major etiological and predisposing factors for OSCC mostly include smoking and alcohol consumption, and ultraviolet radiation (specifically for lip cancer), but several other factors such as human papillomavirus (HPV) and *Candida* infections, nutritional deficiencies, and genetic predisposition have been also associated .[6]

Angiogenesis, the growth of new vessels from existing one is a complex phenomenon which is required for growth and survival of neoplasm. Tumour angiogenesis is mediated by angiogenic factors released by cancer cells, and or by host immune cells. Among the host immune cells, mast cells play an important role in tumor progression via promoting angiogenesis.[7]

Mast cells are mobile secretory cells that demonstrate metachromatic granules and are distributed around the microvascular endothelium in the oral mucosa and dental pulp. They have diverse biological functions which include phagocytosis, antigen processing, production of cytokines, and release of a variety of preformed (e.g., histamine, proteoglycans, and proteases) and newly formed physiological mediators (e.g., leukotrienes and prostaglandins).[4]

They exert their influence locally and systemically by releasing a variety of potent chemicals like histamine, leukotrienes, and cytokines through degranulation and induce neovascularization through angiogenic mediators such as fibroblast growth factor (FGF), transforming growth factor-β (TGF), tumor necrosis factor-α (TNF), and vascular endothelial growth factor (VEGF).[8]

Mast cells are attracted to the lesion site and may turn on an angiogenic switch during tumorigenesis in OSCC. The present study aimed to compare the number, morphology, and distribution of mast cells in different grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) and study their role in tumor growth and pathogenesis.

Materials and Methods

A total of 13 cases of dysplasia (mild, moderate & severe dysplasia) and 26 cases of OSCC (well, moderate and poorly differentiated OSCC) were considered from the archival collection of the Department of Oral Pathology & Microbiology, MCODS Mangalore.

After obtaining the clearance from the Institutional ethical committee, tissue sections from the selected cases were taken and were stained with H&E to confirm the diagnosis while 1% toluidine blue staining was used to evaluate mast cells. Toluidine blue stains mast cell granules purplish-red and nucleus sky blue color. Mast cells were counted manually using an ocular grid under 40x magnification in a stepladder fashion.[Fig 2] The mast cells were counted throughout the tissue sections in 10 representative grid fields (40x magnification). The mean of 10 values was calculated and was expressed as mean (standard deviation) per mm.[3,4]

The following criteria were used to study the morphology of mast cells: [9] [Fig 1]

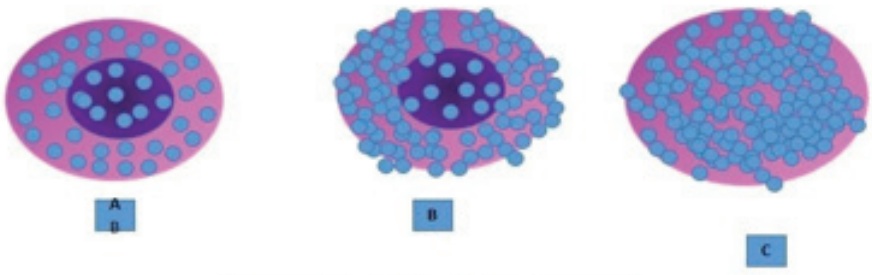


Fig 1: Morphology of mast cells

· Typical (TMC): When the cytoplasmic membrane, the nucleus, and granules of cytoplasm were seen.[A]

· Atypical (AMC): When the nucleus and the granules were seen but the cytoplasm is not defined.[B]

· Granular (GMC): The presence of three or more aggregations of granules from degranulated AMC's without the presence of their nucleus.[C]

Statistical Analysis: The mean mast cell density (TMC, AMC, GMC) was compared between the six groups of dysplasia and OSCC using *One* – way analysis of variance (ANOVA) followed by Post-Hoc Tukey's for group-wise comparisons. A *p*-value of 0.05 or less was considered for statistical analysis.

Results

In the present study mast cells were present in both dysplasia and oral squamous cell carcinoma, the total mast cells decreased with increased grades of dysplasia (40, 38, and 33). The total number of mast cells was increased in mild dysplasia and mast cells were least in poorly differentiated oral squamous carcinoma which showed statistically significant. [Table 1]

OED with mild dysplasia showed the highest number of TMC moderate dysplasia showed an increased number of AMC (18) and severe dysplasia showed a predominance of GMC.[Table 2]

In OSCC cases with well-differentiated OSCC showed a higher proportion of TMC (24.5), moderately differentiated OSCC showed an increased number of AMC (31.86), and poorly differentiated OSCC showed a greater number of GMC (31.92). All these changes were statistically significant.

ROC curve analysis was performed with total mast cells, Typical Mast cells, Atypical mast cells, and Granular mast cells to predict survival. It was found that granular mast cells had the highest area under the curve with a value of 0.896 (95% CI of 0.694-1.000). The other parameters the total mast cells (AUC, 0.250), typical mast cells (AUC 0.271), or atypical mast cells (AUC 0.021) had a lower area under the curve values indicating poor prediction. On the assessment of the coordinates of the cutoff for granular mast cells, it was found that at a cutoff of 13.5 there was 100% sensitivity and 75% specificity.

A Kaplan Meier survival analysis was done using a cutoff of 13.5 of GMC. Both the cases of death had a higher GMC value and survival time of <12 months. This comparison had a chi-square value of 3.635 and a p-value of 0.057. [Table 4]

TABLE 1: Mast cells in oral dysplasia and squamous cell carcinoma

		N	Mean	Std. Deviation	F	P VALUE
TOTAL MAST CELLS	MILD DYSPLASIA	5	40.80	6.301	7.588	0.003
	MODERATE DYSPLASIA	4	38.50	4.359		
	SEVERE DYSPLASIA	4	33.00	7.394		
	WELL DIFFERENTIATED SCC	6	41.83	10.048		
	MODERATELY DIFFERENTIATED SCC	7	57.43	30.506		
	POORLY DIFFERENTIATED SCC	13	27.23	4.024		
	Total	39	38.38	17.041		

Cont... **TABLE 1: Mast cells in oral dysplasia and squamous cell carcinoma**

TYPICAL MAST CELLS	MILD DYSPLASIA	5	21.80	5.119	15.532	<0.001
	MODERATE DYSPLASIA	4	11.75	1.708		
	SEVERE DYSPLASIA	4	10.25	14.221		
	WELL DIFFERENTIATED SCC	6	24.50	11.397		
	MODERATELY DIFFERENTIATED SCC	7	12.14	3.185		
	POORLY DIFFERENTIATED SCC	13	4.46	2.696		
	Total	39	12.49	9.838		
ATYPICAL MAST CELLS	MILD DYSPLASIA	5	13.80	4.604	7.437	0.003
	MODERATE DYSPLASIA	4	18.00	2.449		
	SEVERE DYSPLASIA	4	8.25	6.076		
	WELL DIFFERENTIATED SCC	6	11.50	4.231		
	MODERATELY DIFFERENTIATED SCC	7	31.86	17.846		
	POORLY DIFFERENTIATED SCC	13	8.85	3.412		
	Total	39	14.90	11.623		
GRANULAR MAST CELLS	MILD DYSPLASIA	5	5.00	3.162	8.006	0.002
	MODERATE DYSPLASIA	4	8.75	.957		
	SEVERE DYSPLASIA	4	14.50	6.557		
	WELL DIFFERENTIATED SCC	6	5.83	3.312		
	MODERATELY DIFFERENTIATED SCC	7	13.43	12.421		
	POORLY DIFFERENTIATED SCC	13	13.92	3.121		
	Total	39	10.97	6.941		

TABLE 2: Types of mast cell in oral dysplasia and oral squamous cell carcinoma

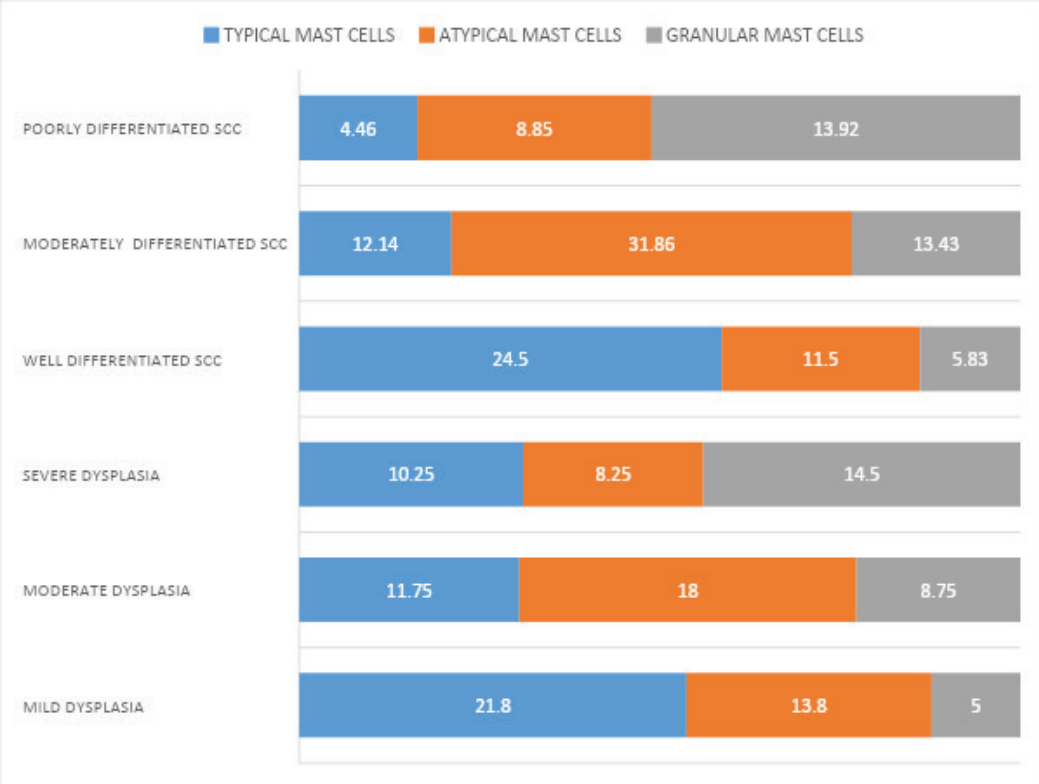


TABLE 3: ROC curve

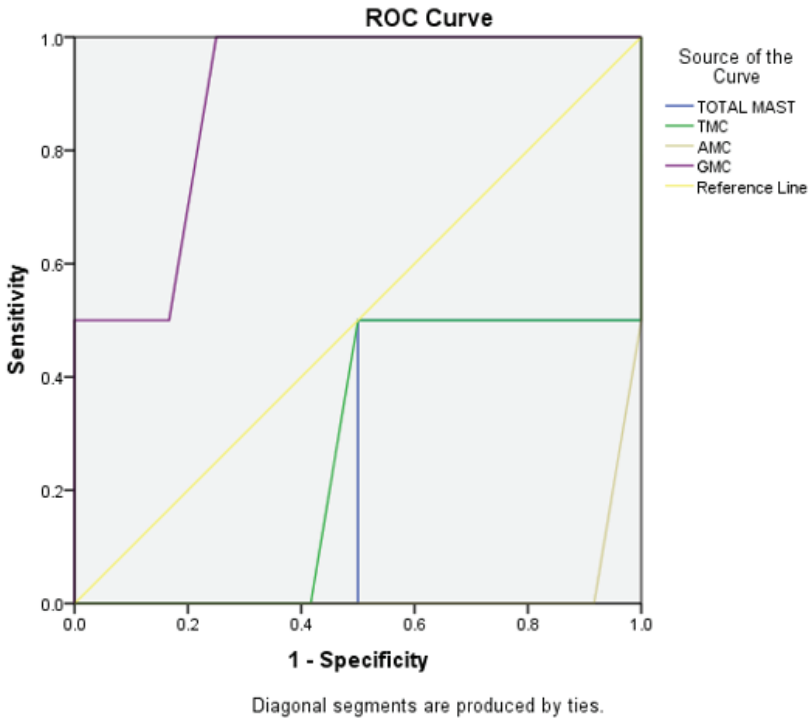
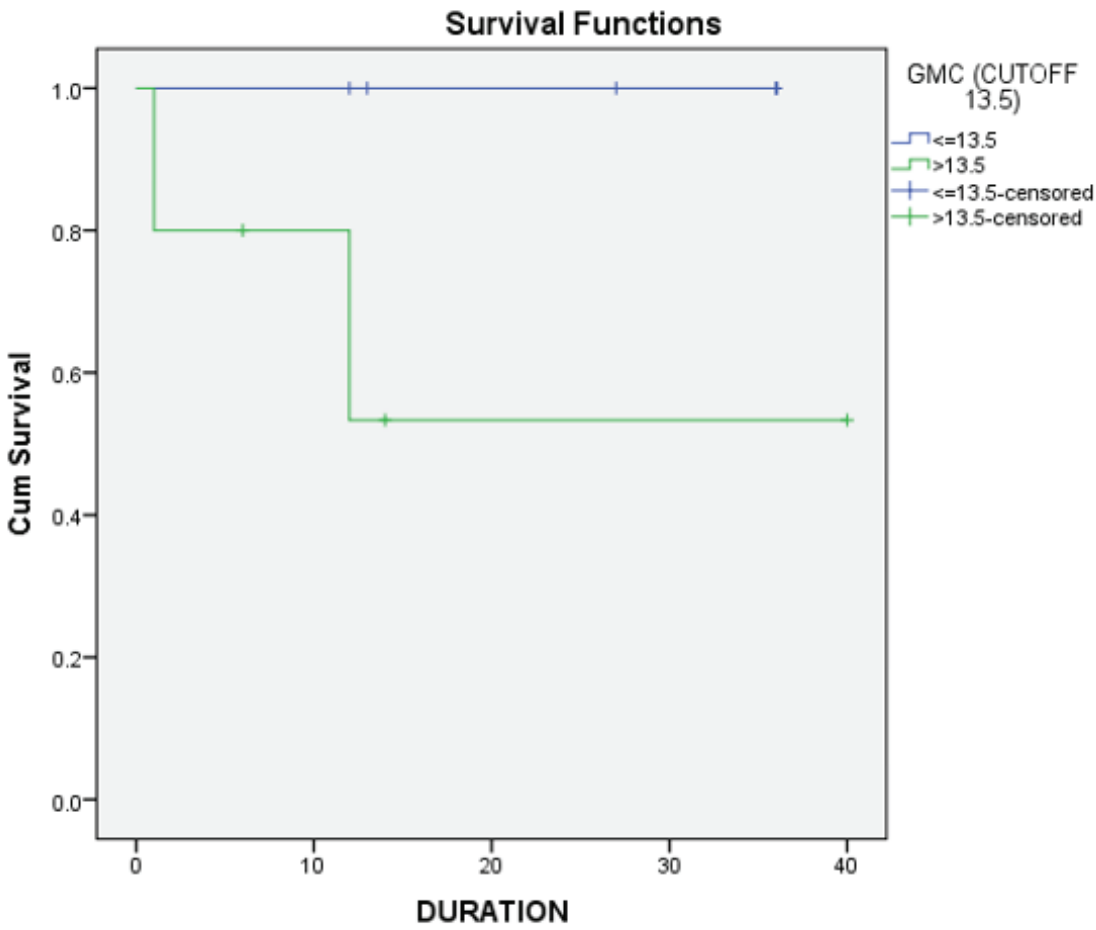


TABLE 4:Survival analysis



Discussion

A mast cell is a type of white blood cell which is a granulocyte derived from the myeloid stem cell which is a part of the immune systems. Mast cells contain many granules rich in histamine heparin and serotonin. They are best known for their role in allergy and anaphylaxis. Additionally, they are also involved in wound healing, angiogenesis, immune tolerance, defense against pathogens, and blood-brain barrier function. Although there are various immunohistochemical markers available for the detection of both OSCC and dysplasias search is still on for cheaper and more widely used methods such as special stains, for example, toluidine blue and crystal violet. Mast cells are easily seen under light microscopy by toluidine blue stain because of the metachromatic granules that fill the cytoplasm.

The pharmacologically active agents in the mast cell granules most likely contribute to the inflammatory reaction seen in epithelial dysplasia. The mast cell degranulation releases IL-1 which may cause increased epithelial proliferation and increased lymphocytic and plasma cell infiltration as seen in dysplasias. Histamine which is released causes increased mucosal permeability and allows the antigens to reach into the underlying connective tissue. Heparin further causes endothelial cell proliferation and migration which results in increased vascularity of the stroma and in epithelial ulceration.[10]

According to Ali Tahir et al [8] mast cells secrete many pro-angiogenic factors such as angiopoietin -1, vascular endothelial growth factor (VEGF), basic Fibroblast Growth Factor (bFGF), Monocyte Chemoattractant

Protein-4 (MCP-4) (chymase), and histamine and has stated that there is evidence of the pro-angiogenic and thus pro-tumor role of mast cells. VEGF is significantly increased in premalignant and invasive oral lesions. Mast cells also release potent angiogenic factors like tryptase which play a significant role in angiogenesis leading to invasion and distant metastasis of OSCC.[11]

The present study was done to assess qualitative and quantitative measurements of mast cells in OED and OSCC and also the role of mast cell number and type in the etiopathogenesis of OED and OSCC. The mean number of mast cells in mild dysplasia was 40.80 in moderate dysplasia was 38.50 and in severe dysplasia was 33. Therefore with an increase in grades of dysplasia, there was a decrease in the number of mast cells.

Ankle et al [7] in his study concluded that biologically and pharmacologically active agents present in the mast cells might contribute to inflammatory reaction seen in leukoplakia. Interleukin-1 contributed to an increase in epithelial proliferation while the release of proangiogenic factors such as histamine, heparin, chymase, VEGF increases the density of microvessels with mild, moderate, or severe dysplasia and OSCC.[9]

Mast cells associated with the tumor have been found to undergo degranulation releasing granular components such as heparin and histamine, which potentiate endothelial cell migration and proliferation and induce adhesion molecule expression on epithelial cells, potentially leading to increased tumor metastasis. Matrix metalloproteinases are also produced by mast cells and may contribute to extracellular matrix degradation. Thus, mast cells may have an impact on both primary tumor development and subsequent tumor progression and metastasis.[3]

Veda Hegade suggested that hypoxia might induce tumor cells to release angiogenic factors which in turn chemoattract mast cells to migrate into the hypoxic areas of the tumor. After migration into the hypoxic areas, mast cells might produce stimulating factors that help further angiogenesis. These angiogenic factors secreted by mast cells either directly promote angiogenesis by stimulating the migration or proliferation of endothelial cells or indirectly through degradation of the extracellular matrix.[6]

Tumors that are rapidly growing may have a high nutritive demand that is provided by the vasculature. When the epithelium is altered as in oral epithelial dysplasia, recruitment of inflammatory cells is seen. The inflammatory and mast cells that migrate to areas of the altered epithelium may stimulate angiogenesis by secreting proangiogenic and angiogenic components either directly or indirectly before the invasion. Once the invasion is established as in OSCC the role of mast cells is probably shifted from angiogenesis to further promoting invasion which might correlate with the decreased mast cell density at the primary tumor site.

Our study showed there was a decrease in the number of mast cells in poorly differentiated OSCC when compared with well-differentiated OSCC which was similar to the findings done by Veda Hegade. Poorly differentiated OSCC and moderately differentiated OSCC are known to be more proliferative and invasive where mast cells may have the dual role of promoting angiogenesis and invasion and the cytotoxic function of mast cells may be too ineffective in such situations.

Mast cells had a role in modifying the stroma for the invasion in OSCC as mast cells either promote or inhibit tumor growth either alone or in association with other cells in the microenvironment and exert an effect on altered tissue. An increased number of TMC's in OED is less inflamed area Increased number of AMCs was observed in OED and OSCC with moderate inflammatory infiltrate. Increased number of AMC's and GMC's in more inflamed areas contribute to the active participation of mast cells in various phases of the inflammatory process manifested by their degranulation.

Mast cells exhibit one of two predominant phenotypes, as defined by staining characteristics and granule enzyme content. Most mast cells in connective tissue sites stain intensively with dyes, probably due to their heparin content. [11]

Tryptase, the most abundant secretory granule-derived neutral serine proteinase contained in mast cells, can degrade components of the extracellular matrix and has been used as a specific marker for mast cells. Tryptase+ mast cells are often among the first

immune cells recruited to tumor sites in response to the chemotactic stimuli and increase in solid tumors. in the study conducted by ***Guoming Hu Etal*** [9] where there was a close association between increased tryptase+ mast cells and decreased survival of patients. He suggested that: tryptase, stored in the secretory granules pre-formed active serine proteases of tryptase+ mast cells, can stimulate the proliferation of endothelial cells, promote the formation of vascular tubes *in vitro*, and also activate plasminogen activator (PA), which induces the release of vascular endothelial growth factor (VEGF) or fibroblast growth factor-2 (FGF-2) from their extracellular matrix-bound state, and is angiogenic *in vivo* in the chorioallantoic membrane (CAM) assay.

Thus mast cells either promote or inhibit tumor growth either alone or in association with other cells in the microenvironment and exert an effect on the altered tissue. Thus mast cells play an important role in mediating the cross-links between the external angiogenic agent and local immunologic factors. Mast cells may induce tumor progression by providing a mitogenic stimulation or angiogenesis-the hallmark of the tumor growth and metastasis through the release of various mediators.

The increase in mast cell density is associated with poor prognosis in different malignant tumors indicating their role in tumor progression. recent studies state that mast cell accumulation at the periphery at the tumor areas and the release of proangiogenic factors may represent a tumor-host interaction that probably favors tumor progression.[12]

Conclusion

Thus mast cells either promote or inhibit tumor growth either alone or in association with other cells in the microenvironment and exert an effect on the altered tissue.

Thus mast cells play an important role in mediating the cross-links between the external angiogenic agent and local immunologic factors. Mast cells may induce tumor progression by providing a

mitogenic stimulation or angiogenesis-the hallmark of the tumor growth and metastasis through the release of various mediators.

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Conflict of Interest - Nil

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