Mast cell, IL-1 Beta and IL-6 for Wound timing and Vitality in Forensic Practice

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Abstract

the application of immunohistochemistry has opened a new era for examining the age of the wound by forensic specialists, The aim of our study was to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase "MCT") as a reliable and promising factor of wounds vitality and to Identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination. The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. The specimens were sera and skin tissues for immunohistochemistry examination, and staining procedures and immunohistochemistry (MCT) ELISA sandwich plate will measure the levels of interleukins. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) .We found that density of Mast cells in sample group was significantly high and higher infiltration that correlates with time passing in the study group as compared by other group in dermis of the sample and control lesions and positive relationship between levels of IL 1-beta & IL-6 level and time progress of wound..

Key words: *forensic, pathology, wound, vitality, IL 1-beta, IL-6 level, Mast cells*

Introduction

During the inflammatory phase, a variety of chemicals are released at the affected site, leading to the recruitment of inflammatory cells, such as neutrophils and plaques Forensic scientists, unlike general pathologists, tend to focus on chronic mapping of the appearance and disappearance of inflammatory cells or substances produced during the inflammatory process[1]. These phenomena - for example, the proportion of positive cells, the level of tissue fibrosis, the distance between inflammatory cells and the free vessels that affected by the degree of injury, which affect the accuracy of the determination of the age of the wound. It is therefore necessary to establish models with varying degrees of injury and to evaluate the parameters involved in wound healing to determine the time of injury[2].

Thus, MCs have been involved in causing many chronic allergic / inflammatory disorders, autoimmune diseases, and cancers. The contributions of MCs in these conditions are the subject of continuous assessment[3]. Acute direct events, the allergic process involves subsequent stages characterized by leukocytes infiltration and initiation of an acquired immune response, followed by a chronic phase involving persistent inflammation, tissue reformation and fibrosis. Thus, the role of MCs at these different stages has gained increasing importance. The dissociation of MCs, in addition to their well-established and widely studied role in IgE-mediated interactions, has been the focus of MCs research in the past decades. However, the determination of the functions of MCs may progress slowly due to difficulties in accessing these cells in vivo and the obstacles encountered when obtained through enzymatic dispersion of tissues or by culture of predators of MCs isolated from the bone marrow or cord or peripheral blood. The culture of predators of mast cells produces a small number of MCs that are often expensive and time-consuming and lead to the emergence of changing phenotypes due to cultural conditions[4].

Several studies reported high levels of IL-1β production by nuclear monocyte, platelet and nuclei cells from active colon lesions in IBD (inflammatory bowel disease) patients. Since IL-1RA levels are only moderately controlled in the colon in patients with IBD, the IL-1RA ratio to IL-1β decreases significantly,

enhancing intestinal inflammation[5]. IL-1β is initially produced as zymogen, but pro-IL-1β does not contain any known functional properties. IL-1β copies are tightly regulated, stimulated by stimulation by TIR or IL-1R stimulation (the first signal required to produce IL-1β) As previously described, IL-1β precursors are bisected in its active form by caspase-1, after inflammatory activation with the NLR (NOD like receptor: NOD for nucleotide-binding domain, Lucien rich repeat containing receptors) pathway (signal 2) [6]. The secretion mechanisms of IL-1β and IL-18 in extracellular space are unclear, but proposed pathways include exocytosis of secretory lysosomes, shedding of membrane micro-vesicles or exososomes, or transfer by membrane vectors, such as ASC (alanine serine cysteine amino-acids transport system) transporters[7].

IL-1β practices a wide range of systemic and local effects. Once in circulation, IL-1β can promote the synthesis of ring-2 oxidation enzymes (COX2) in the vascular network, which stimulates the production of prostaglandin E2 in the brain and fever-mediated. IL-1β also promotes the synthesis of acute phase proteins by liver cells, derives differentiation of macrophages, differentiation of neutrophils and mobilization in bone marrow. Furthermore, IL-1β promotes the recruitment of immune cells to inflammatory sites, by stimulating the expression of adhesion molecules and chemical attractors via endothelial cells[8].

On the other hand Given the IL-6 behavior of restoring the host to the parity state, it is clear that IL-6 acts to control the response of tissue inflammation. In chronic diseases, which are usually manifested by immune stress factors such as intracellular infection and chronic tumors, IL-6 acts not only as a catalyst for acute phase reactions but also as an important player in cellular immune response responses to infected cells and mucosal- The inflammatory reaction is acute, resulting in the destruction of a harmful agent within a short period of time and in a localized area, stimulating an immune response.[8]

IL-6 not only stimulates acute phase reactions but also develops cellular immune responses, including end-stage B cell differentiation, immune globulin secretion and T-cell activation. The main key from acute inflammation to chronic inflammation is the recruitment of monsters to the inflammation area. IL-6 is important for the transition between acute and chronic inflammation. IL-6 plays a somewhat unexpected role in recruiting white cells in vivo. The IL-6 and sIL-6Rα compounds can activate endothelial cells to secrete IL-8 protein and MCP -1, and urge the expression of adhesion molecules[9].

The aim of the study is to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase “MCT”) as a reliable and promising factor of wounds vitality and to identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination.

Methodology

The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. Lacerated skin wounds with a known timing since injury less than 12 hours, regarding time of autopsy all cases were underwent autopsy as soon as possible within a maximum time of 3 hours since arrival to mortality. Only frank lacerated non-contaminated skin wounds were taken in account and the autopsy was done immediately on arrival to MLD. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) [stab-wounds injuries with immediate death]. The specimens are sera and skin tissues for MCT immunohistochemistry.

ELISA sandwich plate will measure the levels of interleukins and specimens for histopathology will be taken from the periphery of wounds for SG and an intact healthy skin from same cadaver (internal control) and some tissue specimens from deaths due immediate death by bullet injury as (external control), stored in 10% formalin for staining procedures and immunohistochemistry (MCT) and the results was analyzed statistically.

Strict exclusion criteria for cases of study include mixed wounds, wounds with unknown timing since injury, contaminated lacerated wounds, wounds with expected timing less than 12 hours, firearm deaths, cases with a known documented history of chronic illnesses, age less than 15 and more than 35 years, pregnant, decomposed cadavers (delayed autopsy),major medical interventions like surgery.

The Quantikine® Human IL-1β/IL-1F2 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human IL-1β in cell culture supernates, serum, and plasma The Quantikine® Human IL-6 Immunoassay is a 4.5 hour solid phase. Both contain E. coli-expressed recombinant human IL-1β and IL 6

and antibodies raised against the recombinant factors.

TB staining was used for presence of "Mast cells" and MCT immunohistochemistry by Elabsceince® is the preferable way for detecting mast cells activation.

Results

The number of wounds in the study sample was determined; most of the bodies had three wounds, 44% of the total study, where 6% of the cases had only one wound. The study group of 156 laceration skin wounds was classified into compression laceration (50%), grinding laceration (20.5%), cut laceration (6.5%), tearing (15.3%) and crush injuries (7.7%)

Mast cells by toluidine blue stain

Mast cells are found in connective tissue and the cytoplasm contains **(heterogeneous)** granules consisting of **heparin and histamine**. Toluidine blue stains mast cells with red-purple color **(metachromatic staining)** and blue background **(orthochromatic staining)** Metachromasia and tissue color staining elements differ according to the features of the dye solution due to the pH, dye concentration and temperature of the underlying dye. Blue or purple dyes will show a red shift while red dyes will show a yellow shift with contrasting tissue elements.

Depending on the number of mast cells stained by toluidine blue, three classes of were estimated, from 2 to 5 cells on 10 HPF the number of mast cells stained was regarded **normal finding**, from 6-8 cells on 10 HPF (+1) and from 9-10 cells on 10 HPF and (+2) and > 10 cells on 10 HPF (+3) The specimens included in the study did not have any histologic abnormality. Using light microscopy, all the mast cells (MC) in the specimens (however fixed) had meta-chromatically stained purple with toluidine blue. The nuclei of all the cells were round or oval. MC were scattered in the dermis, especially along the blood vessels and in the peri-glandular stroma. In the dermis of the Sample and control lesions, Mast cell density in the Sample group was significantly higher (P<0.001) when compared with the other group.

Table 1: Distribution of mast cells

Immunohistochemistry (Mast cells tryptase) for both samples and control

Immunohistochemistry of Mast Cell Tryptase results were recognized and classified into 4 classes according to the mast cells in both control and samples through the period of 6 hours after injury. Tryptase immune reactivity was detected in all of the cells stained by fluorescent avidin fixed either with Bouin or Carnoy's fluid. Some MC, in the dermis of all the examined groups, was immune stained for chymase upon fixation with Carnoy's fluid.

Table 2 correlation between test group and control group number of mast cells

ELISA levels of IL-1 beta and IL-6 in the human model

IL-1 beta standard of the kit measures at 3.9 pg/ml and minimum detectable dose (MDD) of human IL-1 beta is less than 1 pg/ml. The levels of IL-1 beta in the human model shows a significant decrease in relation to the time of the test (1 to 6 hours AMI), as showed in table 2 that the levels of IL-1 beta in the human model samples shows a maximum mean difference of 40.727 at 1 hour of AMI while the control group mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours. The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model.

Table 3: The levels of IL-1 beta in the *h*uman *m*odel

H1, after one hour AMI H4, after four hours AMI c, control

H2, after two hours AMI H5, after five hours AMI

H3, after three hours AMI H6, after six hours AMI

IL-6:

IL-6 standard of the kit measures at 3.13 pg/ml and the MDD of human IL-6 is typically less than 0.70 pg/ml. The IL-6 levels show **increase** with the time of **AMI** progress. The control samples show mean difference of .57436. The sample group shows maximum mean difference of IL-6 level of 353 at five hours of AMI.

Table 4 : The levels of IL-6 in the human model

One-Sample Test						
	Test Value = 0					
	t	df	Sig.	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
c	5.980	10	.000	.57436	.3603	.7884
h1	1.127	10	.286	269.933	263.73	803.59
h2	15.359	7	.000	2.16250	1.8296	2.4954
h3	13.272	9	.000	1.40290	1.1638	1.6420
h4	9.805	5	.000	1.89883	1.4010	2.3966
h5	1.006	3	.389	353.03625	763.9650	1470.0375
h6	6.926	4	.002	.63980	.3833	.8963

H1, after one hour AMI H4, after four hours AMI c, control

H2, after two hours AMI H5, after five hours AMI

H3, after three hours AMI H6, after six hours AMI

Discussion

In this study we found that density of Mast cells in sample group was significantly high (P<0.001) as compared by other group in dermis of the sample and control lesions. This result goes with the results found by Bonilli et al. [10] in the contrary to[11] who noted no significantly different mast cells' number of in normal cutaneous tissue & those in wounds of the sampling which could be explained partly by different techniques & morph-metrical methodology.

In this study we found higher infiltration with mast cells that correlates with time passing in the study group which agrees with the results found by [10] who utilized anti-tryptase & chymase antibodies or avidin to assess density of mast cells in skin wounds through immunofluorescences And found that the MC number in the dermis showed progressive increase within little hours after injury (top at 1 to 3 hours) Matching results was found by [12] who noted a powerful extra-expression of tryptase located in interstitium.

Levels of IL-1 beta in the human model samples show a maximum mean difference of 40.727 at 1 hour of AMI while in control group, the mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours .The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model. This results matches with [13]who found IL-1β, expressed in normal human skin modified in a significant way in vital injuries in epidermal strata, sub-epidermal cellular, vascular and glandular (sweat) and had promoted expression after fifteen and twenty minutes at early increase of reactivity of the epidermis & following thirty to sixty minutes, marked expression was noted, remained many hours & later decreased to a base level again. He commented that it can help as a beneficial method to estimate vitality and aging of wounds, especially during early post-traumatic period before the leukocytic reactions. On another hand this is against what was found by [14] who stated that no wound groups showed a high increase in IL-1b level in comparison to control group. The difference was not significant from statistical view.

In our study the IL-6 levels shows increase with the time of AMI progress which matches with what was found by [15] and the same results was obtained by[16]

Conclusion

Mast cells by toluidine blue stain density in the sample group was significantly higher when compared with the other group in the dermis of the sample and control lesions. The same was shown by Immunohistochemistry (Mast cells tryptase) as there were higher infiltration with mast cells that correlates with time passing in the study group. Regarding ELISA levels of IL-1 beta and IL-6 in the human model the study found a reverse relationship between AMI time and the levels of IL-1 beta in the human model while the IL-6 levels shows increase with the time of AMI progress.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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