Introduction

Multiple myeloma (MM) is a plasma cell dyscrasia that accounts for approximately 10% of all hematologic cancers. The disease is characterized by clonal expansion and accumulation of terminally differentiated B-cells (post germinal centre lymphocytes) in which chromosomal translocations frequently place oncogenes under the control of immunoglobulin enhancers. Mounting evidence indicates that the bone marrow (BM) microenvironment of tumor cells has a pivotal role in myeloma pathogenesis ***(Palumbo et al., 2010).***

Plasma cell infiltration of bone marrow results in the heterogenous clinical manifestations of MM including anemia, impaired hematopoiesis, bone lesions and hypercalcemia. High serum paraprotein may result in hyperviscositywhile high levels of light chain (Bence Jones proteins) in the urine results in renal dysfunction ***(Attal et al., 2010).***

Bone ache is one of the most important characteristics of MM results from osteolytic lesions, pathological fractures and AL amyloidosis. Osteolytic bone lesions cause significant morbidity for patients with multiple myeloma (MM), with approximately 75% complaining of bone pain or fractures ***(Attal et al., 2010)***. The spine, skull and femur are most affected, and 15% of patients have diffuse osteopenia ***(McCarthy et al., 2010)***. The standard diagnostic modality in the detection of skeletal disease is plain radiography, although morpho-metric studies have shown that bony abnormalities can be present even in an absence of visible radiological osteolysis ***(Lokhorst et al., 2010)*.**

The monoclonal plasma cells produce, probably in collaboration with the stromal cells, soluble osteoclast-activating factors (OAF) that stimulate osteoclastic activity ***(Roussel et al., 2010)***. Despite a compensatory increase in osteoblast stimulation, patients with advanced MM disease develop an inhibition of osteoblastic activity, which leads to a bone resorption imbalance and the development of osteolysis ***(Bjorkstrand and Gahrto, 2010).***Increased osteoclast activity manifests clinically with bone pain, pathological fracture and hypercalcaemia ***(Mohty et al., 2010).*** The extent of osteolysis appears to correlate with the degree of BM infiltration with pathological plasmacytes ***(Biver et al., 2010).***

The enhanced and uncontrolled osteoclastic activity is reflected by the increase in markers of bone resorption, including N-or C terminal telopeptide of type I collagen and tartrate-resistant acid phosphatase type 5b (TRACP-5b), in the serum of myeloma patients ***(Silverman et al., 2012)*.**

The degradation product of collagen type I carboxy terminal telopeptide (ICTP) is released as stable fragment representing a new biochemical parameter that reflects the changes in the resorption properties of skeletal system can be measured in the serum as a marker of osteolysis ***(Chopin et al., 2012).***

Aim of the Work

This study aims to determine the significance of collagen type I carboxy terminal telopeptide (ICTP) as a marker of osteolysis, and a predictor of osteolytic lesions compared with the standard prognostic factors.

CHAPTER (1): MULTIPLE MYELOMA

Multiple myeloma (MM) or plasma cell myeloma (previously called myelomatosis, medullary plasmacytoma, or Kahler's disease) is a bone marrow (BM) based multifocal plasma cell neoplasm that characterized by the proliferation of a single clone of plasma cells (PCs) derived from immunoglobulin secreting, heavy-chain class-switched B cells (post germinal) ***(McKenna et al., 2008)***.

The PC clone typically produces a monoclonal (M) protein that can lead to renal failure [due to excess light chains the Bence Jones protein (BJP)] or hyperviscosity. Frequently, there is invasion of the adjacent bone, which destroys skeletal structures and results in bone pain and fractures. Occasionally, PCs infiltrate multiple organs and produce a variety of symptoms ***(McKenna et al., 2008)***.

The diagnosis depends on the identification of abnormal monoclonal PCs in the BM, the existence of M protein in the serum and/or urine, evidence of end organ damage (CRAB: hyper**c**alcemia, **r**enal insufficiency, **a**nemia and **b**one lesion) and a clinical picture consistent with MM ***(Kyle and Rajkumar, 2009)*.**

Epidemiology:

The MM accounts for 1-2% of all cancers, 10-15% of all hematological malignancies and causes 20% of deaths from hematologic malignancies ***(Turesson et al., 2010).*** It represents the second most frequently occurring hematological malignancy in the United States ***(Jemal et al., 2010)*.** Plasma cell myeloma is more common in men

than women with a ratio of 1.4:1, and it occurs as twice as frequently in African Americans as in Caucasians ***(Mckenna et al., 2008)***.

Myeloma is not found in children and only rarely in adults less than 30 years of age, the incidence increases progressively with age thereafter, approximately 90% of cases occurring over 50 years with a median age at diagnosis of 70 years. The risk of MM is 3.7 folds higher for individuals with first degree relative with the disease ***(McKenna et al., 2008).***

Etiologic Factors:

Radiation Exposures:

Myeloma risk was considered to be two times higher among radiologists exposed to low doses of radiation than among physicians not exposed to radiation ***(Linet et al., 2010).*** Exposure of patients to diagnostic X-ray has not been linked with the development of MM in most epidemiologic studies,but there was evidence of increasing risk with exposure to increasing numbers of radiographic procedures***(Linet et al., 2010).***

Workplace Exposure:

Several epidemiologic studies have evaluated the risk of MM among agricultural workers, with positive association reported particularly among farmers who use herbicides and insecticides ***(Kushi et al., 2012).***Workers in various metal occupation and industries have been reported to have an increased myeloma risk especially inpeople exposed to benzene and other organic solvents.

However, a meta-analysis by **Wong** and **Raabe** of more than 350,000 petroleum workers similarly showed no increased risk ***(Wongand Raabe, 2000).***

Life style Factors:

Tavani et al. (2000), found a dietary link for MM with higher risk among people consuming large quantities of liver and butter, and a lower risk among people consuming large amounts of vegetables. No association of MM and coffee, alcohol or red meat intake has been found. Moreover, elevated risks were associated with obesity in comparison to people of normal weight ***(Dispenzieri et al., 2009).***

Medical Conditions:

The monoclonal gammopathy of undetermined significance (MGUS) is considered a potential risk for MM. The risk of progression of MGUS to MM-related disorders is, thus, 1% per year ***(Kyle, 2010)***. Repeated or chronic antigenic stimulation of the immune system may lead to MM. Several case-controlled studies have suggested that MM is associated with past-history of infections, inflammatory conditions, connective tissue disorders, autoimmune illnesses, and allergy-related disorders ***(Lindqvist et al., 2011)***. Patients with the human immunodeficiency virus (HIV) may have an increased likelihood of developing MM ***(Shiels et al, 2011).***

Pathogenesis of Multiple Myeloma:

Before discussing the pathogenesis of malignant plasma cell clone, it is important to review normal plasma cell origin, development and maturation.

Normal plasma cell development [Fig. 1]:

In postnatal life the BM is the primary lymphoid organ where B lymphocytes develop. Committed precursor B cells (pro-B cells) undergo functional variable diversity joining (VDJ) rearrangement of the immunoglobulin heavy chain (IgH) and immunoglobulin light chain (IgL) genes in the BM [under the influence of interleukin 3 (IL3) and stem cell factor (SCF)], before exiting as naïve mature B cells ***(Yang et al., 2013).***

Naïve mature B cells in the peripheral blood (PB) migrate through post-capillary venules into the substance of the lymph node or into the spleen (secondary lymphoid organs). After antigen encounter, these cells differentiate into short-lived PCs during early immune response, and secrete mainly immunoglobulin M (IgM). Later in the antigenic response, some antigen-activated B cells enter a germinal center in a T-cell–dependent immune process and undergo somatic hypermutation and IgH isotype switching. These result in PCs that are able to secrete all the different classes of Ig. Subsequently, the B cells develop into either long-lived PCs or memory B cells ***(Yang et al., 2013).***

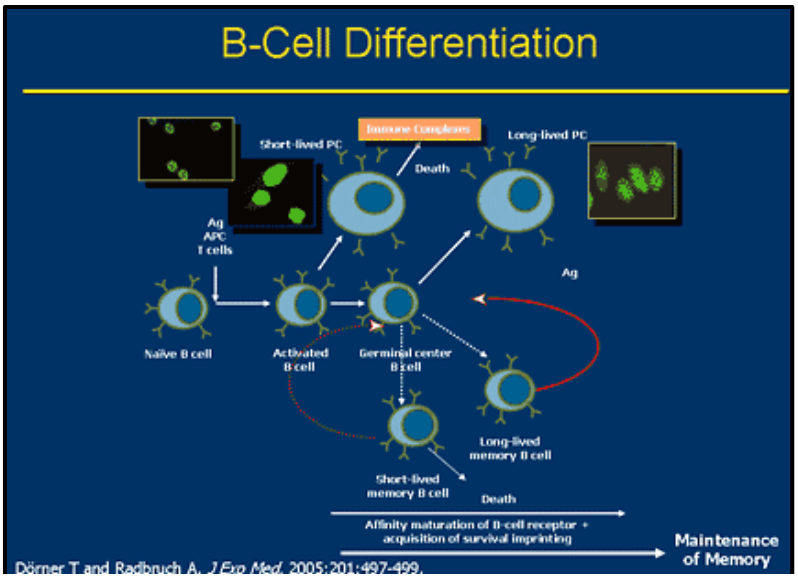

Fig. (1): The development of normal plasma cells
(Dorner& Radbruch, 2005)

A variety of signals have been identified as being important in the final steps of PC differentiation. When T cell contact is required, the CD40-CD40L (CD154) interactions play a dynamic role and are essential for antibody production. CD40 activated B cells in turn produce numerous cytokines, such as IL-1β, IL-6, IL-10, and tumor necrosing factor (TNFα), which may act as autocrine differentiation factors for Ig production***(Elgueta et al., 2009).***

Development of malignant plasma cell clone:

In order to understand the pathogenesis of MM, it's important to review not only the molecular changes involved in the development of the malignant clone, but also the mechanisms responsible for the interaction between the malignant plasma cells and their microenvironment, since they play a relevant role in bone destruction, tumor cell growth, survival, migration and drug resistance.

1. Genetic abnormalities:

To date, no single molecular defect can account for the pathogenesis of MM. A multitude of abnormalities has been identified in signaling pathways, apoptotic mechanisms and the cell cycle***(Dispenzieri et al., 2009)***.

a. Increased karyotypic instability

Increasing evidence suggests that the development of myeloma is a multistep process that includes the progressive occurrence of multiple structural chromosomal changes. Chromosomal abnormalities are detected by conventional cytogenetics in about one third of myelomas, while fluorescence in situ hybridization (FISH) increases the proportion of chromosomal abnormalities to>90%***(McKenna et al., 2008).***

- **IgH translocations:**

In MM, IgHtranslocation may be classified into ***primary*** or ***secondary***. Primary IgHtranslocations occur as initiating events during the pathogenesis of MM, whereas secondary translocations are involved in disease progression. The breakpoints occur mainly within or immediately adjacent to IgHswitch regions or JH regions juxtaposing oncogenes to the proximity of the powerful IgH enhancer, resulting in up-regulation of theses oncogenes ***(Zingone et al., 2010).***

There is a marked diversity of chromosomal loci involved in IgH translocations as shown in **Figure (2)**. About 40% of MM tumors have IgH translocations involving five recurrent chromosomal patterns: 11q13 (CCND1), 4p16 (FGFR/MMSET), 16q23 (MAF), 6p21 (CCND3) and 20q11 (MAFB) as shown in **Table (1).**

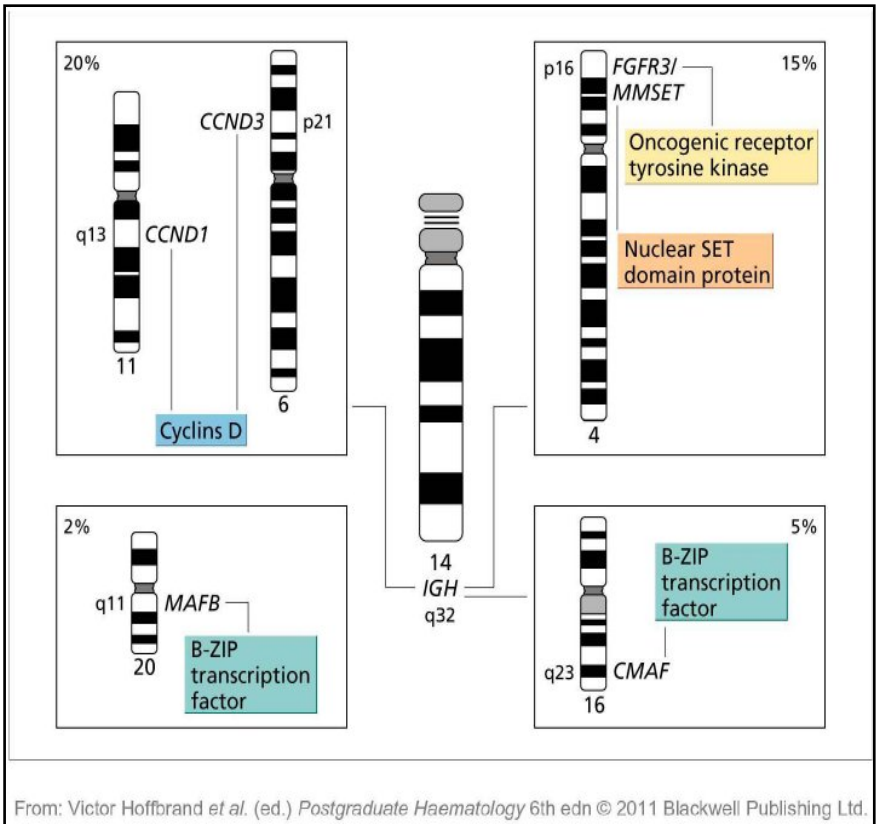

Fig. (2): IgH translocations in multiple myeloma ***(San-Maguel& Blade, 2011).***

Table (1): Frequent chromosomal partners and candidate oncogenes in which translocations t(partner chromosome; 14q32) involved in the pathogenesis of MM:

Chromosome partner	Freq.	Oncogene	Localization	Distance From breakpoint	Function
11q13	15-20%	Cyclin D1	Der (14)	100- 330 kb	Cell cycle Regulator
		Myeov	Der (11)		Unknown
4p16	12-17%	FGFR3	Der (14)	50- 100 kb	Growth factor receptor tyrosine kinase
		MMSET/ WHSCI	Der (4)		Chromatin Remodeling
16q32	5-10%	c-maf	Der (14)	550- 1350 kb	B-ZIP Transcription factor
6p21	5%	Cyclin D3	Der (14)	65 kb	Cell cycle Regulator
6p25	< 5%	MUMI/IRF4	Der (14)	Immediately Adjacent	Transcriptional regulator of IFN and IFN-stimulated Genes
20q11	<2%	MAFB	Der (14)		B-ZIP Trancriptional Factor

(Kuehl& Bergsagel, 2002).

- **Gains and losses of chromosomal material**

Although IgHtranslocations are a hallmark in many cases of MM, there are other chromosomal abnormalities also involved in pathogenesis and prognosis that result in changes in chromosomal copy number. The loss of chromosome 13 is the most common monosomy in MM (40-50% of newly diagnosed patients). This abnormality shows a strong association with t(4;14) and t(14;16), deletion of 17p and gains on 1q ***(Walker, 2010).***

Chromosome 17p deletion, which includes loss of *TP53,* occurs at a lower frequency (5-10% of newly diagnosed MM), but its prognostic influence seems to be more important. Classical cytogenetics, FISH and comparative genomic hybridization analysis (CGH) have all demonstrated that gains on 1q are some of the most common abnormalities in MM. Mostly they are the result of tandem duplications and jumping segmental duplications of the chromosome 1q band. The increased expression of *CKS1B* (1q21) detected in MM with gains of 1q has been suggested as the cause of increased proliferation in these cases ***(Munshi et al., 2009).***

- **Aneuploidy:**

Hyperdiploidy, with chromosome counts of greater than 50, has been reported in 30-45% of abnormal cases with gains in the odd number chromosomes 3, 5, 7, 9, 11, 15, 19 and 21by conventional cytogenetics and CGH ***(Torris, 2008).***

Hypodiploidy is also a common finding, with loss of chromosomes 8, 13, 14 and X observed by conventional cytogenetics ***(Smadja et al., 2001).***

b. Late genetic events

Some genetic changes in MM, such as secondary translocations, mutations, deletions and epigenetic abnormalities, are considered late oncogenic events and are associated with disease progression ***(Chng, 2011).***

Dysregulation of MYC is a paradigm for secondary translocations in MM. Activating RAS mutations are considered molecular markers of disease progression with a prevalence of 75% in MM cases at relapse. Moreover, TP53 inactivation, via either deletion or mutation, seems to be more frequently associated with disease progression. In addition, epigenetic changes like methylation of the tumor suppressor genesCDKN2B andCDKN2A leading to their inactivation has been described in advanced MM and extramedullary forms ***(Chng, 2011).***

2. BM microenvironment and homing of myeloma cells:

Contact between myeloma cells and BM stromal cells (BMSC) is critically as important as genetic lesions in inducing both the tumor growth and the development of myeloma bone disease, also it plays a key role in the abnormal regulation of many factors implicated in MM. There is a synergistic, pathologic relationship between myeloma cells and the cells comprising the BM microenvironment, including fibroblasts, osteoblasts, and osteoclasts ***(Dispenzieri et al., 2009)***.

a. Adhesion and homing of myeloma cells to BM microenvironment is mediated via a series of adhesion molecules including: the integrin family (VLA-4, VLA-5 and VLA-6), intracellular adhesion molecules (ICAM-1) and vascular cell adhesion molecules (VCAM-1) in addition to stromal derived factor-1 alpha (SDF-1α). Homing of PCs is facilitated via expression of adhesion molecules such as CD138, CD38, CD44 and CD106.

- **VCAM-1**: Localization may also be achieved by the interactions between tumor cell surface integrin (alpha4-beta1) and vascular cell adhesion molecule 1 (VCAM-1) expressed on marrow endothelial and stromal cells. These interactions have been demonstrated to increase the production of osteoclast stimulating activity ***(Pivonka et al., 2008).***
- **CD56:**A cell adhesion molecule belonging to the Ig superfamily, CD56 (N-CAM), is strongly expressed in most PCs of myeloma patients and is believed to play a role in myeloma homing and cell adhesion to the marrow ***(Kraj et al., 2008*)**.
- **SDF-1α:** it is a CXC chemokine expressed by BM stromal cells and endothelial cells, and binds to CXCR4 receptors which are expressed on hematopoeitic stem cells and lymphocytes, as well as malignant cells and osteoclast precursors.SDF-1/CXCR-4 plays an important role in hematopoietic stem cell homing and tumor migration and proliferation. Recent data showed that SDF-1 not only mediates the migration and homing of

myeloma cells**,** but also increases osteoclast motility and bone resorbing activity. This was associated with an over-expression of osteoclast activation related genes, including RANKL, TRAP, MMP-9, CA-II and Cathepsin K ***(Tzeng et al., 2011).***

Adhesion of myeloma cells to BM microenvironment induces a cell mediated drug resistance by the following mechanisms:

i. Cell cycle arrest at G1(associated with up regulation of p27 the inhibitor of CDK).

ii. Apoptosis inhibition via up regulation FLIP-L, an endogenous inhibitor of FAS (CD95)

iii. Protection of tumor cells from initial drug induced DNA damage by reducing telomerase II activity.

b. Cytokines production:

The binding of MM cells to the BM micro-environment induces the transcription and secretion of cytokines such as: interleukins 6 and 21 (ILs), transforming growth factor (TGF), tumor necrosis factor (TNF-α), SDF-1, vascular endothelial growth factor (VEGF), macrophage inflammatory protein-1α (MIP-1α) and other cytokines by both PCs and stromal cells this will trigger signaling pathways (e.g. RAF/MEK/MAPK, PI3K/AKT and JAK/ATAT) leading to promotion of proliferation and inhibition of apoptosis in addition to production of additional adhesion molecules which in a vicious circle enhances cell adhesion as shown in **Figure (3)*(Lust et al., 2009)***.