INTRODUCTION

Plasma cell myeloma (multiple myeloma) is a neoplastic plasma cell disorder that is characterized by clonal proliferation of malignant plasma cells in the bone marrow microenvironment, monoclonal protein in the blood or urine, and associated organ dysfunction ***(Palumbo and Anderson, 2011)***. It accounts for 1% of all cancers and approximately 10% of all hematologic malignancies ***(Rajkumar, 2011).***

Multiple myeloma is associated with a diverse set of clinical manifestations ***(Greenberg et al., 2015).*** The diagnosis of myeloma requires 10% or more clonal plasma cells on bone marrow examination or a biopsy proven plasmacytoma plus an evidence of end-organ damage (hypercalcemia, renal insufficiency, anemia, or bone lesions) that is felt to be related to the underlying plasma cell disorder. In addition, the presence of 60% or more clonal plasma cells in the marrow should also be considered as myeloma regardless of the presence or absence of end-organ damage ***(Rajkumar, 2014).***

When multiple myeloma is suspected clinically, patients should be tested for the presence of M proteins using a combination of tests that should include a serum protein electrophoresis (SPEP), serum immunofixation (SIFE), and the serum free light chain (FLC) assay ***(Katzman et al., 2006).***

The prognoses of patients with multiple myeloma are highly variable. While a portion of patients survive over a decade after their diagnosis, a small subset at the other end of the spectrum exhibits a highly aggressive course. Prognostic factors for multiple myeloma have been suggested, and the International Staging System (ISS) is most widely applied. It stratifies patients by their albumin and β2-microglobulin (β2M) levels ***(Greipp et al., 2005).***

Chromosome aberrations in multiple myeloma are complex and represent a hallmark of the disease, involving many chromosomes that are altered both numerically and structurally. Researches have proved that the detection of these chromosomal aberrations is of crucial importance not only because of their association with clinical prognosis, but also because they can be used as measurable targets for response to treatment ***(Yang et al., 2012).*** Although many genetic abnormalities are amenable to detection by conventional cytogenetics, their detection is impaired by the relatively low rate of proliferation and, consequently, the low proportion of cells in metaphase. Some aberrations are cryptic and cannot be detected by conventional cytogenetics. These limitations can be overcome in part by interphase fluorescence *in situ* hybridization (FISH) ***(Jekarl et al., 2013).***

Translocations involving the IGH locus at 14q32 have been reported to be the most common genetic lesion in multiple myeloma and are demonstred by FISH in up to 50–60% of

cases. More than 20 different partner chromosomes have been described. These translocations include t(4;14) (p16;q32), t(6;14) (p25;q32), t(8;14) (q24;q32), t(9;14) (p13;q32), t(11;14) (q13;q32) and t(14;16) (q32;q22) ***(Turkmen et al., 2014)***.

AIM OF THE WORK

The aim of the study is to:

- Detect IgH 14q32 rearrangements {t(4;11) and t(4;14)}by interphase FISH in Egyptian patients with plasma cell myeloma.
- Determine the relationships of these chromosomal abnormalities with standard prognostic parameters.

MULTIPLE MYELOMA

I. Definition of Multiple Myeloma

Plasma cell myeloma, multiple myeloma (MM), or myelomatosis is a disease resulting from the proliferation in the bone marrow (BM) of a clone of neoplastic cells that are closely related, both morphologically and functionally, to plasma cells (PCs) **(Figure 1)**. The World Health Organization (WHO) classification uses the term 'plasma cell myeloma'. In the great majority of cases, the neoplastic cells secrete a protein that is either a complete immunoglobulin (Ig) or an immunoglobulin light chain (Bence– Jones myeloma). Clinical features result either directly from the effects of the neoplastic proliferation or indirectly from effects of the protein, often designated a paraprotein, which the myeloma cells produce ***(Mckenna et al., 2008).***

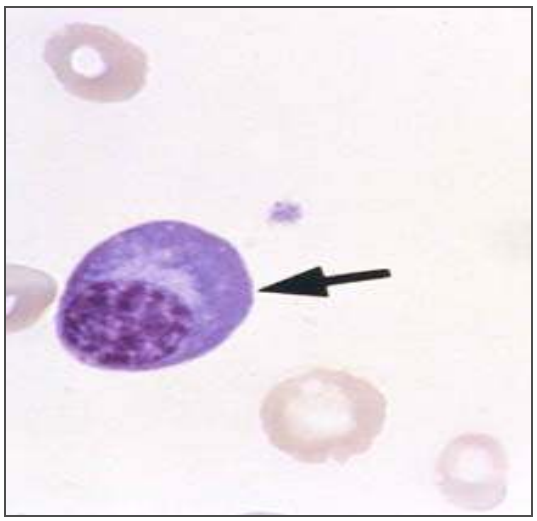

Figure (1): Plasma cell, Wright stain, x400 (http://pathology.mc.duke.edu/research/PTH225.html).

II. Epidemiology

The MM accounts for 1-2% of all cancers, 10-15% of all hematological malignancies and causes 20% of deaths from hematologic malignancies ***(Rajkumar, 2009).*** 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 ***(McKenna et al., 2008).***

III. Etiologic Factors

A. *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).***

B. 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 in people exposed to benzene and other organic solvents ***(Wong and Raabe, 2000).***

C. Genetic element:

Multiple myeloma has been reported in familial clusters of two or more first-degree relatives and in identical twins. Thus, a genetic element may exist in some patients ***(Kyle, 1996*)**. However, the incidence is too low to postulate genetic predisposition ***(Segel and Lichtman, 2004).***

D. Life style Factors:

There were 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. Moreover, elevated risks were associated with obesity in comparison to people of normal weight ***(Dispenzieri et al., 2009).***

E. 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 ***(Lindqvist et al., 2011)***. Patients with the human immunodeficiency virus (HIV) may have an increased likelihood of developing MM ***(Shiels et al., 2011).***

IV. 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.

A. Normal plasma cell development (Figure 2):

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).***

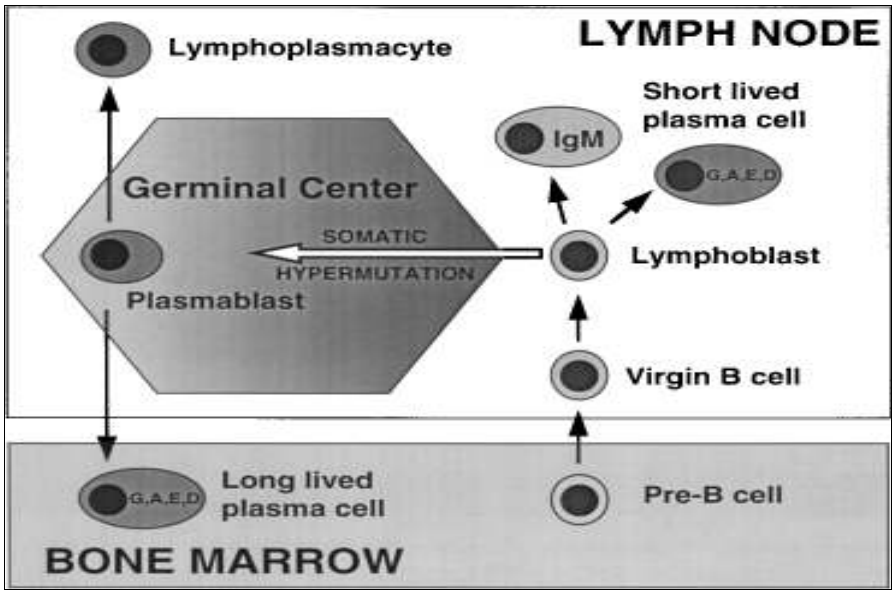

Figure (2): Normal plasma cell development ***(Hallek et al., 1998).***

B. *Development of malignant plasma cell clone:*

1. Genetic abnormalities:

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

i. IgH translocations:

In MM, IgH translocation may be classified into ***primary*** or ***secondary***. Primary IgH translocations 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

IgH switch regions or JH regions juxtaposing oncogenes to the proximity of the powerful IgH enhancer, resulting in up-re gulation of theses oncogenes ***(Zingone et al., 2010).***

There is a marked diversity of chromosomal loci involved in IgH translocations as shown in **figure 3** and **table 1.**

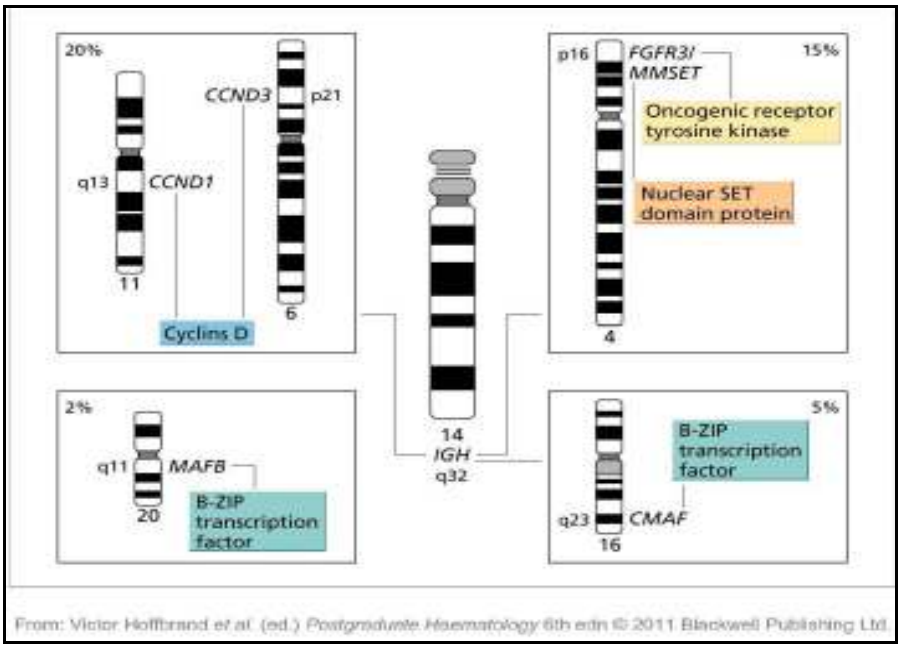

Figure (3): IgH translocations in multiple myeloma ***(San-Maguel and Blade, 2011).***

Table (1): Primary translocations in MGUS and MM

Chromosomal translocation	Gene dysregulated by translocation	Functions
t(11;14)	CCND1 (cyclin D1)	Cell cycle regulator
t(4;14)	FGFR-3 and MMSET	Growth factor receptor tyrosine kinase Transcription factor
t(6;14)	CCND3 (cyclin D3)	Cell cycle regulator
t(14;16)	C-maf	Transcription factor
t(14;20)	MafB	Transcription factor

(Lin, 2009 and Rajkumar, 2009)

ii. <u>Aneuploidy:</u>

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 21 by conventional cytogenetics ***(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).***

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 (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. 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 ***(Tzeng et al., 2011).***

b. Cytokines production:

The binding of MM cells to the BM micro-environment induces the transcription and secretion of cytokines such as: interleukin 6 (IL-6) and IL-21, transforming growth factor (TGF), tumor necrosis factor (TNF-α), SDF-1, 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/STAT) 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 4** ***(Lust et al., 2009)***.

- **IL-6:** high levels of IL-6 are produced by BM stromal cells of myeloma patients. IL-6 serves as a growth and survival factor for both benign and malignant PCs, with subsequent production of IL-1β, VEGF, and MIP-1α. In turn, IL-1β and MIP-1α regulate and activate BM osteoclasts ***(Lust et al., 2009)***.

- **MIP-1α:** it is a chemokine that is produced by myeloma cells, and act as an important mediator of their localization to the BM. In addition, it induces osteoclasts formation ***(Roodman, 2009).***
- **TGF-β:** It is a ubiquitous, multifunctional growth factor that is released from the bone matrix during osteoclastic bone resorption and acts to inhibit osteoblast differentiation ***(Giuliani et al., 2011).***
- **IL-3:** Recent evidence suggests that IL-3 may play a role in regulation of bone formation in myeloma bone disease ***(Roodman, 2009)***.
- **Hepatocyte growth factor (HGF):** is produced by myeloma cells and is increased in the serum of patients with MM, where levels of HGF were correlated with a poor prognosis ***(Du et al., 2007*)**.

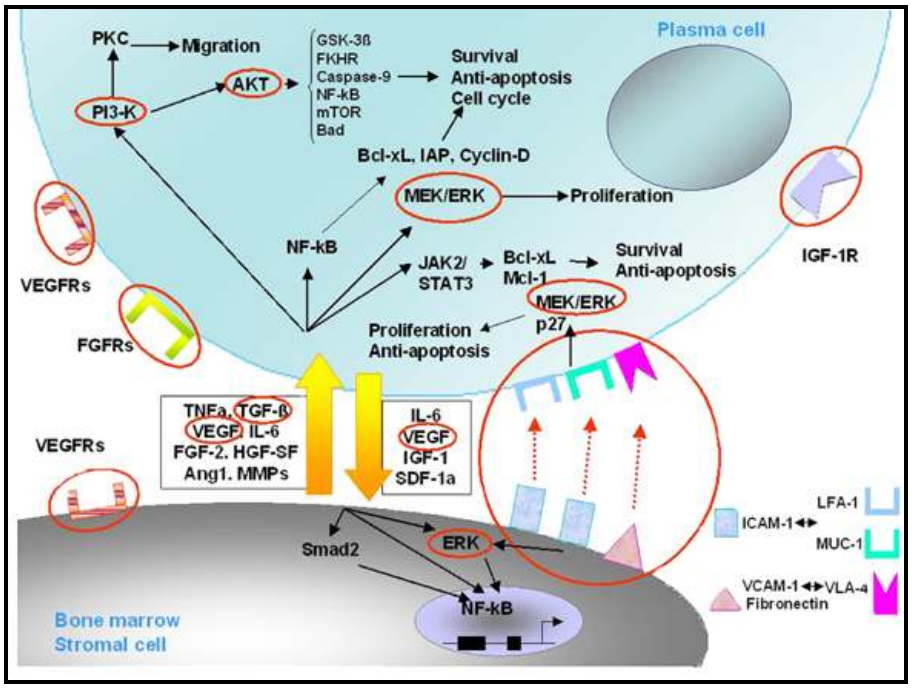

Figure (4): Interaction between myeloma cells and bone marrow microenvironment and signalling pathways involved in pathogenesis of MM ***(Ribatti et al., 2006).***

3. Biology of Bone Disease:

Bone destruction in myeloma is related to increased osteoclastic activity which is not accompanied by a comparable increase in osteoblast formation. This uncoupling of resorption and formation leads to rapid bone loss, osteoporosis, lytic lesions and fractures. The enhanced and uncontrolled osteoclastic activity has been confirmed consistently in several animal models and is also reflected by the increase in markers of bone resorption, including N- or C-terminal cross-linking telopeptide of type I collagen (NTX or ICTP/CTX respectively) and tartrate-resistant acid phosphatase type 5b (TRACP-5b), in the serum of myeloma patients ***(Edwards et al., 2008).***