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Isoprenoid Pathway Related Cascade in Multiple Myeloma

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This study assessed the changes in the isoprenoid pathway and its metabolites digoxin, dolichol and ubiquinone in multiple myeloma. The following parameters were assessed: isoprenoid pathway metabolites, tyrosine and tryptophan catabolites, glycoconjugate metabolism, RBC membrane composition and free radical metabolism. There was elevation in plasma HMG CoA reductase activity, serum digoxin and dolichol and a reduction in RBC membrane Na^+-K^+ ATPase activity, and serum ubiquinone levels. Serum tryptophan, serotonin, nicotine, strychnine and quinolinic acid were elevated while tyrosine, dopamine, noradrenaline and morphine were decreased. The total serum glycosaminoglycans and glycosaminoglycan fractions, the activity of GAG degrading enzymes and glycohydrolases, carbohydrate residues of glycoproteins and serum glycolipids were elevated. The RBC membrane glycosaminoglycans, hexose and fucose residues of glycoproteins, cholesterol and phospholipids were reduced. The activity of all free radical scavenging enzymes, concentration of glutathione, iron binding capacity and ceruloplasmin decreased significantly while the concentration of lipid peroxidation products and NO increased. Hyperdigoxinemia related altered intracellular Ca^{++} mediated oncogene activation, dolichol induced altered glycoconjugate metabolism and ubiquinone deficiency related mitochondrial dysfunction can contribute to the pathogenesis of multiple myeloma. The biochemical findings reported could be the cause or the consequence of multiple myeloma. (Pathology Oncology Research Vol 9, No 2, 107–114, 2003)

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Introduction

Changes involving the isoprenoid pathway have been described in neoplasms. The isoprenoid pathway produces 4 key metabolites important in cellular function – digoxin (an endogenous Na^+-K^+ ATPase inhibitor), dolichol (important in N-glycosylation of proteins), ubiquinone (a component of the mitochondrial electron transport chain and membrane antioxidant) and cholesterol.[1] Previous work has demonstrated a pathogenetic role for hypothalamic digoxin in essential hypertension, syndrome X, bipolar mood disorder and vasculitis.[2,3,4]

Alteration in membrane Na^+-K^+ ATPase has been described in oncogenesis suggesting a possible role for endogenous digoxin.[5] An important feature of malignant transformation is loss of the cholesterol feedback inhibition mechanism that regulates cholesterol synthesis. Cancer cells seem to require an increased concentration of cholesterol and cholesterol precursors.[6] Prevention of tumor-cell growth can be achieved by restricting either cholesterol availability or cholesterol synthesis. In vivo-and-cell-culture experiments have shown that lowering the plasma cholesterol concentration or intervening in the mevalonate pathway with HMG CoA reductase inhibitors decreases tumor growth. Another key protein in the internal signalling pathway that triggers cell growth is ras. Ras is activated by hooking a 15 carbon farnesyl chain to ras by the enzyme farnesyl transferase.[7] Farnesyl transferase inhibitors are used to block K-Ras-driven tumors.

Alteration in ubiquinone which is a component of the mitochondrial electron transport chain and a membrane antioxidant, can also lead to mitochondrial dysfunction and free radical generation. Defects in structure and function of mitochondria have been described in neoplasms. Free radical

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mechanisms have been implicated in tumorogenesis.[8] Free radicals are required for the action of oncogene coded growth factors. Altered glycoproteins and dolichol have been described in neoplasms. The dolichol pathway is important in N-glycosylation of protein. Altered glycosylation of serum transferrin has been reported in neoplastic lesions. Abnormal glycoconjugates have been described in neoplastic disorders. A number of fucose and sialic acid containing carbohydrate ligands are important in malignant cell transformation.[9] Glycosylation inhibitors are used to treat neoplasms. In multiple myeloma, interaction of tumor and host cells with adhesion and extracellular matrix molecules like heparan sulphate proteoglycan and syndecan are important.[10]

Digoxin has been reported to regulate the transport of aminoacids, especially the neutral aminoacids.[11] Tryptophan metabolism has also been implicated in neoplastic disorders and immune activation. Interferons act by inducing the enzyme indoleamine – 2,3 – dioxygenase which catalyses the catabolism of tryptophan along the kynurenine pathway.[12] This leads on to tryptophan depletion and increase in level of its metabolites kynurenine and quinolinic acid. Cachexia related to cancer has also been related to indoleamine 2,3 digoxygenase induction and depletion of tryptophan by enhancing its catabolism. The kynurenine pathway can also contribute to oncogenesis. Neurotransmitters could contribute to the regulation of the immune response. Elevated serotonin and reduced dopamine levels have been related to immune activation and immunoproliferation.[13] Tryptophan and tyrosine catabolism could be important in this respect with regard to immunoproliferative neoplasms like multiple myeloma. Studies from our laboratory have demonstrated that tryptophan is a precursor for endogenous nicotine and strychnine and that tyrosine is a precursor for endogenous morphine.[14] Since tryptophan metabolites have a role in immunomodulation, strychnine and nicotine were also studied. Since digoxin can modulate the neuronal availability of tryptophan by regulating the neutral aminoacid transport system, it can also control the levels of the tryptophan catabolites- strychnine and nicotine.

This study was undertaken to assess the following parameters in freshly diagnosed cases of multiple myeloma (1) The isoprenoid pathway (2) The tryptophan/tyrosine catabolic patterns (3) Glycoconjugate metabolism and (4) RBC membrane changes as a reflection of neoplastic cell membrane change (the isoprenoid pathway produces four metabolites which can regulate membrane function and structure-dolichol, digoxin, cholesterol and ubiquinone).

Materials and Methods

15 freshly diagnosed cases of multiple myeloma were chosen randomly for the study from the orthopaedics and hematology wards of Medical College, Trivandrum and the Regional Cancer Centre, Trivandrum over a 3 year period. The age of the patients ranged from 50-60 years. The diagnosis of multiple myeloma was made by standard criteria. Major criteria (1) Plasmacytoma by biopsy (2) >30% plasma cells by bone marrow examination (3) M protein > 3.5g/dl of IgG, >2.0g/dL of IgA, or >1g / 24h urine of kappa or lambda light chains. Minor Criteria (1) 10-30% plasma cells in bone marrow (2) M protein less than above (3) Lytic bone lesions (4) Normal IgM<50mg/dL, IgA < 100mg/dL, or IgG <600mg/dL. Diagnosis of myeloma was made when one major +one minor, or three minor criteria including the first two are present. The staging of myeloma was done according to Durie and Salmon Staging. All the patients were in stage 1 disease. Stage I disease fulfilled the following criteria. (1) Hb>10g/dL (2) Ca^{++} normal (3) Radiologically normal or only osteolytic lesion (4) Low concentration of paraprotein- IgG<50g/L, IgA<30g/L and Bence- Jones protein in urine <4g/24h. This produces a tumor cell load ($x10^{12}/m^2$)- of <0.6 (low). The study was conducted in freshly diagnosed cases before treatment was started. Therefore other prognostic factors were not included in the study. Informed consent was obtained from all the patients. The permission of the Ethics committee of the institute was also obtained. All the patients included in the study were non-smokers (active and passive). They were free of systemic diseases like hypertension and diabetes and were also not on any treatment for the same. Each patient had age and sex matched healthy normal control.

Fasting blood was removed from each of the patients for various estimations. RBCs were separated within 1 hour of collection of blood for the estimation of membrane Na^+-K^+ ATPase. Serum was used for the estimation of HMG CoA reductase activity. Plasma/serum was used for the estimation of the other parameters. All biochemicals used in this study were obtained from M/s.Sigma Chemicals, USA. Activity of HMG CoA reductase of the plasma was determined using the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate.[15] For the determination of Na^+-K^+ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used.[16] Digoxin in the plasma was determined by the procedure described by Arun et al.[17] For estimation of ubiquinone and dolichol in the plasma, the procedure described by Palmer et al was used.[18] Tryptophan was estimated by the method of Bloxam and Warren and tyrosine by the method of Wong, O'Flynn and Innoye.[19,20] Serotonin was estimated by the method of Curzon and Green and catecholamines by the method of Well-Malherbe.[21,22] Quinolinic acid content of plasma were estimated by HPLC (C_{18} column micro Bondapak™ 4.6 x 150 mm), solvent system 0.01 M acetate buffer (pH 3.0) and methanol (6:4), flow rate 1.0 ml/minute and detection UV 250-nm). Nicotine, morphine and strychnine were estimated by the method described by Arun et al.[17] Details of the procedures used for the estimation of total and individual GAG, carbohydrate components of glycoproteins,

activity of enzymes involved in the degradation of GAG (beta glucuronidase, beta-N-acetyl hexosaminidase, hyaluronidase and cathepsin-D), activity of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) have been described before.[23] Serum glycolipids (gangliosides, glycosyl diglycerides, cerebrosides and sulphatides) were estimated as described.[24] Cholesterol was estimated by using commercial kits supplied by Sigma Chemicals, USA. SOD was assayed by the method of Kakkar, Das & Viswanathan.[25] Catalase activity was estimated by the method of Maehly and Chance, glutathione peroxidase by the method of Paglia and Valentine and glutathione reductase by the method of Horn and Burns.[26, 27,28] MDA was estimated by the method of Will and conjugated dienes and hydroperoxides by the procedure of Brien.[29] Reduced glutathione was estimated by the method of Beutler, Duran and Kelley.[30] Extraction of erythrocytes for vitamin E was carried out according to the procedure described by Colin, Rammel, Cunlifee and Keiboom and Vitamin E estimated in the extract by HPLC (Waters HPLC, Nova Pak C_8 column (4.6 x 150 mm) – solvent-acetonitrile: methanol: water (63:33:4), flow rate – 2 ml/min, detection – UV 280 nm). For Vitamin E, the retention time was 3.5 min under these conditions.[31] Nitric oxide was estimated in the plasma by the method of Gabor and Allon.[32] Iron binding capacity in the plasma was estimated by the method of Wootton and ceruloplasmin by the method of Henry, Chiamori , Jacobs & Segalov.[33, 34] Free fatty acid were estimated by the method of Falholt , Lund & Fatholt.[35] Statistical analysis was done by 'ANOVA'.

Results

The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in multiple myeloma when compared with controls. The concentration of serum ubiquinone and the activity of erythrocyte membrane Na^+-K^+ ATPase were decreased (*Table 1*). The concentration of serum tryptophan, quinolinic acid and serotonin was increased in the plasma while that of tyrosine, dopamine and noradrenaline was decreased in multiple myeloma. (*Table 1*) Nicotine and strychnine could be detected in the plasma of patients with multiple myeloma but was not detectable in control serum. Morphine was not detected in the plasma of these patients. (*Table 1*)

The concentration of total glycosaminoglycans (GAG) increased in the serum of multiple myeloma patients. The concentration of heparan sulphate (HS) heparin (H), chondroitin sulphates (ChS), hyaluronic acid and dermatan sulphate was increased in multiple myeloma. The concentration of total hexose, fucose and sialic acid was increased in the glycoproteins of the serum in these patients. The concentration of gangliosides, glycosyl-diglycerides, cerebroside and sulphatide showed significant increase in the serum of these patients (*Table 2*).

Table 1. **Serum isoprenoidal metabolites and neurotransmitters in multiple myeloma patients**

Groups	*Control (1)*	*Multiple myeloma (2)*
HMG CoA Reductase		
HMG CoA / Mevalonate	1.15 ± 0.12	0.86 ± 0.075**
Digoxin (ng / dl)	12.80 ± 1.09	23.54 ± 1.47 **
Dolichol (µg / dl)	39.1 ± 2.36	79.8 ± 4.19 **
Ubiquinone (µg / dl)	144.2 ± 8.65	94.40 ± 5.92 **
Na^+-K^+ ATPase		
(µg / p_i / mg protein)	5.04 ± 0.221	0.346 ± 0.26**
Tryptophan (mg / dl)	1.11 ± 0.08	2.01 ± 0.07**
Tyrosine (mg / dl)	1.14 ± 0.09	0.934 ± 0.06**
5HT (µg / dl)	20.9 ± 1.9	51.8 ± 4.8**
Dop (ng / dl)	12.89 ± 0.67	7.92 ± 0.51**
Norepi (ng / dl)	45.15 ± 2.35	32.54 ± 1.78**
QA (ng / ml)	370.60 ± 21.07	652.15 ± 44.93**
Morphine (µg / dl)+	2.56 ± 0.02	ND
Strychnine (µg / dl)+	ND	3.22 ± 0.01
Nicotine (µg / dl)+	ND	4.53 ± 0.2

**P less than 0.01. + Mean of the values from 15 samples ± SD. ND: not detectable, 5 HT: serotonin, Dop: dopamine, Norepi: norepinephrine, QA: quinolinic acid.

The activity of glycosaminoglycan (GAG) degrading enzymes, beta glucuronidase, beta-N-acetyl hexoseaminidase, hyaluronidase was increased in multiple myeloma patients. Similarly to the activity of beta galactosidase, beta fucosidase and beta glucosidase and the protease, Catepsin D increased in multiple myeloma patients (*Table 2*).

The concentration of total GAG, hexose and fucose residues of glycoproteins in the RBC membrane decreased significantly in multiple myeloma. The concentration of RBC membrane cholesterol increased in multiple myeloma while that of phospholipid decreased. The ratio of RBC membrane cholesterol:phospholipids increased in multiple myeloma (*Table 2*).

The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes decreased significantly in multiple myeloma while the concentration of MDA, hydroperoxides, conjugated dienes and NO increased significantly. The concentration of glutathione was decreased but alpha tocopherol was unaltered in multiple myeloma. Lastly, iron binding capacity, ceruloplasmin and albumin levels were significantly decreased in the serum of multiple myeloma patients (*Table 3 and 4).*

Discussion

The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The increase in endogenous digoxin is due to

increased synthesis as documented by increased HMG CoA reductase activity. In multiple myeloma there was significant inhibition of the RBC membrane Na^+-K^+ ATPase. The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+-Ca^{++} exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores.[36] Increased intracellular calcium activates phospholipase C beta which results in increased production of diacyglycerol (DAG) with resultant activation of protein kinase C.[37]

Digoxin, apart from affecting cation transport is also reported to influence the transport of various metabolite across cellular membranes, including aminoacids and various neurotransmitters.[11] Two of the aminoacids in this respect are important, tryptophan, a precursor for nicotine and strychnine and tyrosine a precursor for morphine. We had already shown the presence of endogenous nicotine and strychnine in the brain of rats loaded with tryptophan and morphine in the brain of rats loaded with tyrosine.[14] The results now obtained showed that the concentration of tryptophan, quinolinic acid and serotonin was higher in the plasma of patients with multiple myeloma while that of tyrosine, dopamine and noradrenaline was lower. Serum of patients with multiple myeloma showed the presence of high amounts of nicotine and strychnine in their serum. Morphine was absent in the serum of these patients. Thus there is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the patients serum. This could be due to the fact digoxin can regulate neutral aminoacid transport system with preferential promotion of tryptophan transport over tyrosine.[11] The decrease in membrane Na^+-K^+ ATPase activity in the disorder studied could be due to the fact that the hyperpolarising neurotransmitters (dopamine and noradrenaline) are reduced and the depolarising neuroactive compounds (serotonin, nicotine and quinolinic acid) are increased.

Table 2. **Serum glycoconjugates, lysosomal enzymes and RBC membrane composition in multiple myeloma**

Groups	*Control (1)*	*Multiple myeloma (2)*
Total GAG (mg uronic acid / dl)	4.57 ± 0.408	11.63 ± 0.99**
HA (mg uronic acid / dl)	0.525 ± 0.041	1.72 ± 0.112**
HS (mg uronic acid / dl)	0.318 ± 0.022	2.031 ± 0.143**
H (mg uronic acid / dl)	0.284 ± 0.019	1.812 ± 0.038**
DS (mg uronic acid / dl)	2.83 ± 0.232	3.23 ± 0.229**
ChS (mg uronic acid / dl)	0.587 ± 0.043	3.61 ± 0.201**
hexose (mg / g protein)	13.55 ± 1.26	26.5 ± 2.48**
fucose (mg / g protein)	1.65 ± 0.149	2.18 ± 0.191**
sialic acid (mg / g protein)	6.85 ± 0.617	9.27 ± 0.822**
ganglioside (µg / dl)	26.5 ± 1.2	32.08 ± 1.9**
glycosyl diglyceride (µg / dl)	12.5 ± 0.72	21.22 ± 1.66**
cerebrosides (µg / dl)	16.25 ± 1.10	22.42 ± 0.965**
sulphatides (µg / dl)	5.25 ± 0.61	7.94 ± 0.628**
β glucuronidase (µg p–nitrophenol / hr / g protein)	59.52 ± 5.26	102.90 ± 5.26**
β N-acetyl hexosaminidase (µg p–nitrophenol / hr / g protein)	2273 ± 78.6	3219 ± 79.50**
hyaluronidase (µg N-acetyl glucosamine / hr / g protein)	62.9 ± 4.1	192.2 ± 4.3**
cathepsin-D (µg tyrosine / hr / g protein)	90.9 ± 8.9	303.4 ± 9.8**
β galactosidase (µg p-nitrophenol / hr / mg protein)	52.8 ± 3.75	76.23 ± 6.38**
β fucosidase (µg p-nitrophenol / hr / mg protein)	23.63 ± 1.65	37.38 ± 2.87**
β glucosidase (µg p-nitrophenol / hr / mg protein)	27.36 ± 2.46	46.96 ± 2.21**
GAG (µg / mg protein) – RBC membr	6.62 ± 0.71	4.53 ± 0.29**
hexose (µg / mg protein) – RBC membr	145.09 ± 11.85	63.57 ± 5.82**
fucose (µg / mg protein) – RBC membr	63.33 ± 4.60	46.5 ± 2.5**
cholesterol (nmol / mg protein) – RBC membr	704.33 ± 63.09	876.31 ± 42.27**
phospholipid (nmol / mg protein) – RBC membr	717.57 ± 67.36	540.2 ± 48.97**
cholesterol: phospholipid – RBC membr	0.861 ± 0.095	1.229 ± 0.12**

** P less than 0.01.

The neurotransmitter pattern of reduced dopamine and noradrenaline, and increased serotonin can contribute to cancer related psychosis. This neurotransmitter pattern is common in multiple myeloma and schizophrenia.[38] A schizoid state of mind predispose the patients to the development of neoplasms. Alteration in natural killer cell activity has been reported in psychiatric disorders.[39] Serotonin and acetyl choline promote cell proliferation and dedifferentiation by inhibiting adenyl cyclase or by activating phospholipase-C (PLC).[40] Nicotine by binding to the nicotinic receptor promotes cholinergic transmission. Dopamine and noradrenaline elevate cyclic AMP levels and inhibit cell proliferation and differentiation.[40] Increased quinolinic acid can lead on to cancer related

Table 3. **Serum free radicals and free radicals scavenging enzymes in multiple myeloma**

Groups	*Control (1)*	*Multiple myeloma (2)*
MDA µm / ml RBC	10.830 ± 0.432	12.633 ± 0.291**
hydroperoxide µm / ml RBC	253.60 ± 10.18	267.33 ± 5.30**
conjugated dienes µm / ml RBC	49.33 ± 2.53	54.94 ± 2.81**
nitric oxide µm / g protein	2.835 ± 0.207	3.341 ± 0.158**
glutathione µg / ml RBC	256.60 ± 10.96	233.23 ± 12.99**
superoxide dismutase units / mg protein	43.14 ± 1.94	32.50 ± 1.52**
catalase $x10^{-2}$units / mg protein	3.486 ± 0.117	2.125 ± 0.084**
GSH peroxidase units / g protein	48.10 ± 1.64	41.52 ± 1.29**
GSH reductase units / g protein	8.370 ± 0.487	6.651 ± 0.312**

** P < 0.01

cachexia.[12] Serotonin, dopamine and noradrenaline receptors have been demonstrated in lymphocytes. It has been reported that during immune activation, serotonin is increased with the corresponding reduction in dopamine and noradrenaline in the brainstem monoaminergic nuclei.[13] Thus elevated serotonin and reduced noradrenaline and dopamine can contribute to the immune activation and immunoproliferation in multiple myeloma. Decreased morphine levels can lead to increased metastatic property of tumors as morphine has a suppressing effect on tumor metastasis and tumor growth.[13]

Digoxin can promote NMDA excitotoxicity. The increased presynaptic neuronal calcium can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling.[41] Increased intracellular calcium in the post synaptic neuron can also activate the calcium dependent NMDA signal transduction. The plasma membrane neurotransmitter transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a sodium gradient which is disrupted by the inhibition of Na^+-K^+ ATPase, resulting in decreased clearance of glutamate by presynaptic and glial uptake at the end of synaptic transmission. By these mechanisms, inhibition of Na^+-K^+ ATPase can promote glutamatergic transmission.[41] The elevated levels of quinolinic acid and serotonin can also contribute to NMDA excitotoxicity. Quinolinic acid and serotonin are positive modulators of the NMDA receptor.[41] Glutamate excitotoxicity has been implicated in the pathogenesis of neuronal degeneration. This could explain the increased incidence of paraneoplastic motor neuron disease in multiple myeloma. Increased glutamatergic transmission resulting in excitotoxicity has been implicated in cellular proliferation. Excitatory aminoacids, such as glutamate, can act as trophic factors and promote cellular proliferation.[40]

Table 4 . **Concentration of alpha tocopherol acetate, iron binding capacity, ceruloplasmin and albumin in multiple myeloma.**

Groups	*Control (1)*	*Multiple myeloma (2)*
alpha tocopherol acetate (µg / ml RBC)	5.253 ± 0.328	3.216 ± 0.308
ironbinding capacity (µg / dl)	261.80 ± 16.00	219.64 ± 14.21**
ceruloplasmin (mg / dl)	35.81 ± 1.53	30.26 ± 1.79**
albumin (µg / dl)	4.78 ± 0.05	3.21+0.01**

** P < than 0.01

The elevation in the level of dolichol may suggest its increased availability for N- glycosylation of proteins. Previous studies have shown that digoxin administration can upregulate glycosaminoglycan and glycolipid synthesis (unpublished report). The results now obtained show an increase in the concentration of serum total GAG, individual GAG fractions, glycolipids and carbohydrate components of glycoproteins in multiple myeloma. The increase in the carbohydrate components, total hexose, fucose and sialic acid in multiple myeloma was variable, suggesting qualitative changes in glycoprotein structure. The activity of GAG degrading enzymes and that of glycohydrolases showed significant increase in the serum of multiple myeloma patients. The increase in the activity of glycohydrolases and GAG degrading enzymes could be due to reduced lysosomal stability and consequent leakage of lysosomal enzymes into the serum. The increase in the concentration of carbohydrate components of glycoproteins and GAG despite of increased activity of many glycohydrolases may be due to their possible resistance to cleavage by glycohydrolases consequent to qualitative changes in their structure.[42] Proteoglycan complexes formed in the presence of altered calcium/magnesium ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate. In multiple myeloma, interaction of tumor and host cells with adhesion and extracellular matrix molecules like heparan sulphate proteoglycan and syndecan are important.[10] Elevated levels of heparan sulphate may favor an upregulated interaction between tumor and host cells with adhesion and exracellular matrix molecules. This interaction is

important in the pathogenesis of myeloma. Accumulation of structurally abnormal glycoproteins leading to amyloid deposition has been described in myeloma. The abnormal glycoconjugate metabolism and lysosomal instability reported here may be important in amyloid deposition. Abnormal glycoconjugates accumulation can lead to neuronal degeneration such as motor neuron disease described in myeloma.

The protein processing defects observed can result in defective glycosylation of tumor antigens and viral glycoprotein antigens with consequent defective formation of MHC-antigen complex.[43] This results in defective transport of MHC class-1 glycoprotein antigen complex to the antigen presenting cell's surface for recognition by CD_4/CD_8 cell/NK cell.[37] Defective presentation of exogenous viral antigens can produce immune evasion by the virus leading to herpes viral persistence and oncogenesis in multiple myeloma.[44] Kaposi's sarcoma associated herpesvirus (KSHV) was found in the bone marrow dendritic cells of multiple myeloma patients but not in malignant plasma cells or bone marrow dendritic cells from normal individuals or patients with other malignancies. In addition, the virus was detected in bone marrow dendritic cells from two out of eight patients with MGUS. Viral interleukin-6, the human homolog of which is a growth factor for myeloma was found to be expressed in the myeloma bone marrow dendritic cells. KSHV may be required for transformation from MGUS to myeloma and perpetuate the growth of malignant plasma cells.[44] Defective presentation of endogenous tumor antigens can lead to the loss of NK cell immunosurveillance and oncogenesis.[43] Altered cell surface glycoproteins, glycolipids and GAG can lead to defective contact inhibition and oncogenesis.[37] A number of fucose and sialic acid containing natural ligands have been implicated in neoplastic transformation and metastasis as well as in immune activation and lymphocytic proliferation too.[9]

The upregulation of isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipase A_2 and D. The RBC membrane cholesterol was increased while the phospholipids were reduced resulting in a increased cholesterol:phospholipid ratio. The concentration of total GAG, hexose and fucose residues of glycoprotein decreased in the RBC membrane and increased in the serum suggesting their reduced incorporation into the membrane and defective membrane formation. The change in membrane structure produced by alteration in glycoconjugates and cholesterol:phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase resulting in further membrane Na^+-K^+ ATPase inhibition. Similar changes can affect the structure of organalle membranes. This results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum. Lysosomal instability can contribute to abnormal glycoconjugate metabolism important in paraneoplastic neuronal degeneration and amyloidogenesis. Defective peroxisomal membranes lead to catalase dysfunction which has been documented in multiple myeloma.

The concentration of ubiquinone decreased significantly in most of the cases which may be the result of low tyrosine levels, reported in multiple myeloma consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine.[11]. The aromatic ring portion of ubiquinone is derived from the tyrosine. Ubiquinone, which is an important component of mitochondrial electron transport chain, is a membrane antioxidant and contributes to free radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore causing a collapse of the hydrogen gradient across the inner membrane and uncoupling of the respiratory chain.[45] All this leads to a defect in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of superoxide ion which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free radical scavenging. The increase in intracellular calcium may lead to increased generation of NO by inducing the enzyme nitric oxide synthase which combines with superoxide radical to form peroxynitrite. Increased calcium also can activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Increased generation of free radicals like the superoxide ion, and hydroxyl radical can produce lipid peroxidation and cell membrane damage which can further inactivate Na^+-K^+ ATPase triggering the cycle of free radical generation again. The free radicals and scavenging enzymes were estimated this disorder. There was an increase in lipid peroxidation as shown by the increase in the concentration of MDA, conjugated dienes, hydroperoxides and NO with decreased antioxidant protection as indicated by decrease in ubiquinone and reduced glutathione in multiple myeloma. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in multiple myeloma suggesting reduced free radical scavenging. Alpha- tocopherol values were unchanged in this neoplasm. In our study, the iron binding capacity and serum ceruloplasmin are reduced, suggesting increased amounts of free iron and copper, promoting free radical generation. Ceruloplasmin is a 132 KD monomeric copper oxidase which has been implicated in iron metabolism because of its catalytic oxidation of Fe^{2+} to Fe^{3+} (feroxidase activity).[46] In the presence of iron in Fe^{2+} form, the conversion of H_2O_2 to hydroxyl radical is greatly increased. Low ceruloplasmin results in more of the iron to be in Fe^{2+} form. It has been shown that ceruloplasmin

increases iron uptake by cells increasing the apparent affinity for the substrate by a factor of 3. Low ceruloplasmin levels can result in decreased iron uptake and this results in increased amount of free iron. The peroxisomal membrane is defective owing to membrane Na^+-K^+ ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Glutathione is synthesised by the enzyme glutathione synthetase which needs ATP. The low ATP levels consequent upon digoxin-induced mitochondrial dysfunction can result in decreased synthesis of glutathione. Glutathione peroxydase, a selenium containing enzyme oxidises reduced glutathione (GSH) to oxidised glutathione (GSSG) which is then rapidly reduced to GSH by glutathione reductase. There is also a concomittant conversion of H_2O_2 to H_2O. Thus the glutathione system of free radical scavenging is defective in the presence of membrane Na^+-K^+ ATPase inhibition. Superoxide dismutase exists in a mitochondrial and cytoplasmic form. Opening of the mitochondrial PT pore produces hyperosmolality and matrix expansion rupturing the outer membrane producing loss of the mitochondrial dismutase and a decrease in its activity.[45] The reduction in catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase suggests reduced free radical protection. Mitochondrial dysfunction related free radical generation has been implicated in the oncogenesis. Free radicals are required for the action of growth factors and promote cellular proliferation. Mitochondrial dysfunction and free radical generation can also contribute to neuronal a degeneration like motor neuron disease described in multiple myeloma.

The increased intracellular calcium and ceramide related opening of the mitochondrial PT pore also lead to volume dyregulation of the mictochondria causing hyperosmolality of the matrix and expansion of the matrix space.[45] The outer membrane of the mitochondria ruptures and release cytochrome C into the cytoplasm. This results in activation of caspase-3. Caspase 3 activation can cleave and inactivate p21 involved in oncogenesis.[45]

Increased intracellular calcium activates calcium dependent calcineurin signal transduction pathway which can produce T cell activation and secretion of Interleukin-6 and TNF alpha.[47] Interleukin-6 can stimulate the growth of myeloma cells by functioning as a autocrine growth factor. IL-6 was found to induce in vitro growth of myeloma cells. Myeloma cells spontaneously produced IL-6 and expressed IL-6 receptor.[48] This can explain the immune activation in multiple myeloma and related paraneoplastic syndromes, snch as motor neuron disease. Membrane Na^+-K^+ ATPase inhibition can produce immune activation and is reported to increase CD4/CD8 ratios as exemplified by the action of lithium. Defective presentation of glycoprotein neuronal antigens to the CD4 and CD8 cell can explain the immune dysregulation and autoimmunity describe in paraneoplastic syndromes.

Thus the defective isoprenoid pathway is important in the pathogenesis of multiple myeloma. It can be a cause or a consequence of multiple myeloma. (i). Altered intracellular calcium can promote ras oncogene activation (ii). Protein processing defect and defective presentation of tumor antigens and defective immunosurveillance. This can also lead to defective contact inhibition. Defective processing of viral glycoprotein antigens and their presentation can lead to viral persistance and oncogenesis. (iii). Mitochondrial defect and free radical generation. This also leads to caspase-3 activation and cleaving of p21 protein which couples cell division to DNA duplication. (iv) Digoxin related tryptophan/tyrosine transport defect leading to increase in neurotransmitters that promote cell proliferation (nicotine and serotonin) and decrease in neurotransmitters that inhibits cell proliferation (dopamine and noradrenaline). This also leads to quinolinic acid related cachexia and immunoproliferation (v) Increased production of farnesyl phosphate leading on to farnesylation of ras oncogene and its activation.

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