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Editorial

National Health Policy 2017 (NHP 2017): Biomedical Research and Technology

It was after a gap of nearly 15 years that revised National Health Policy has been finally approved by the Union Cabinet in March this year. It envisages to 'achieve the highest possible level of good health and well-being for all Indians through a preventive and promotive healthcare orientation in all developmental areas, and to achieve universal access to good quality health care services without anyone having to face financial hardship as a consequence.' Though the policy will be subjected to further debate by various stakeholders and organizations for action plans development over a period of months, policy in itself was based on four assumptions. First, the health priorities are ever changing. Maternal and child mortality have rapidly declined but there is growing burden on account of non-communicable diseases and some infectious diseases. The second is the emergence of a robust health care industry estimated to be growing at double digit. The third is the growing incidences of catastrophic expenditure due to health care costs, which are presently estimated to be one of the major contributors to poverty. Fourth, is a rising economic growth enables enhanced fiscal capacity.

The Policy envisages providing larger package of assured comprehensive primary healthcare through the 'Health and Wellness Centres' and denotes important change from very selective to comprehensive primary health care package, assuring availability of free, comprehensive primary health care services, for all aspects of reproductive, maternal, child and adolescent health and for the most prevalent communicable, non-communicable and occupational diseases in the population.

The policy recommends an expansion of scope of interventions to include detection and response to early childhood development delays and disability, adolescent and sexual health education, behavior change with respect to tobacco and alcohol use, screening, counseling for primary and secondary prevention from common chronic illnesses –both communicable and non-communicable diseases.

Schools may act as a site for primary health care incorporating health education as a part of the curriculum, thereby promoting hygiene and safe health practices starting from school environs itself.

This issue of Annals reflects some of the subjects touched upon in NHP 2017 and resonate the concern that non-communicable diseases are emerging and posing a big important public health problem in India. There is an ever increasing demand on research into multifaceted aspects of Diabetes mellitus and Cardio-metabolic disorders. High incidence of infections in diabetes perplexed the physicians as well as biomedical researcher. Whether or not hyperglycemia imposes an independent risk for infection is an unresolved question till date. Several epidemiologic studies have shown that diabetics receive treatment for infections more often than non-diabetics. However, the magnitude of the effect of diabetes on the risk of infection remains an active research question. Studies have explored host factors and found that neutrophil chemotaxis and adherence to vascular endothelium, phagocytosis, intracellular bactericidal activity, opsonization, and cell-mediated immunity are all depressed in

diabetics with hyperglycemia. Chawla *et al* have studied the circulating LL-37 antimicrobial-peptide (also referred as Cathelicidin) and compared groups with short and long term glycemic status and published their findings in this issue of Annals. The research further increases our curiosity in this very important disease and a call for finding alternative in research in this NCD domain.

Pande, Kaur and Sachdev in their retrospective-cum-prospective cohort study published in this issue of Annals have shown that prevalence of obesity, hypertension, diabetes mellitus and metabolic syndrome is high in our community. Very rightly, the NHP 2017 articulates the need for the development of strategies and institutional mechanisms in seven areas, to create Swasth Nagrik Abhiyan –a social movement for health. It recommends setting indicators, their targets as also mechanisms for achievement in each of these areas. These seven areas are –

- The Swachh Bharat Abhiyan,
- Balanced, healthy diets and regular exercises,
- Addressing tobacco, alcohol and substance abuse,
- Yatri Suraksha – preventing deaths due to rail and road traffic accidents,
- Nirbhaya Nari –action against gender violence,
- Reduced stress and improved safety in the work place, and
- Reducing indoor and outdoor air pollution.

Another very important area in NHP 2017 is mental health programs with due recognition to National Mental Health Policy 2014. The policy suggests training community members to provide psychological support to strengthen mental health services in the country. Collaboration with government would be an important plank to develop a sustainable network for community/locality towards mental health. Creating network of community members for support and leveraging digital technology in a context where access to qualified psychiatrists is difficult is suggested to be adopted to fill gaps in mental health services. It poses additional responsibilities for psychiatrists and psychologists to explore and find solutions to common morbid conditions and improving awareness among public at large and health professionals in particular.

Chadda *et al*, in their article in this issue of Annals, have estimated that nearly 20-40 % of medical-surgical patients have comorbid psychiatric or psychosocial problems, often unrecognized by treating physicians. Using a cross-sectional, descriptive, online questionnaire-based study on Consultation Liasion Psychiatry, study noted deficiencies including stigmatization and suggested need for better teamwork, training and manpower development to provide optimal care. It is a high time that NHP suggestions be implemented across continuum from primary to tertiary care.

Recognizing the integral role of technology (eHealth, mHealth, Cloud, Internet of things, wearables, etc) in the healthcare delivery, a National Digital Health Authority (NDHA) has been proposed to be set up in NHP 2017 to regulate, develop and deploy digital health across the continuum of care. The policy advocates extensive deployment of digital tools for improving the efficiency and outcome of the healthcare system.

Apart from using technology for improving healthcare, there is a need to integrate technology in all

aspects of public health care. There is a need for exploration of technology in education, device development, and optimization of surgical gadgets, linking with community and creating awareness. This will not be possible working in-silos but involving technocrats in healthcare teams at all levels of implementation to reap the benefits.

Saxena *et al* have demonstrated value of non-invasive uro-flowmetry in diagnosing urinary tract dysfunction at an earlier stage. This research also throw light on using technology in patient care, improving patient satisfaction and decreasing morbidity associated with delayed diagnosis.

Use of high level technology has also been demonstrated by the work of Natarajan *et al* and by Singh *et al* in the issue. While Natarajan and coworkers in their study have shown value of custom mega prosthesis in patients with metachronous osteosarcoma, Singh et al have reviewed in detail the role of human mesenchymal stem cells in tissue repair and regeneration.

We do visualize a future where the health delivery will leverage on optimal use of technology creating more demands on health professionals to keep themselves abreast with the newer developments. It will challenge medical educationists to evolve strategies to prepare the current generation of physicians learn differently and prepare them future ready to face and tackle appropriately newer challenges as and when they arise. The NHP 2017 though has touched upon this aspect with suggestion to review present PG entrance examination, has given little thought with focused attention on actions at entry level for raw students who are beginning their journey in the medical field lacking appropriate maturity and aptitude. Studying under a highly competitive education system prevent the present day health professionals from developing resilience and are vulnerable to stress of highly demanding healthcare system which is inconsiderate to the working environment for physicians. There is a need for a change in overall education system keeping a balance between Indian values and evidence-based pedagogy from West. This also require culturally appropriate research on teaching technologies in Indian settings and scientific evidence thus derived must be used for policy enunciation and should not be based merely on biased opinions.

Lastly, as the NHP 2017 concludes that a policy is as good as its implementation, one should start implementing right away as far as the resources permit. Small incremental steps, as is said, fetch big dividends in a long run.

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Circulating Antimicrobial Peptide LL-37 Status in Type 1 Diabetes Mellitus and its Relation with Glycemic Control

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ABSTRACT

Antimicrobial-peptides are important molecules of constitutive innate immunity. Though patients with diabetes mellitus are generally prone to infections, there is limited information on their antimicrobial-peptide status. We assessed the circulating LL-37 antimicrobial peptide (also referred as cathelicidin) levels in patients with type 1 diabetes mellitus and its relation with their glycemic status. The LL-37 mRNA expression was assessed in the peripheral blood mononuclear cells (PBMC) by quantitative RT-PCR using *ß-actin* and *cytochrome-C1* as the reference genes in 154 subjects (Type 1 diabetes, n=111 and healthy subjects, n=43). Serum LL-37 was quantified using sandwich-ELISA. Average HbA1c over last 2 years and current HbA1c were used to determine long-term and short-term glycemic status. LL-37 mRNA expression and serum LL-37 levels were correlated with the glycemic status. The LL-37 mRNA copies were comparable between type 1 diabetes and healthy subjects [median (IQR) = 6.7 (1.8–15.28) vs. 7.2 (2.23–21.86), respectively, P = 0.42]. There was no significant difference in serum LL-37 levels between the two groups [median (IQR) = 3.9 (2.88–7.52) vs. 5.0 (3.19–9.05) ng/ml, respectively, P = 0.52]. The LL-37 mRNA and its protein concentration showed no significant correlation with the average or current HbA1c values. The constitutive circulating antimicrobial peptide LL-37 status is not significantly altered in patients with type 1 diabetes mellitus and also not affected by their glycemic status.

Keywords: Innate-immunity, diabetes mellitus, antimicrobial peptide LL-37, cathelicidin, glycosylated hemoglobin.

Introduction

Recurrent bacterial and fungal infections of genitourinary tract, skin, and lungs are common in patients with type 1 diabetes mellitus (T1DM) (1-3). The reasons of increased susceptibility to infections in diabetes are not clear but could involve hyperglycemia mediated abnormalities in innate immunity involving polymorphonuclear cell dysfunction and various antimicrobial peptides (4-5). The LL-37 antimicrobial peptide (cathelicidin) is an essential component of the innate immunity. It is produced by several cells such as leukocytes, keratinocytes and mucosal epithelial cells to promote phagocytosis in macrophages, dendritic cell differentiation, chemokine production, keratinocyte migration and wound healing. LL-37 also inhibits apoptosis of leukocytes and biofilm formation (6-9).

Though there is increasing interest in the role of LL-37 in bacterial infections, HIV and tuberculosis, the information on LL-37 expression in diabetes is limited. Since patients with uncontrolled diabetes may be particularly

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prone to infections, LL-37 expression might be altered in them (10-13). Earlier studies involving limited number of patients showed variable status of LL-37 in diabetes. Gonzales *et al* and Santiago *et al* reported reduced LL-37 mRNA expression in circulating leukocytes in DM and peri-ulcer skin biopsy of patients with diabetic foot, respectively (11, 12) and Brauner *et al* showed reduced LL-37 levels in T1DM as compared to patients with type 2 diabetes (13). The relationship of glycemic control with circulating LL-37 has not been studied till date. Present study investigated LL-37 mRNA expression in peripheral blood mononuclear cells (PBMC) and serum LL-37 concentration in a cohort of 111 patients with T1DM and assessed its relation with their glycemic status.

Materials and Methods

The study included 111 patients with T1DM attending 'Diabetes of young' clinic at the All India Institute of Medical Sciences, Delhi. Pregnant, lactating women and patients with overt bacterial infections were excluded. Their details including age at onset of diabetes, nephropathy, retinopathy, pulmonary tuberculosis and serial HbA1c values during previous two years were noted from clinical records. Forty three healthy subjects with normal HbA1c who consented for the study were included for comparison.

Quantitative Real Time Reverse Transcription PCR (qRT-PCR) for LL-37 mRNA Expression

Ten ml blood was collected in fasting state in nuclease-free heparinized tube for isolating PBMC by Ficol and RNA extraction using Trizol (Invitrogen, Carlsbad) (14). RNA was quantified using UV spectrophotometer (GeneQuant, Amersham) and its quality was assessed by agarose gel electrophoresis and RNA integrity number (Bio-analyser 2100, Agilent Technologies Inc.). First strand of cDNA was prepared using 2.0µg of total RNA, random hexamer nucleotides and M-MuLv reverse transcriptase in 20µl reaction incubated at 25˚C for 10 min followed by 42˚C for 1 hour (14).

The qRT-PCR for LL-37 mRNA was carried out using SYBR-mix (Biorad, Hercules, USA) in CFX96 cycler (Bio-Rad) in 20µl reaction using LL-37 gene-specific primers (sense-5*'gcggtggtcactggtgctcctgctgct3'* and antisense-5*'gaagaaatcacccagcagggcaaatc3'*) (14). The RNA expression of housekeeping-genes (*ß-actin* and *cytochrome C1*) was used as control. The sense and antisense primers for *ß-actin (5'catgtacgttgctatccaggc3' and 5'ctccttatgtcacgccacgat3')* and *cytochrome C1 (5'agctgccaacaacggagcat3' and 5'gactgaccacttgtgccgct3')* were designed using 'Primer-3-software' (http://www.ncbi.nlm.nih.gov/tools/primer-blast). The PCR conditions were 94°C x 3 min, 39 cycles of 94°C x 30s, 55°C for *ß-actin* and 60°C for LL-37 and *cytochrome C1* each x 30s, 72°C x 30s , 86°C x 20s, final extension 72°C x 10 min. The intra-assay and inter-assay variation were 0.9-1.7% and was 3-5%, respectively. All the reactions were performed in duplicates, and specificity of amplified products was checked by post-PCR melt-curve analysis and agarose gel electrophoresis (Fig.1). The specificity of amplified products of LL-37 was checked by DNA sequencing and that of *ß-actin* and *cytochrome C1* by RFLP using Eco0109I and TaqI restriction enzymes, respectively. The mRNA copy number of LL-37 was determined per 10^3 copies of geometric mean of *ß-actin* and *cytochrome C1.*

ELISA for Serum LL-37 Concentrations

A sandwich ELISA with LL-37 specific antibody precoated on to 96 well microplate was used (MyBiosource Inc., USA). Briefly, blood was allowed to clot at room temperature, centrifuged at 1000xg for 15 minutes at 4°C and serum was stored at -80°C. Assay was carried out using 100 µl of serum along with seven standards ranging from 1.56-100 ng/ml. Avidin-HRP was used in conjunction with biotinylated detection antibodies as second step reagent for indirect enzymatic labeling. Serum samples

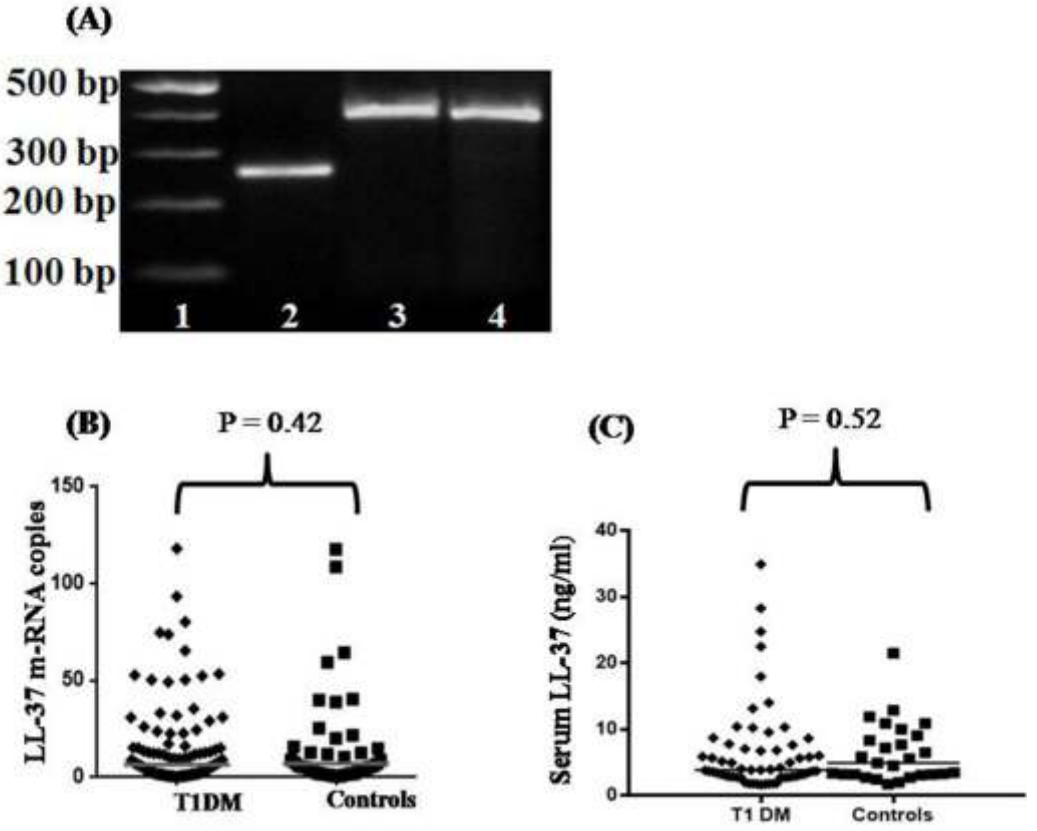

Fig.1: Agarose gel (1.5%) electrophoresis showing the PCR products of *ß-actin*, CYC1 and LL-37 (lanes 2,3,4, respectively), Lane 1: 100 bp DNA ladder (A); LL-37 expression in T1DM and controls, mRNA copies/10^3 reference genes (B) and serum LL-37 concentration (C)

from patients and healthy subjects were put in the ELISA plates in 2:1 ratio to minimize assay-related variation. The ELISA was carried out as per the kit protocol with optical density measured at a wavelength of 450 nm using i-Mark reader (Biorad). The specific absorbance was plotted by four-parameter logistic curve fitting. The assay had minimum detection limit of 0.94 ng/ml of natural and recombinant LL-37, coefficient of variation < 10% and reportable range between 1.56-100.0 ng/ml.

The study was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, New Delhi. Written informed consent was obtained from all subjects.

Statistical Analysis

Data are shown as mean ± SD, frequencies and median with interquartile range. The differences in the study parameter between patients with diabetes and healthy subjects were compared using Student's 't' test or by Mann' Whitney 'U' test as appropriate. Differences in the frequency of subject with serum LL-37 value below the detection range of the assay (<1.56 ng/ml) between diabetes and controls were analyzed using chi Square test. Pearson's correlation coefficient was used to assess relationship between LL-37 mRNA expression with average HbA1c over last two years and current HbA1c. Similar analysis was also carried out for serum LL-37. All the statistical analyses were implemented on SPSS 11.5 (SPSS Inc., USA). A two-tailed P value of < 0.05 was

considered significant.

Results

Table 1 shows the clinical and biochemical characteristics of the study subjects. The mean age and male: female ratio of patients (28.6 ± 11.68 years and 56:55) and healthy subjects (29.6 ± 5.86 years and 23:20) were comparable. The mean BMI was significantly less in the diabetes group than healthy subjects (20.6 ± 4.0 kg/m^2 and 25.1 ± 2.79 kg/m^2, respectively, P <0.001). The mean HbA1c was higher in diabetes than healthy subjects [9.2 ± 1.98% (77.1 ± 21.78 mmol/mol) and 5.4±0.33% (35.1 ± 3.62 mmol/mol), P <0.001]. The mean duration of diabetes was 15.4 ± 9.12 years and history of diabetic ketoacidosis was present in 76 (68%) patients. GAD_{65} auto-antibodies were positive in 58.6% of the 87 patients tested. Retinopathy and proteinuria >150 mg/24 hours were present in 22.5% and 5.4%, respectively. The mean hemoglobin and leukocyte counts were in normal range in both patients and healthy subjects.

LL-37 mRNA Expression and its Serum Concentration in Diabetes

The mean LL-37 mRNA expression was comparable between diabetes and healthy groups. In view of the skewed distribution of LL-37 mRNA expression, the two groups were compared using non-parametric test. There was no difference in the median copy number of LL-37 mRNA) between the two groups (Table 1). The median serum LL-37 protein concentration were comparable between diabetes and healthy subjects [median (IQR) = 3.9 (2.88–7.52) vs. 5.0 (3.19–9.05) ng/ml, respectively, P = 0.52]. Similarly, the frequency of subjects with serum LL-37 below the detection range were comparable between diabetes and control groups (45.9% vs. 37.2%, respectively, P = 0.42).

Table 1: Clinical and biochemical characteristics of study subjects

Parameter	Diabetes (n=111)	Healthy subjects (n=43)	t and U-values	P
Age (years)	28.6 ± 11.68	29.6 ± 5.86	U= 2152.0	0.35
Male: Female (n)	56:55	23:20	-	0.86
Body mass index (kg/m^2)	20.6 ± 4.00	25.1 ± 2.79	U = 803	<0.001
HbA1c (mmol/mol)	77.1 ± 21.78	35.1 ± 3.62	U = 1.0	<0.001
Total leukocytes (count/mm^3)	7462 ± 1920	7039 ± 2041	t = 1.2	0.24
Polymorphs (%)	59.1 ± 9.71	58.7 ± 6.05	U = 1051	0.98
Lymphocyte (%)	30.4 ± 8.47	29.9 ± 4.14	U = 1014	0.79
Erythrocyte sedimentation rate (mm/hr)	17.6 ± 13.36	15.7 ± 9.3	U = 2333	0.90
LL-37 mRNA/10^3 housekeeping copies Median (IQR)	6.7 (1.8 – 15.28)	7.2 (2.23 – 21.86)	U = 2186	0.42
Serum LL-37 (ng/ml) Median (IQR)	3.9 (2.88–7.52) (n = 60)	5.0 (3.19–9.05) (n= 27)	U = 740.5	0.52

Correlation of LL-37 mRNA Expression and Serum LL-37 Protein with Glycemic Status

The LL-37 mRNA expression showed no significant correlation with the current HbA1c ($r = -0.014$, $P = 0.88$) and the average HbA1c during previous two years ($r = 0.105$, $P = 0.27$). Similarly, there was lack of significant correlation between the serum LL-37 concentration and current HbA1c ($r = 0.05$, $P = 0.72$) and average HbA1c ($r = 0.03$, $P = 0.83$).

Eleven of 111 T1DM patients had *M.tb* positive sputum culture. The LL-37 mRNA expression and its serum concentration showed no significant differences between patients with and without sputum culture positive for *M.tb*.

Discussion

The reasons of susceptibility to infections in patients with diabetes and their relation to hyperglycemia are under investigation (7-9, 15-17). Antimicrobial peptides are a key component of the first and second layer of defense of the body. Its presence in the skin and mucosal epithelial barrier supplement the first layer of defense. Polymorphonuclear cells secreting LL-37 pass through various compartments of the body to provide defense against various pathogenic organisms (9, 18). Recently, there has been interest in the altered expression of circulating LL-37 in diabetes (11, 13). However, results have been variable, which could be due to the limited number of subjects in these studies (11, 13). The present study has been carried out to assess possible alteration in the LL-37 expression among patients with diabetes. The strength of the study are a large cohort of patients with T1DM, age matched healthy subjects, measurement of both mRNA expression and serum LL-37 concentration and the availability of HbA1c data of patients during previous two years.

The present study showed no significant alteration in the LL-37 mRNA expression and circulating LL-37 protein concentration in patients with diabetes. Interestingly, both LL-37 mRNA and LL-37 protein concentration also showed no significant relationship with current or average HbA1c indicating that circulating LL-37 expression is not altered with severity of hyperglycemia. There have been only two previous studies reporting mRNA expression in the PBMC and serum concentration of LL-37 in patients with diabetes. Gonzalez *et al* reported significantly lower mRNA expression of LL-37 in 30 patients with T2DM compared to healthy subjects ($P < 0.001$) (11). Brauner *et al* assessed the serum concentration of LL-37 in 58 patients with diabetes (27 T1DM and 31 T2DM) and 19 healthy subjects (13). The study revealed significantly lower serum LL-37 concentration T1DM compared to those with T2DM. However, the LL-37 levels among patients with T1DM were comparable to the control group. Interestingly, in the current study we did not find any significant difference in LL-37 expression between patients with and without sputum *M.tb* positivity. These preliminary findings need to be further assessed in future studies targeting larger cohort of diabetes patients with and without active tuberculosis.

Thus, the lack of significant difference in the serum LL-37 concentration between patients and healthy subjects in current study coupled with no significant relationship of LL-37 with glycemic control suggest that constitutive LL-37 expression is not altered in patients with diabetes at least in the circulation. Further, the expression of LL-37 peptide is not affected by adverse glycemic status in patients with T1DM.

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Declaration of Interest

The authors have nothing to disclose and there is no conflict of interest.

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Ethics

The study was carried out after approval from the Ethics Committee of the All India Institute of Medical Sciences, New Delhi (Reference IESC/T-296/08.08.2014 vide letter 23-8-2014). Written informed consent was obtained from all the patients and controls.

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Custom Mega Prosthesis in Metachronous Osteosarcoma

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ABSTRACT

Background: The objective of the current study was to determine the incidence, clinical and pathologic characteristics, and outcome of patients with conventional osteosarcoma who developed metachronous tumours and treated by limb salvage surgery with Custom Mega Prosthesis.

Methods: Among 1198 osteosarcoma patients who were treated with limb salvage surgery and implantation of custom mega prosthesis, 6 patients were found to have metachronous lesions. The absence of pulmonary metastases was confirmed by chest radiographs and computed tomography while radionuclide bone scan and biopsy were used to confirm the absence of skeletal metastases. The patients were treated by limb salvage surgery with custom mega prosthesis for the metachronous tumour and functional outcome was evaluated by MSTS scores.

Results: Index primary tumours involved the femur (n=3) and the tibia (n=3) and were treated with limb salvage surgery using endoprosthetic reconstruction. Single metachronous tumours developed in the all of these patients with the interval between identification of the primary tumour to development of the metachronous tumours varying from 18 months to 41 months. All metachronous tumours were treated with neoadjuvant chemotherapy and limb salvage surgery. We obtained excellent functional outcome for primary tumours and good functional outcome for metachronous tumours. Two patients succumbed to disease due to pulmonary and cerebral metastasis during follow-up.

Conclusions: With advances in survival rate in the multidrug chemotherapy era in the post tumours-resection period, advanced diagnostic modalities help in diagnosing metachronous osteosarcoma. It should be recognized as important sequelae in long-term survivors. Meticulous follow-up is required to permit early detection and successful therapeutic intervention. Limb salvage surgery has provided consistent good results in metachronous osteosarcoma patients.

Keywords: Metachronous tumours, osteosarcoma, skeletal metastases, limb salvage surgery.

Introduction

Eighty percent of patients with osteosarcoma are known consistently to harbour micro-metastases in the lungs at the time of diagnosis (1). These metastases are undetected in imaging studies. Untreated, they are detected 6–9 months later and usually are responsible for the patient's mortality. In a rather smaller group, skeletal metastases with soft tissue invasion were present with pulmonary metastasis.

In contrast, skeletal lesions may appear later, after treatment of the primary tumours in

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the absence of pulmonary metastasis. These lesions are designated as metachronous tumours. They did not metastasize from the primary tumours or the lungs but developed later and spontaneously in other parts of the skeleton.

Materials and Methods

During the past 28 years, 1198 patients of osteosarcoma were treated by the senior author (MVN). All these patients had osteosarcoma at one site at the time of initial diagnosis. They were treated with neoadjuvant multidrug chemotherapy, tumour resection and implantation of custom mega prosthesis. Henceforth they were serially monitored for the presence of detectable pulmonary and skeletal metastases. Six of 1198 patients were identified with single post-therapy metachronous osteosarcomas. Before the discovery of the metachronous tumours, the lungs and skeleton had been continually free of disease.

Results

The primary lesions in these 6 patients were located in the following sites: distal femur (n=3), proximal tibia (n=2) and distal tibia (n=1). These metachronous tumours appeared as single lesions in all these 6 patients. They were located in the proximal humerus (n=2), proximal tibia (n=1), tibial diaphysis (n=1), proximal femur (n=1) and distal radius (n=1). The metachronous tumours occurred at long bones in 83% (5 out of 6 sites). Among the 6 patients who developed metachronous osteosarcoma, the mean age was 24.2 years and 5 out of 6 patients were males. The interval between the diagnoses of the primary tumours and the metachronous tumours varied from 18 to 41 months. Selected illustrations of primary and metachronous tumours in individual patients are depicted in Fig. 1 and 2.

The metachronous tumours, presented with characteristics similar to those of the primary tumours. Histologic characteristics of the primary tumours were as follows: osteoblastic (n=3), chondroblastic (n=2) and periosteal (n=1). The diagnosis of all 6 metachronous tumours were confirmed by biopsy. The histology in 6 metachronous tumours were concordant with the corresponding primary tumours detected prior. The average survival period of these patients post-treatment of the metachronous tumours was 2.45 years, with 2 succumbing to disease at 8th and 18th month of follow-up. The mean

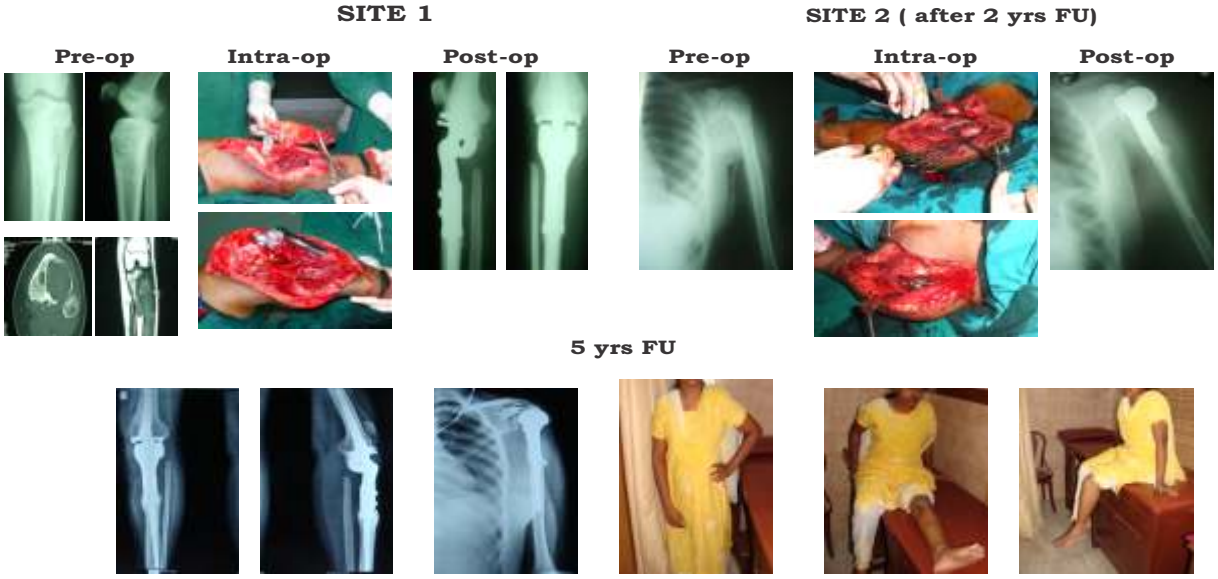

Fig. 1: Metachronous osteosarcoma (PT - PH)

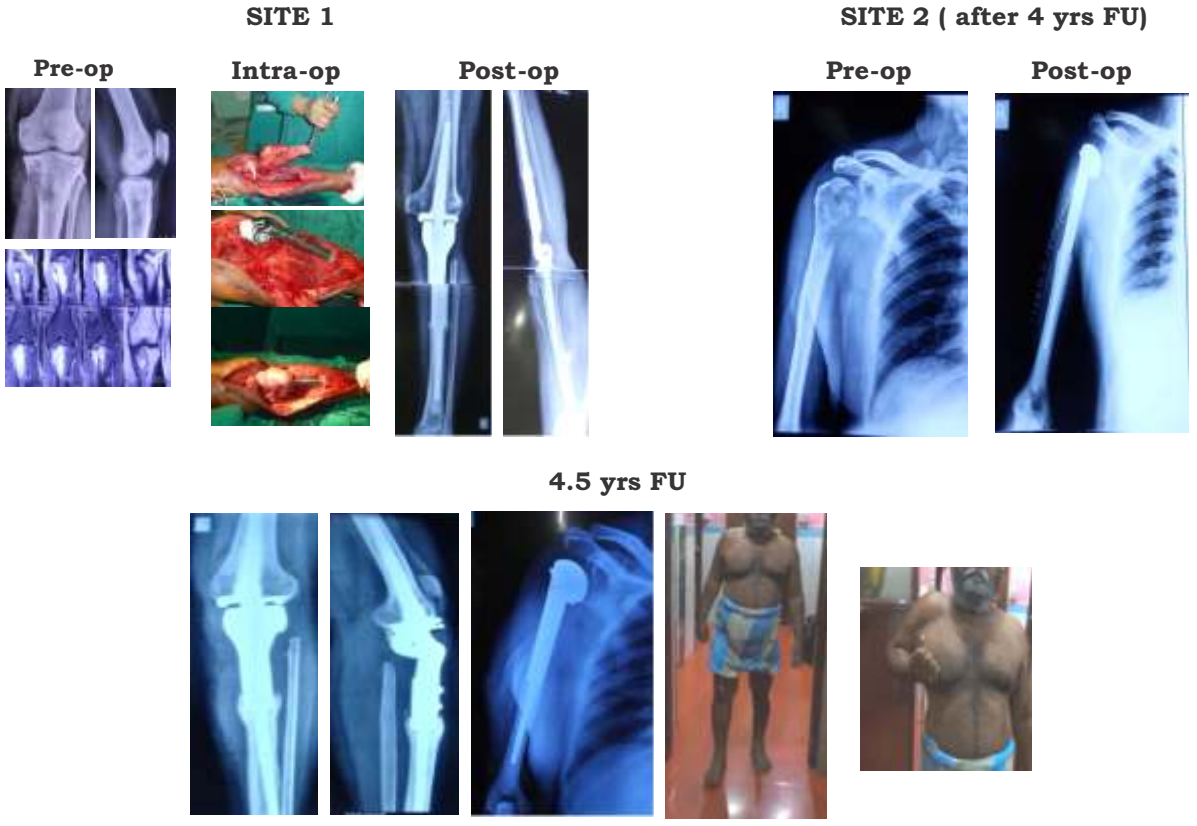

Fig. 2: Metachronous osteosarcoma (PT - PH)

Musculoskeletal Tumour Society Score (MSTS) score for functional outcome of primary tumours was 22.9 while for metachronous tumours was 19.5 with poorer outcome noted at proximal humerus and distal tibia. The patients' details are depicted in Table 1.

Discussion

Lungs are the most common site for the development of metastases in patients with osteosarcoma. Skeletal metastases although less common, about 3.6-10%, also were reported in early studies and are generally in association with pulmonary metastases (1). Lockshin and Higgins (2) similarly reported a 41% incidence of bone metastases among 22 osteosarcoma patients who were dying in hospital. These metastases were detected radiologically or at autopsy.

Compared with the detection of pulmonary and skeletal metastases (concurrently or later), the detection of single or multiple skeletal lesions without pulmonary involvement subsequent to the initial presentation of the primary tumour has been relatively uncommon. These lesions were termed as metachronous lesions (3). Several early reports suggested a multicentric origin and reported most lesions discovered in the absence of pulmonary metastases (4-13). This finding is in concurrence with our definition. These metachronous skeletal sites generally were similar to the sites affected by primary osteosarcoma. Corradi *et al* (14) reported 26 patients with 59 metachronous lesions with 76% of sites at long bones and 62% male predisposition. We report 6 cases of metachronous tumours in 1198 osteosarcoma patients (incidence: 0.5%) with 83% sites at long bones and 83% male predisposition.

Table1: Demographic details of limb salvage surgery for metachronous osteosarcoma (OS)

Sl. No.	Name/Patient (Pt. No.)	Age / Sex	Site 1 Diagnosis (A)	Site 1 Treatment	Site 2 Diagnosis (B)	Interval duration (Yrs)	Site 2 Treatment	MSTS Score	Outcome
1.	Pt1: ML	30/ M	Right Distal Femur (DF) OS with Metases -2004	CMP Right DF	Right Distal Radius (DR) OS- 2007	3	CMP Right DR	A: 24 B: 21	1.5yrs; Recurrence of Metases/ Spine/Pelvis; died of disease
2.	Pt2: SH	18/F	Left Proximal Tibia (PT) OS- 2005	CMP Right PT	Left Proximal Humerus (PH) OS -2007	1.75	CMP Left PH	A: 22 B: 20	8yrs; Disease free interval; continues to be disease free
3.	Pt3: SS	19/ M	Left Distal Femur (DF) OS- 2006	CMP Left DF	Right Proximal Femur (PF) OS- 2008	1.9	CMP Right PF	A: 24 B: 21	0.6yrs; Disease free interval-REC-died of pulmonary and cerebral Metases
4.	Pt4: VA	11/ M	Right Distal Femur (DF) OS- 2011	CMP Right DF	Right Tibia Diaphysis OS- 2012	1.75	CMP Right Tibia	A: 23 B: 18	3.5yrs; Disease free; alive
5.	Pt5:MP	17/ M	Left Distal Tibia (DT) OS -2013	CMP Left DT	Left Proximal Tibia (PT) OS- 2015	2.1	Total Tibia	A: 23 B: 18	0.5yrs; Disease free interval; alive; infection
6.	Pt 6: N	50/ M	Left Proximal Tibia (PT) OS -2011	CMP Left PT	Right Proximal Humerus (PH) OS– 2015	3.75	CMP Right PH	A: 21 B: 19	0.5yrs; Disease free interval; continues to be disease free

The etiology and pathogenesis of metachronous osteosarcoma are unknown. Batson *et al* (15) postulated that the metastases migrated through the vertebral venous system and by passed the portal, caval, and pulmonary circulations. It is now known that metachronous osteosarcoma may occur as a single lesion (16, 17) or as multiple lesions (7, 16, 17). Fitzgerald *et al* (10) described 12 patients with multiple metachronous osteosarcoma with 4 patients developing a third metachronous tumour and 2 patients developing a fourth metachronous tumour. It was not possible to determine whether the metachronous sarcomas represented late metastases or new 'primary' tumours. We report 6 cases of single metachronous osteosarcoma in our study. There are two classifications for metachronous osteosarcoma (12, 18).

The clinical and radiographic features generally resemble those of primary 'classic' osteosarcoma (1, 2). Howat *et al* reported a case of the multifocal metachronous periosteal variety (7). Because the blood supply to the periosteum is poor compared with the blood supply to intramedullary bone, they considered their cases of periosteal osteosarcoma of primary origin. We found all our metachronous lesions having same clinical, radiological and histopathological resemblance to the index primary tumour.

Speculation has been raised that other pre-existing conditions, such as Rothmund–Thomson syndrome, Paget disease, Fanconi anaemia, thyroid adenoma, childhood retinoblastoma, carcinoma of bladder and other genitourinary tumours, may contribute to the development of metachronous osteosarcoma. These diseases and cancerous conditions are known to be associated with the development of osteosarcoma and other malignant tumours, and its occurrence with osteosarcoma has been noted (17-21). We report all 6 cases, resembling the previously detected primary tumour. None of 6 patients in our series had bilateral retinoblastoma as all patients were screened and were evaluated thoroughly using PET scan.

In one of the large reported studies of metachronous osteosarcoma by Fitzgerald *et al* (10), the interval between discovery of the primary and metachronous lesions varied from 9 months to 14 years. Aung *et al* (13) reported the median latency interval between the diagnosis of the primary and the metachronous tumours was 1.5 years. Corradi *et al* (14) reported the interval

between diagnosis of primary osteosarcomas and metachronous tumour, ranging from 7 to 171 months (median: 21 months). In our series, the interval between discovery of the primary tumours and the metachronous tumors varied from 20 to 41 months (mean: 27 months, i.e. 2.4 years).

In the study by Fitzgerald *et al* (10), the survival post-metachronous tumour excision and endoprosthetic replacement varied from 5 months to 11 years. Aung *et al* (13) reported that survival was found to be correlated with time to development of the metachronous tumour and longer time interval from diagnosis of the primary to the metachronous osteosarcoma correlates with the prognosis. In study by Jaffe *et al* (22), the 5-year post-metachronous survival rate in patients who developed metachronous tumours 24 months from and after diagnosis of the primary osteosarcoma were 8% and 61%, respectively. In our study, the survival varied from 8 months to 8 years with two patients succumbing to metastasis at 8th and 18th month. However, we could not conclude that time interval from diagnosis correlated with prognosis or survival.

Conclusion

With improvement in the cure rate, metachronous osteosarcoma should be recognized as important sequelae in long-term survivor. Serial surveillance is required to permit early detection and successful therapeutic intervention. Upon the discovery of metachronous osteosarcoma, it should be treated always with curative intent. Limb salvage surgery using custom endoprosthetic replacement provides predictable results in the management of metachronous tumours with good to excellent functional outcome.

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Burden of Cardiometabolic Disorders among Subjects Undergoing Preventive Health Check-up: A Follow-up Study

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ABSTRACT

Objective: To study the incidence of hypertension (HTN), diabetes mellitus and coronary artery disease (CAD) in a cohort of adult subjects.

Methods: A total 2159 participants (mean age: 48.6±11.6 years; 1342 males and 817 females) who attended the hospital for a comprehensive health check up were recruited and followed-up as a cohort for a mean period of 3.5 years. Their baseline and follow-up evaluation included clinical examination, biochemical investigations and cardiac check-up.

Results: At baseline, 64% participants were overweight or obese, 44.3% had HTN (grade I or above), 16.7% had diabetes mellitus with additional 29.3% having impaired fasting glucose or glucose intolerance, and 46.6% had metabolic syndrome. The prevalence of CAD at baseline was 6% in males and 3% in females. The incidence (per 1000 person-years of follow-up) of HTN, diabetes mellitus and CAD was 72.2, 26.3 and 12.2, respectively.

Conclusion: The burden of cardiometabolic disorders and their risk factors is high in India. Urgent remedial public health preventive measures are required to curtail the emerging epidemic of cardiometabolic disorders.

Keywords: Hypertension, diabetes mellitus, coronary artery disease, cardiometabolic syndrome.

Introduction

India has a large burden of cardiovascular disease (CVD) due to high prevalence and suboptimal management of several risk factors including diabetes mellitus (DM), hypertension (HTN), obesity and dyslipidemia (1-3). There are few cohort studies reported from India, which provide data on incidence of coronary artery disease (CAD), HTN and DM in susceptible middle aged and elderly subjects.

A large number of apparently healthy subjects undergo preventive health check-up at Sitaram Bhartia Institute of Science and Research, New Delhi. Their evaluation includes detailed clinical history, physical examination and several investigations to assess their cardiovascular risk status. Many subjects undergo repeat evaluation after a variable period of time to assess changes in their status following life-style modifications and pharmacotherapy as prescribed at the time of the first visit. We utilized this opportunity to

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establish a follow-up cohort to determine the incidence of CAD, DM and HTN.

Methods

Study Design

Retrospective-cum-prospective cohort study.

Setting and Selection of Participants

Records of 5611 apparently-healthy adult subjects (>20 years) participating in preventive health check-up (PHC) at Sitaram Bhartia Institute of Science and Research, New Delhi from January 2000 to September 2009 were recruited as potential participants. Those with complete baseline information and fulfilling the eligible minimum follow-up of 150 days or more were invited to participate for follow-up (n=2453). Of those successfully contacted, 2098 (85.6%) consented for second evaluation, 13.7% refused and 0.7% were reported to have died (cause not known). Seventy one of these 2098 subjects were excluded from analysis on account of non-eligibility: age<20 years, period of follow-up<150 days, incomplete information and certain outliers.

Another 132 subjects were enrolled prospectively from October 2009 onwards, if the follow-up data for a minimum of 150 days was available. Thus, 2159 individuals formed the cohort and were included for analysis (Fig. 1). Clinical details were recorded in a structured form by a consultant in medicine. Information was recorded about the present symptoms, if any, with a detailed system review on direct questioning. Tobacco consumption, alcohol

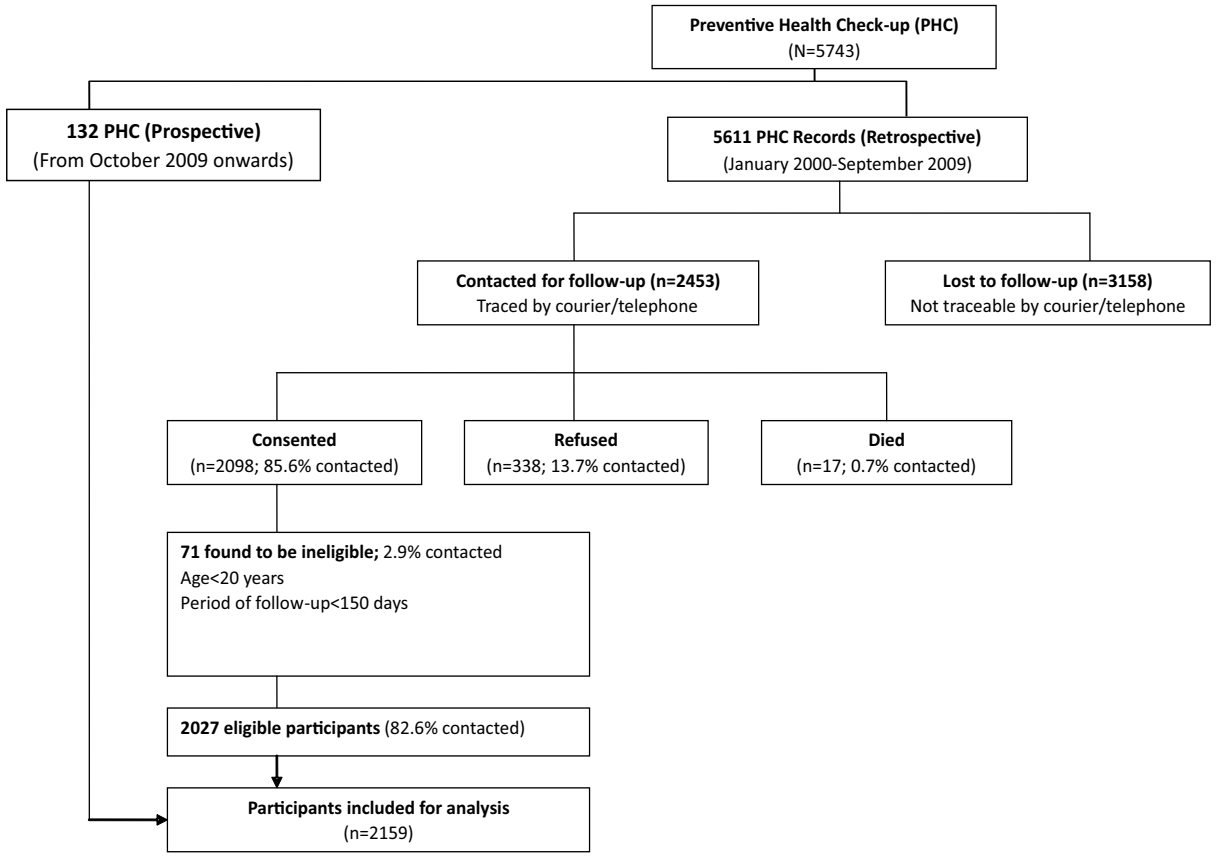


Fig. 1: Participant flow chart

intake, anthropometry (height and weight) and blood pressure were recorded. Biochemical and other clinical investigations included plasma glucose (fasting and 2 hours after 75 g glucose), lipid profile, resting 12 lead electrocardiogram (ECG) and treadmill stress test (TMT)/ echocardiography. Details of current medications, if any, were recorded. Similar observations were recorded on follow-up. Ethical clearance was obtained from the local Institutional Ethics Committee. A written informed consent was obtained from each subject for the follow-up evaluation.

Statistical Methods

Statistical analysis was undertaken using STATA (Ver 9.0). Appropriate log transformations were done for variables having skewed distributions (plasma glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c). Significance of differences between mean of continuous variables at baseline and during follow-up were determined using Student's t-test for paired values. Student's t-test was used for assessing significance of difference in the means of continuous variables in different categories of subjects. Chi-square test was used to compare proportions in two groups.

Results

Baseline Characteristics

Of the 2159 participants, 1342 were males (mean age 48.5 ±12.0 years) and 817 females (mean age 48.8±11.0 years). Their weight, height, body mass index (BMI) and important physiological and biochemical characteristics are summarized in Table 1. One hundred and twenty eight of 817 women (15.6%) and 724 of 1342 men (54.0%) consumed alcohol (<120 G/week), the difference being highly significant ($p<0.001$). Three hundred and sixteen (14.4%) were current smokers while 185 (8.5%) had quit smoking. Prevalence of current or past smoking was significantly higher amongst men.

The men were taller and heavier as compared to women, but the BMI was significantly higher in women. The subjects were considered to be overweight and obese if their BMI was ≥25 kgm^{-2}. The age specific prevalence of overweight and obese was significantly higher in females as compared to males after the age of 60 years (Fig. 2). Only 35.8% of the participants were in the normal weight range (BMI <25.0 kgm^{-2}), whereas 47.5% were overweight and 16.4% were obese (≥ 30 kgm^{-2}) (4). If WHO recommended criteria for Asian population (5) were used (BMI 23 kgm^{-2}) for classification of normal and overweight, 84.5% were overweight or obese.

Subjects were labeled hypertensive if their systolic blood pressure was ≥140 mmHg and/or the diastolic pressure was ≥90 mmHg, or if they were receiving anti-hypertensive medications (6). HTN grade I or above (6) was noted in 955 (44.3%) of the participants (Table 2). The prevalence of HTN increased with age and was higher in males as compared to females (Fig. 2), particularly in the age groups below 50 years.

Fifty-four percent of the subjects had normal fasting and postprandial plasma glucose values (<100 and <141 mg/dl, respectively), whereas another 29.3% had pre-diabetes (impaired fasting glucose: fasting plasma glucose between 100 and 125 mg/dl or impaired glucose tolerance: postprandial glucose between 140 and 199 mg/dl). Three hundred and fifty-eight subjects (16.7%) had DM; the diagnosis was made if their fasting or postprandial plasma glucose was ≥125 or ≥200 mg/dl, respectively, or they were on antidiabetic medications. The prevalence of DM increased with age in both males and females (Fig. 2).

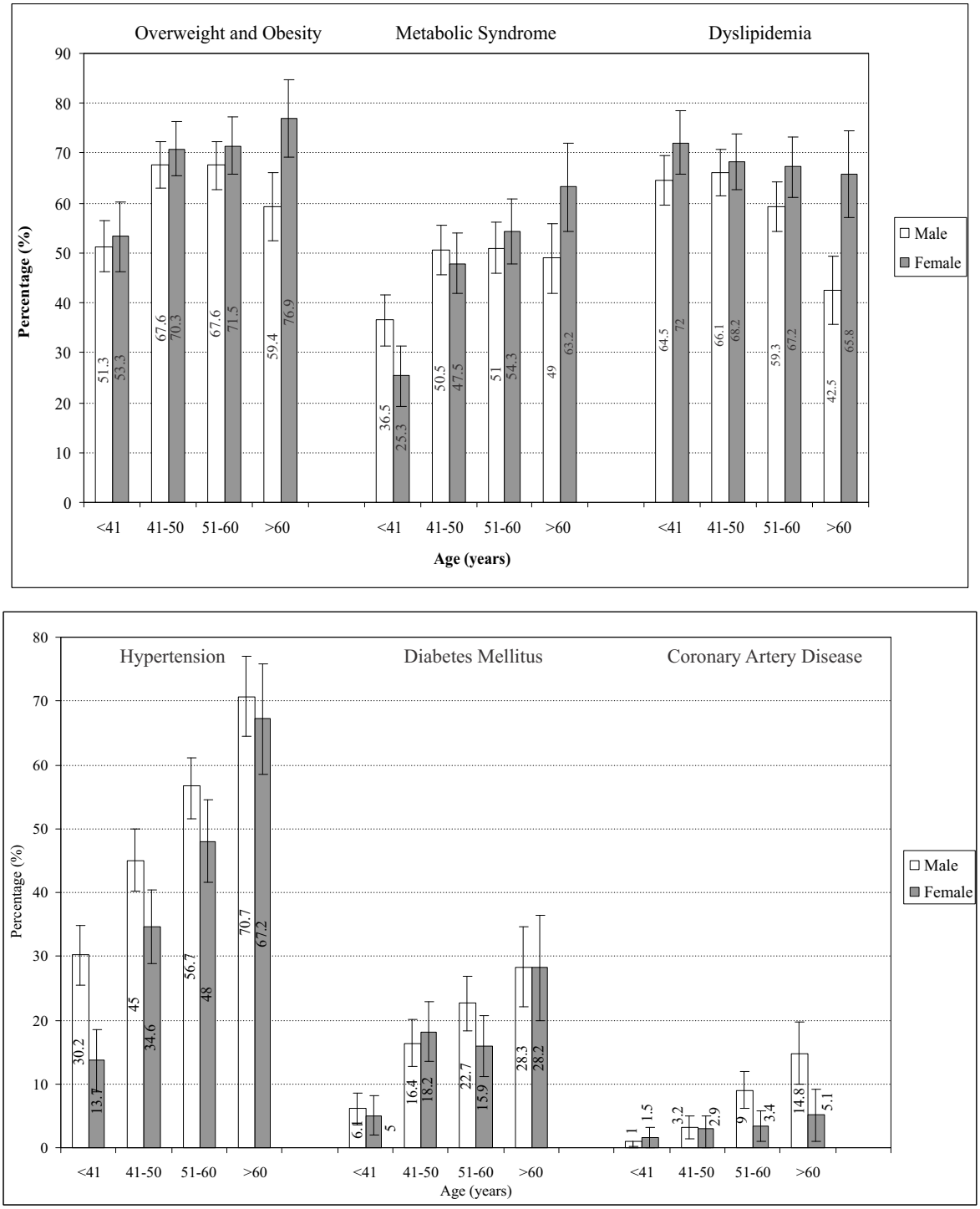
Participants were considered to have dyslipidemia if serum triglyceride was ≥ 150mg/dL and/or if HDL-c was <40 mg/dL in males and <50 mg/dL in females. Using this criterion, dyslipidemia was present in more than

Table 1: Comparison of gender specific cardiovascular risk factors between baseline and follow-up

Variable	Males					Females				
	Baseline		Follow-up		P value*	Baseline		Follow-Up		P value*
	N	Mean (S.D.)	N	Mean (S.D.)		N	Mean (S.D.)	N	Mean (S.D.)	
Anthropometry										
Weight (kg)	1342	75.3 (11.4)	1342	75.6 (11.7)	**0.0222**	817	66.8 (11.2)	817	67.5 (11.5)	**0.0000**
Height (cm)	1342	169.6 (6.5)	1342	169.6 (6.6)	0.8335	817	156.6 (6.2)	817	156.4 (6.3)	**0.0000**
BMI (kg/m^2)	1342	26.1 (3.4)	1342	26.2 (3.5)	**0.0172**	817	27.2 (4.4)	817	27.6 (4.5)	**0.0000**
Blood Pressure (mm Hg)										
Systolic	1329	131.5 (18.3)	1329	130.6 (19.0)	0.1076	804	128.0 (19.0)	804	127.8 (18.9)	0.7516
Diastolic	1329	82.8 (10.2)	1329	81.1 (10.6)	**0.0000**	804	79.5 (9.5)	804	78.1 (9.9)	**0.0001**
Glucose Profile (mg/dl)**										
Fasting Plasma Glucose	1292	104.7 (1.2)	1292	109.4 (1.2)	**0.000**	779	101.9 (1.2)	779	105.6 (1.2)	**0.000**
Post Prandial Plasma Glucose	1234	107.8 (1.5)	1234	105.5 (1.5)	**0.0369**	747	107.9 (1.4)	747	105.0 (1.4)	**0.0186**
Lipid Profile (mg/dl)**										
Total Cholesterol	1272	180.9 (1.2)	1272	176.3 (1.2)	**0.0000**	765	184.3 (1.2)	765	182.6 (1.2)	0.2139
Triglycerides	1274	133.4 (1.6)	1274	134.0 (1.6)	0.7316	760	112.5 (1.6)	760	118.1 (1.6)	**0.0008**
HDL-c	1269	41.1 (1.2)	1269	38.3 (1.2)	**0.0000**	756	47.0 (1.2)	756	45.4 (1.2)	**0.0000**
LDL-c	1262	110.1 (1.4)	1262	107.1 (1.4)	**0.0132**	753	111.2 (1.3)	753	110.1 (1.3)	0.4379

*P value between baseline and follow-up by paired Student's t-test; #P value between baseline and follow-up by two sample test of proportion;

** Geometric means using log transformation. BMI- Body Mass Index; HDL-c- High Density Lipoprotein Cholesterol; LDL-c- Low Density Lipoprotein Cholesterol



Number of subjects in different age groups: <41 years=561 (M: 364; F: 197), 41-50 years=673 (M: 402; F: 271), 51-60 years=606 (M: 374; F: 232), >60 years= 319 (M: 202; F: 117); Error bar represents 95% CI; Gender specific trend analysis significant for all ($p<0.01$), except dyslipidemia and coronary artery disease among females

Fig. 2: Age and gender-specific prevalence of overweight/obesity, metabolic syndrome, dyslipidemia, hypertension, diabetes mellitus and coronary artery disease at baseline

Table 2: Gender-specific baseline prevalence of hypertension, diabetes mellitus, pre-diabetes and coronary artery disease

Disease	Males			Females			Total		
	N	% (n)	95% CI	N	% (n)	95% CI	N	% (n)	95% CI
Hypertension	1340	48.1 (645)	45.4-50.8	815	38.0 (310)	34.6-41.3	2155	44.3 (955)	42.2-46.4
Diabetes Mellitus	1330	17.2 (229)	15.1-19.2	815	15.8 (129)	13.3-18.3	2145	16.7 (358)	15.1-18.2
Pre-Diabetes	1330	31.7 (422)	29.2-34.2	815	25.4 (207)	22.4-28.3	2145	29.3 (629)	27.3-31.2
Coronary Artery Disease	1342	6.0 (81)	4.7-7.3	817	3.0 (25)	1.8-4.2	2159	4.9 (106)	3.9-5.8

Table 3: Incidence of hypertension, diabetes and CAD during follow-up

Disease	Males		Females		Total	
	n/N*	Incidence (per 1000 person years) (95% CI)	n/N*	Incidence (per 1000 person years) (95% CI)	n/N*	Incidence (per 1000 person years) (95% CI)
Hypertension	192/692	75.4 (70.1-81.8)	118/500	66.8 (61.7-72.8)	310/1192	72.2 (68.2-76.2)
Diabetes Mellitus	113/1070	29.0 (27.4-30.6)	49/659	21.5 (20.1-22.9)	162/1729	26.3 (25.0-27.2)
Pre-Diabetes[#]	229/632	97.1 (89.9-105.3)	103/450	64.0 (59.2-69.9)	332/1082	83.8 (79.2-88.9)
Pre-Diabetes to Diabetes Mellitus[+]	86/306	81.4 (73.7-90.9)	40/150	78.5 (67.3-94.2)	126/456	81.0 (74.5-88.7)
Coronary Artery Disease	56/1261	12.2 (11.5-12.7)	34/792	12.4 (11.4-12.8)	90/2053	12.2 (11.6-12.5)

*n=number of subjects developing the event at the end of follow-up; N=number of subjects at risk for the development of event at baseline; # Incidence of Pre-diabetes amongst normoglycemic; +Incidence of diabetes amongst pre-diabetics

half of the subjects. There was a trend for decline in the prevalence of dyslipidemia with advancing age in male subjects only (Fig. 2).

Metabolic syndrome (MS) was diagnosed if any three of the following 5 were present (modified from Adult Treatment Panel III (6) criteria as waist circumference was not recorded in all subjects): (i) BMI ≥25.0 kgm^{-2} which was used as a surrogate for increased waist circumference; (ii) Systolic blood pressure >130 mmHg or diastolic pressure >85 mmHg; (iii) Serum triglycerides ≥150 mg/dL; (iv) Serum HDL-c <40 mg/dL in males or <50 mg/dL in females; and (v) Fasting plasma glucose >100 mg/dL. Using these criteria for clinical diagnosis, 46.6% of the subjects had metabolic syndrome. There was no gender difference in prevalence of MS. The prevalence of MS increased with age in male subjects only (Fig. 2).

CAD was defined to be present if an individual gave a history of pre-existent disease diagnosed by a cardiologist or had undergone an intervention for the same (angioplasty/coronary artery bypass grafting). It was also diagnosed if on the current evaluation, the echocardiography, ECG and/or TMT were compatible with the diagnosis in the opinion of a consultant cardiologist. The prevalence of CAD increased with age. It was significantly higher in males compared to females above 50 years of age (Fig. 2).

Changes in Baseline Characteristics on Follow-up

Gender specific changes in important parameters are shown in Table 1. Notably, there was a mild increase in weight, BMI and fasting plasma glucose in both males and females on follow-up. Height decreased significantly in females only. Diastolic blood pressure and post-prandial glucose decreased significantly in both males and females, whereas LDL-c decreased significantly in males only. The number of patients taking antihypertensive and anti-diabetic medication and lipid lowering drugs was significantly greater on follow-up (results not included in the Table).

Incidence of HTN, DM and CAD

Patients were followed-up for a variable period of time with a mean duration of 3.7±2.5 years for males and 3.5±2.3 years for females. The age-specific incidence of diseases was not calculated because of small number of subjects. The incidence of HTN, DM and CAD per 1000 person years follow-up was 72.2, 26.3 and 12.2, respectively (Table 3). The incidence of HTN and DM was lower in females but difference was not statistically significant except for incidence of pre-diabetes. It is noteworthy that the incidence of CAD in men and women was similar in this cohort of middle aged and elderly subjects, despite higher prevalence of disease amongst the males. On univariate analysis, the incidence of hypertension, CAD and DM increased with age, BMI, diastolic blood pressure and post-prandial glucose (Table 4). The proportion of subjects taking medications for HTN, DM and dyslipidemia increased significantly on follow-up (data not shown).

Discussion

The prevalence of overweight and obesity, even by the western standards (BMI ≥25 kg m-2), was high in this sample of subjects drawn from a relatively higher socioeconomic status. This, however, is comparable to several studies from India in urban populations. In conformity with earlier reports, we also observed higher prevalence of obesity amongst women compared to men (7-9). High BMI is associated with increased cardiovascular mortality globally. It has been reported that this association is rather weak in data from India (10, 11). This may in part be due to paucity of long term cohort studies examining this relationship. Pednekar *et al* (12) reported lowest mortality from all causes in Mumbai in the BMI group 25 to <30 kg m-2; only obese men >60 years of age had increased mortality. In our study, BMI was not significantly associated either with the prevalence of CAD at baseline or incident CAD during follow-up. Abdominal obesity did not emerge as a significant predictor of myocardial infarction in Interheart Modifiable Risk Score (13, 14).

The prevalence of HTN in the present study (44.3%) is higher than other reports on urban Indian subjects. In a meta-analysis of 25 studies on urban subjects from different parts of India, Anchala *et al* (15) reported a prevalence of 33.8% (95% CI 29.7-37.8). This, however, included all age groups above 18 years. Prevalence of HTN increases significantly with advancing age as noted by us as well (Fig. 2); this explains the higher overall prevalence of HTN in the present sample of subjects. The prevalence of HTN was significantly higher in males. Many authors have not reported gender-specific

Table 4: Risk factor for developing incident hypertension, CAD and diabetes mellitus (Univariate Analysis)

Parameters	Incident Hypertension					Incident CAD					Incident Diabetes				
	No		Yes		P-value	No		Yes		P-value	No		Yes		P-value
	N	Mean (S.D.)	N	Mean (S.D.)		N	Mean (S.D.)	N	Mean (S.D.)		N	Mean (S.D.)	N	Mean (S.D.)	
Age (years)	882	44.0 (10.8)	310	48.9 (10.5)	**<0.001**	1963	47.9 (11.4)	90	53.3 (10.1)	**<0.001**	1567	47.0 (11.5)	162	51.5 (10.7)	**<0.001**
BMI (kg/m^2)	882	25.5 (3.5)	310	26.9 (4.0)	**<0.001**	1963	26.2 (3.9)	90	26.1 (3.3)	0.2708	1567	26.2 (3.7)	162	27.7 (4.2)	**<0.001**
Diastolic Blood Pressure (mm Hg)	882	75.5 (6.1)	310	77.6 (6.2)	**<0.001**	1959	81.7 (9.9)	90	81.8 (11.8)	0.9207	1564	80.9 (9.9)	162	84.1 (10.0)	**<0.001**
Post Prandial Plasma Glucose (mg/dL)	859	104.0 (45.7)	301	117.2 (56.6)	**0.0001**	1909	117.4 (59.1)	89	134.1 (74.2)	**0.0098**	1535	96.5 (27.7)	161	134.1 (45.2)	**<0.001**

CAD- Coronary Artery Disease; BMI- Body Mass Index

prevalence of HTN as they did not document significant differences. The incidence of HTN in the current cohort was 75.4 per 1000 person years for males and 66.8 per 1000 person years for females. However, in the community-based New Delhi Birth Cohort (NDBC) (16), over an average follow-up period of 7 years in a relatively younger group of subjects (mean age 29 years), the incidence of HTN was 42.4 per 1000 person years for men and 18.0 per 1000 person years for women. This lower incidence in NDBC is related to the age difference. Only 54.7% of the hypertensive subjects were aware of HTN at baseline. This lack of awareness of HTN is fairly common all over the world. Thus, overall estimates for the awareness of high blood pressure were 25.3% (95% CI 21.4–29.3) for rural Indians; and 42.0% (95% CI 35.2– 48.9) for urban Indians in a meta-analysis (15).

The prevalence of DM in 16.7% of the subjects with an additional 29.3% having pre-diabetes is high, but in consonance with numerous other reports from India (2). The incidence of DM in the present study (26.3 per 1000 person-years) is comparable to the finding reported by Anjana *et al* (17) who observed the incidence to be 22.2 per 1000 person-years in a larger cohort of subjects from rural and urban areas of Chennai followed-up for 9.1 years (11,629 person-years of follow-up). Again, the incidence of DM amongst subjects with pre-diabetes at baseline in the present study (83.8 per 1000 person-years) is similar to the finding reported by Anjana *et al* (17) (78.9 per 1000 person-years). In the relatively younger NDBC the incidence for DM were 10.4 per 1000 person years for men and 5.2 per 1000 person years for women (16).

Several authors have reported the prevalence of CAD in India ranging from 3.2-12.6% over the past two decades. In a community-based survey, overall age-adjusted prevalence of CAD was reported as 3.5% (4.8% in men and 2.6% in women) in a recent report from Kerala (18). Chadha *et al* in 1990 reported the overall prevalence of 3.19% (3.95% in men and 2.53% in women) in a random urban sample of 13,723 adults in Delhi (19). The prevalence of CAD amongst women has been reported to be higher by certain authors (20-22). However, the electrocardiographic criteria for the diagnosis of CAD are inaccurate in women (23). The prevalence observed in our study (6.0% in men and 3.0% in women) is somewhat higher compared to the prevalence noted in community-based surveys (18, 19). There are few studies reporting the incidence of CAD in Indian subjects. The present study shows that with advancing age the incidence of CAD in women catches up with the incidence in men. This has been emphasized in a recent review on the subject (24). The incidence of CVD in a cohort of subjects with DM was reported to be 5.6 per 1000 person-years follow-up (25). This compares with the incidence of CAD as 12.2 subjects per 1000 person years noted in the present study after detailed evaluation with ECG, TMT and echocardiography for diagnosis of CAD.

The risk factors for the development of HTN, DM and CAD noted in the present study are well recognized. Apart from age, overweight/obesity was the single most important factor predicting leading to the HTN and DM. Further, increasing age in itself was also related to obesity (Fig. 2). Increasing age and dysglycemia were noted to be the most important factors related to development of CAD. These observations emphasize the urgent need for controlling the epidemic of overweight/obesity in India by instituting life-style interventions in susceptible populations.

This study is based on observations in subjects attending a tertiary care hospital, and is not community-based. Further, the subject population is derived from the relatively higher socio-economic status and this may not be the representative of the community at large. To this extent, the findings of the present study will need to be corroborated by larger community-based cohort studies representing various socio-economic strata. We present the findings of the

present study considering that there are few cohort studies looking at the incidence of HTN, DM, and CAD from India. The baseline observations are mostly retrospective and the period of follow-up is short and variable. Further, the follow-up has been done after usual interventions for the risk factors noted at the time of the initial evaluation. The ascertainment of CAD in this study was not always based on angiographic evidence of critical arterial narrowing or definitive procedures such as angioplasty or Coronary Artery Bypass Grafting (CABPG). Thus, a positive TMT or stress echo or stress thallium was considered consistent with CAD as opined by a senior cardiologist. Considering that these investigative procedures have significant false positive and false negative rates, our estimates of prevalence and incidence of CAD could be in some error.

The high prevalence of MS in the present study is similar to other reports. It is reported to vary from 8%-43% in men and from 7% to 56% in women around the world (26). Increase in prevalence of MS with increasing age has been reported by others (27). Gender had no influence on prevalence of MS, similar to the observations in the present study.

The present study for definition and follow-up of the cohort was not merely an observational study. After baseline evaluation, all subjects were individually counseled regarding diet, exercise, abnormalities detected, diagnosis made, pharmacotherapy if required, and need for follow-up. The observations made at the time of follow-up, therefore, are influenced by compliance with recommendations made at baseline. It was noted that both systolic and diastolic mean blood pressures showed a significant decline in both men and women on follow-up. The number of subjects using anti-hypertensive medications also increased significantly. It is well known that HTN in early stages results in few symptoms and therefore remains undetected. Similarly, post-prandial plasma glucose showed a significant decrease on follow-up, likely because of better control of DM by appropriate interventions. These findings suggest that routine health check-up in middle aged subjects may help in controlling some of the risk factors for cardiometabolic disorders.

Conclusions

The prevalence of obesity, HTN, DM and MS in this hospital-based sample of subjects undergoing preventive health check-up was high but comparable to other published reports. Urgent remedial public health preventive measures are required to curtail the emerging epidemic of cardiometabolic disorders.

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Role of Uroflowmetry in Children with Ano-Rectal Malformation in Anticipating Upper Urinary Tract Damage

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ABSTRACT

Background: The lower urinary tract dysfunction (LUTD) has high incidence in children with ano-rectal malformation (ARM) which if left untreated leads to upper tract damage.

Aim: To determine role of uroflowmetry in early diagnosis of LUTD in children with ARM.

Methods: This prospective study included twenty consecutive patients of ARM and every patient underwent uroflowmetry at-least 6 weeks after definitive procedure.

Results: The mean age of patients was 3.015±0.86 years. Of the twenty patients, there were 12 (60%) males and 8 (40%) females; 11 (55%) were high ARM, 4 (20%) were intermediate and 5(25%) were low ARM. Lower urinary tract symptoms (LUTS) was present in 8/20 (40%) patients but uroflowmetric abnormalities were present in 11/20 (55%) patients. Forty five percent (5/11) patients with abnormal uroflowmetry were asymptomatic and 25% (2/8) symptomatic patients had normal uroflowmetry. The incidence of uroflowmetric abnormalities was significantly higher in patients with spinal anomalies ($p=0.03$; $\chi 2=4.1$) and those with high ARM ($p=0.004$; $\chi 2=8.1$).

Conclusion: Uroflowmetry is a noninvasive method that may help in early detection of neurovesical dysfunction in asymptomatic children and subsequent cystometric analysis in patients with uroflowmetric abnormalities can be done for early definitive diagnosis and prevention of upper urinary tract damage.

Keywords: Ano-rectal malformation, lower urinary tract dysfunction, neurovesical dysfunction, uroflowmetry.

Introduction

The patients of Ano-rectal Malformation (ARM) have high incidence of urogenital abnormalities. Upto 50-60% incidence is reported for high/intermediate ARM and 15-20% incidence for Low ARM. The incidence of lower urinary tract dysfunction (LUTD) is high in ARM, as these patients have associated spinal abnormalities (1-3). LUTD is defined as any functional anomaly of the bladder and/or urethra that has negative influence on voiding function. In patients with ARM, voiding dysfunction usually is neuropathic in origin and is commonly caused by associated defects of the lumbosacral spinal column (e.g. sacral agenesis) or abnormalities in the spinal cord (e.g. tethered spinal cord). Less commonly, iatrogenic pelvic nerve damage acquired during reconstruction of the ARM causes voiding dysfunction but

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posterior sagittal anorectoplasty (PSARP) causes minimal injury to nerve supply of genito-urinary system as there is limited rectovesical dissection (3-5). Urological abnormalities can result in severe deterioration of the upper urinary tract when treated inadequately (6) or there is delay in identification of LUTD (7, 8). LUTD are more common and severe in complex ARM but less complex ARM are also not free from risk to develop LUTD and therefore urodynamic study is recommended to diagnose LUTD in patients of ARM (9). The maximum flow rate and the type of flow curve obtained on non-invasive uroflowmetry may guide us for the requirement of more invasive urodynamic testing (10). With this premise in mind, the role of early uroflowmetry in anticipating the LUTD and future deterioration of upper urinary tract functioning/damage has been investigated in the present study.

Material and Methods

This prospective study conducted at a tertiary health care centre during the period from February 2012 to January 2013 which included 20 patients of ARM. The patients who had urinary tract infection (UTI) were treated before including into the study. This study aimed to determine the role of uroflowmetry in early diagnosis of LUTD in patients of ARM. The parents of patients were asked about the presence of any of the lower urinary tract symptoms (LUTS) like crying while urination, urinary hesitancy, and straining weak stream, intermittency, frequency, urgency and incontinence, wherever applicable as per age of patient and were investigated thoroughly. Hemogram, renal function test, X-ray spine, ultrasound abdomen, micturating cystourethrogram (MCU) and uroflowmetry were done in every patient atleast 6 weeks after definitive procedure. Uroflowmetry was done using Wireless Portable Urodynamic System, model Delphis. Software used is Delphis basic Urodynamic Software to display curve nomogram by Hospimedica International Ltd.

Uroflowmetry was done after ensuring good hydration and repeated in same sitting if reasonable volume was not expelled. The maximum flow rate (Qmax) was considered to be normal when the square of it was equal or more than voided volume. In addition, the maximum flow rate was considered normal only when it persisted for at least 2 seconds; other sharp peaks of shorter duration were considered artefacts. Bell shape curve of uroflowmetry was considered normal.

Twenty patients with the mean age of 3.015±0.86 years and suffering with ARM were recruited. Out of these 12 (60%) were male and 8 (40%) female; 11 (55%) were of high ARM, 4 (20%) intermediate and 5(25%) were of low ARM.

The document associated congenital anomalies noted at birth were present in 10 (50%) patients. Incidence of congenital anomalies in high ARM was 64% (7/11), in Intermediate ARM was 50% (2/4), and in Low ARM it was 25% (1/5). Vertebral anomalies was present in 7/20 (35%) and all had partial sacral agenesis. Urogenital anomalies were present in 6/20 (30%) patients (M:F=1:5). One male child had right undescended testis. Solitary kidney was present in one, hydronephrosis (HDN) in 3 and vesico-ureteric reflux in 1 patient. CVS anomalies were present in 2/20 (10%) patients and both had VSD. One patient (5%) had esophageal atresia with tracheo-esophageal fistula (Table 1).

Statistical Analysis

The continuous variables were described by mean and standard deviation. Number and/or percentage was described as categorical variables. Statistical analysis was conducted by using Statistical Package for social sciences (SPSS 21, IBM, SPSS Inc., Chicago, IL, USA). The P value < 0.05 was considered as significant. Categorical variables were compared by Chi-square test.

Table 1: Patient characteristics

Patient Characteristics	n=20 (%)
Sex	
Male	12/20(60%)
Female	8/20(40%)
Type of ARM	
High	11/20 (55%)
Intermediate	4/20 (20%)
Low	5/20 (25%)
Associated Anomalies	10/20 (50%)
High ARM	7/11 (64%)
Intermediate	2/4 (50%)
Low	1/5 (20%)
Vertebral Anomalies	7/20 (35%)
High ARM	6/11 (55%)
Intermediate	1/4 (25%)
Low	0
Urogenital Anomalies	6/20 (30%)
Right UDT	1 (5%)
Hydronephrosis (R:L)	3 (1:2) (15%)
VUR	1 (5%)
Single Kidney	1 (5%)
Gastrointestinal (EA+ TEF)	1 (5%)
Cardiovascular (VSD)	2 (10%)

ARM- Anorectal malformation, UDT- Undescended testis,
EA+TEF- Esophageal atresia + tracheo-esophageal fistula, VSD- Ventricular-septal defect

Results

LUTS were present in 8/20 (40%) cases of which straining was most common followed by weak stream and crying while micturition. Straining was present in 8(40%) patients, crying and weak stream in 7 (35%) each and recurrent UTI in 3(15%) patients. The mean Qmax was 7.18 ±3.95 ml/sec and the mean voided volume was 67.85±46.88 ml. The maximum flow was normal in 9(45%) patients and abnormal in 11 (55%) patients. The mean voiding time was 10.45±3.08s and mean time to maximum flow was 2.55±0.75s. The curve was Bell shape in 9 (45%) patients and abnormal in 11 (55%) patients. The pattern was staccato in 7 patients, interrupted in 1, plateau in 3 patients. Thus, the uroflowmetric abnormalities were present in 11/20 (55%) patients.

LUTS was present in 8/20 (40%) patients but uroflowmetric abnormalities were present in 11/20 (55%) patients. Forty five percent (5/11) of patients with abnormal uroflowmetry were asymptomatic and 25% (2/8) symptomatic patients had normal uroflowmetry.

On follow-up Ultra-sonograph (USG), 11/20 (55%) patients had significant post-void residual urine (PVRU) volume; 5/5 asymptomatic patients with uroflowmetry abnormalities had significant PVRU: 3 of them had previously known co-existent HDN and 1 had bladder wall thickening (BWT). Patients with HDN were evaluated to assess the renal function. Among those six symptomatic patients who had abnormal uroflowmetry, 5 (83%) had significant PVRU; one had co-existent hydrourteronephrosis (HDUN) that was non-

refluxing on MCU and other had BWT with HDUN who demonstrated left side vesico-ureteric reflux (VUR) on MCU (Table 2).

One patient who was asymptomatic and not having uroflowmetric abnormality had significant PVRU on USG, 2 symptomatic and six asymptomatic patients who had normal uroflowmetry had no abnormality in USG.

The incidence of uroflowmetric abnormalities was significantly higher in patients with spinal anomalies ($p=0.03$; $\chi 2=4.1$). Out of twenty, seven patients had spinal anomalies and out of them 6 (84%) had uroflowmetric abnormalities. Out of 13 patients with normal x-ray spine, 5(38.5%) had uroflowmetric abnormalities. Similarly, the incidence of uroflowmetric abnormalities was significantly higher in patients with high ARM ($p=0.004$; $\chi 2=8.1$). Uroflowmetric abnormalities were present in nine out of 11 patients (82%) with high ARM, 2/4 (50%) of intermediate ARM and none who had low ARM (Fig. 1).

Discussion

The association of neurovesical dysfunction (NVD) with ARM is commonly neurogenic and caused by neuropathological disorders associated with sacral and spinal abnormalities (1, 4) and less commonly due to iatrogenic pelvic nerve damage (3, 8). The LUTS are categorically classified according to their relation to storage or voiding phase of bladder function. The storage symptoms are increased or decreased frequency, incontinence, urgency and nocturia, while voiding symptoms are hesitancy, straining, weak stream and intermittency (10). Other symptoms include holding manoeuvres like standing on tiptoe, forcefully crossing the legs or squatting with the heel pressed into the perineum (11), feeling of incomplete emptying, post-void dribbling and genital or lower urinary tract pain. Most of these

Table 2: Ultrasound findings of patients with uroflowmetric abnormalities

Asymptomatic patients with uroflowmetric abnormalities	
Associated anomaly	Follow up USG
1. Single Kidney	PVRU
1. RHDN	RHDN/PVRU
1. LDHN	LHDN/PVRU
1. LHDN	LHDN/PVRU
1. None	BWT/PVRU
Symptomatic patients with Uroflowmetric abnormalities	
Associated anomaly	Follow up USG
1. Right UDT	PVRU
1. EA+TEF	PVRU
1. Cardiac anomaly -VSD	PVRU
1. Left VUR	BWT+HDUN+PVRU
1. None	HDUN+PVRU
1. None	NAD

PVRU-Post void residual volume, RHDN-right hydronephrosis, LHDN-Left hydronephrosis, BWT-Bladder wall thickening, UDT-Undescended testis, HDUN-hydroureteronephrosis, NAD-No abnormality detected

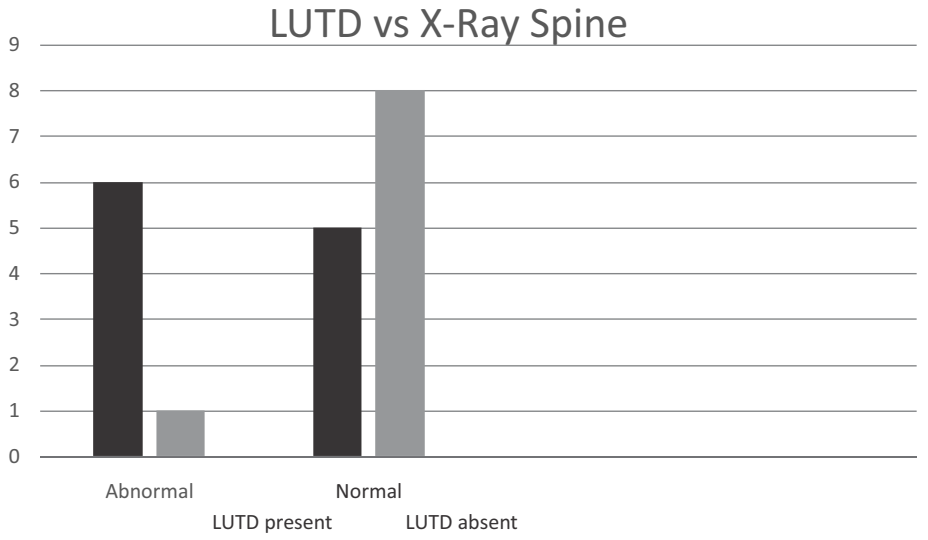

Fig. 1: Correlation of lower urinary tract dysfunction in patients with abnormalities in X-ray spine

symptoms, can be appreciated after age of 5 years, except straining which is applicable in all ages, weak stream that is relevant from infancy and intermittency which is considered physiological until 3 years of age (10). In our study since most of the patients were less than 5-year old, symptoms which were focussed upon were straining, weak stream and crying while micturition.

The PVRU is considered significant when it is more than acceptable limit of 10% of bladder capacity in adults but it is not relevant in infants and children. A range of 5 to 20 ml may be associated with insufficient emptying, so that the examination should be repeated. More than 20 ml residual urine found on repetitive occasions indicates abnormal or incomplete emptying, if time gap between urination and ultrasound should not be more than 5 minutes and child should not have waited over ambitiously for urination so that he has achieved a state of bladder fullness in excess of what is normal for the patient (10). In our study, PVRU volume was high in eleven patients.

Measurement of urine flow and residual urine (with ultrasound) as a stand-alone examination is by far the most common procedure in paediatric urodynamic practice. To a large degree, the results of the flow/residual examination decide whether the child requires an invasive urodynamic investigation. The Qmax is most important parameter when assessing bladder outflow and is considered normal when the square of it was equal or more than voided volume (10). There is linear correlation of square of maximum flow and voided volume in normal individuals (12).

In our study, 5/11 patients with uroflowmetric abnormality were asymptomatic but all of them showed radiologic abnormality suggestive of bladder outlet obstruction. The 2/8 patients with LUTS did not show any uroflowmetric abnormality but none of these patients were found to have any radiologic finding suggestive of bladder outlet obstruction. These findings suggest that uroflowmetry is a more sensitive investigation to detect functional or anatomical bladder outlet obstruction than LUTS alone. Being a non-invasive investigation, it can be used as a screening test to identify the patients who require urodynamic study.

Jindal *et al* (8) reported 70% incidence of neurovesical dysfunction in ARM and in our

series, uroflowmetric abnormalities were present in 11 (55%) cases. Emir and Soylet (13) had reported a 45.4% incidence of neurovesical dysfunction, 70% with supra-levator anal atresia and 34.7% in patients with infra-levator anal atresia. Mosiello *et al* (14) had reported, overall a 35% incidence of neurovesical dysfunction, 51% with high ARM and 40% with low ARM and Kakizaki *et al* (1) reported a 100% incidence of NVD in patients with high ARM. We have also observed that uroflowmetric abnormality was present in significantly higher number of patients with supra-levator ARM with presence of uroflowmetric abnormality in 82% of High ARM and 50 % of Intermediate ARM.

Goosens *et al* (9) reported 43% incidence of LUTD in ARM with vertebral anomalies and 8% with no vertebral anomalies and in present study 6/7 (84%) of patients with vertebral anomalies had LUTD but it was also present in 5/13 (38.5%) patients with normal X-ray spine.

Borg *et al* (15) studied LUTD longitudinally and assessed the bladder function at ages 5, 10 and 15 years. The LUTD was classified as neurogenic and non-neurogenic and they found that dysfunction in neurogenic group was permanent while non-neurogenic LUTD was often transient and mild. The number of children with non-neurogenic LUTD was higher in the lower age group at 5 years. The only predictor of LUTD overall (neurogenic and non-neurogenic LUTD together) was spinal cord malformation at both 5-year and 10-year follow-up. In our study, we also found that uroflowetric abnormalities are significantly higher in patients with vertebral anomalies.

Limitations of Study

In our study due to restricted duration, the sample size is small. MRI spine was not done in patients with NVD and cystometry and specific management strategy were not included in study.

Conclusion

Uroflowmetric abnormalities are present in significantly higher number of patients with vertebral anomalies; however normal X-ray spine does not rule out NVD and also their incidence is significantly higher in high ARM. All patients after PSARP should be followed clinically and radiologically for protection of upper urinary tract damage. Uroflowmetry is a non-invasive method that may help in early detection of NVD in asymptomatic patients and subsequently cystometric analysis can be performed earlier in the patients with uroflowmetric abnormalities that may help in ascertaining with early definitive diagnosis and planning of management strategy to prevent upper urinary tract damage.

Conflict of Interest

None

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Need Assessment of Consultation Liaison Psychiatry amongst the Clinical Faculty

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ABSTRACT

Nearly 20-40% of patients with medico-surgical illnesses in general hospitals have a co morbid psychiatric illness or psychosocial issues, which interfere in improvement of the primary illness. It is important to assess the attitudes and awareness of non-psychiatrist clinicians about the co-existing psychiatric morbidity in their patients and their felt needs, which can help in mitigating this morbidity. The present study attempts to gauge the non-psychiatrist clinician's perception, felt needs and barriers to referral/ intervention in a tertiary care teaching hospital. A cross-sectional, descriptive, online questionnaire-based method was used. Of the 239 clinical faculty members, only 45 responded. Responses indicated that clinicians were aware of the existence and significance of psychological problems in their patients, but could do with further increased levels of awareness and more specific training in evaluation and intervention. Stigma, lack of awareness of available services, and lack of detailed understanding regarding psychological problems were the important barriers to referral/ intervention. Better teamwork, training and more manpower were the specific suggestions for improvement in the future.

Keywords: Psychosomatic medicine, stigma, general hospital psychiatry.

Introduction

Rapid changes in social structure and lifestyles have resulted in a surge in the prevalence of psychological conditions, particularly common mental disorders. Depression is now the second commonest cause of morbidity worldwide (1). In a general hospital setting, most of such patients initially present to medical and surgical departments, with only a small number presenting directly to psychiatry. Nearly 20-40% of patients attending various out-patients and in-patients clinical services for various medico-surgical problems also suffer from psychiatric illnesses like anxiety and stress-related disorders, depression, functional somatic syndromes, and substance use disorders or have psychosocial issues complicating their primary illness. Many of these remain unidentified and have a potential to increase physical morbidity, prolonging hospital stay and incurring increased cost to the system. Consultation liaison (CL) psychiatry is a speciality of psychiatry, in which a psychiatrist works in liaison with the primary clinical team and helps in diagnosis and management of patients with psychiatric problems in non-psychiatric settings. It includes collaborative teaching and research activities with health professionals in non-psychiatric divisions (2). Taken together, CL psychiatry thereby provides the interface for collaborative management,

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training and research between psychiatry and other clinical departments and can, therefore, be of significant value in a general hospital setup. CL psychiatry derives its roots from psychosomatic medicine, a discipline concerned with the interplay of biological and psychosocial factors in the causation, course and outcome of various diseases (3).

Worldwide, although various models of CL services were developed in the 1970s and early 80s (4) evaluative research in training of CL psychiatry is currently lacking (5). In India too, though the first general hospital psychiatric units (GHPUs) was set up as early as 1933 and there may be more than 500 GHPUs in the country, there are very few CL psychiatry units in the country (6). The number of papers published on this topic from India are also few and leave much to be desired.

A survey in a major teaching hospital in Delhi found that 87% of physicians and surgeons were of the opinion that they would have been benefitted if their undergraduate training in psychiatry had been better (7). Similar findings were noted in another study involving 86 general practitioners and specialists (8). Several studies have found that timely referral and care of psychiatric problems in patients admitted in other wards improves quality of life and promotes early discharge. Alhuthail (9) demonstrated that nature of comorbid psychiatric diagnosis influenced the duration of stay in the ward. The referral time was noted as crucial and accounted for 22% variance of duration of ward stay. de Jonge *et al* (10) assessed the impact of psychiatric interventions on both the duration of ward stay as well as the quality of life of patients admitted in a medical ward. Psychiatric interventions were found to reduce duration of ward stay, particularly in the elderly. The quality of life was also enhanced by such interventions.

A recent study from Iran exploring the roadblocks in CL psychiatry reported that the most common reasons of physicians for not requesting psychiatric consultation were lack of time, access to psychiatrist and belief in the need for psychiatric consultation (11). A similar study from Saudi Arabia reported that poor psychiatric knowledge of medical doctors negatively influenced the referral rates to psychiatry and reflected the lack of integration of psychiatry and medicine at the training level (12). Poor psychiatric knowledge is also reflected in another study where majority of the medical specialists believed that the main task of the visiting psychiatrist was to advise them on psychosocial issues, while leaving clinical responsibility in their hands (13). Even in the Western countries like the UK, clinicians consider emotional assessment of routine patients impractical and referrals are avoided because of 'stigmatisation' (14). Underestimation of psychiatric morbidity by clinicians from different specialities is common and there is a general reluctance to refer patients to a psychiatrist for varied reasons (15).

A major problem for lack of integration of psychiatric services is considered to be the focus on brief consultations only in absence of liaison activities. Indeed, literature suggests that psychiatric liaison on medical wards produce a more positive attitude towards psychiatry and a higher consultation request rate. A study comparing CL as against consultation only model found that liaison activities were more favourably received by consultees than consultation alone and increased the consultation rate (16). Such liaison services have found more benefit in focussed departments like geriatrics and critical care medicine where delirium, dementia and psychiatric symptoms occur very frequently (17, 18), and also in oncology services where end of life care, depression and anxiety form a major focus of patients (19, 20). For most other clinical departments, specific conditions like suicidality, aggression and depression might be topics of focussed liaison discussions.

In India, we have very few CL psychiatry units. It is important to know the awareness,

attitude and perceived needs of non-psychiatric clinicians about the need of CL psychiatry services in GHPUs. The present study was planned in this background to assess the perception of non-psychiatric clinical faculty regarding the nature and extent of mental health problems in their patients; to understand the factors influencing referral to a mental health specialist and, to assess their perceived needs for CL psychiatry services.

Methods

The Department of Psychiatry, All India Institute of Medical Sciences, New Delhi has started a dedicated CL psychiatry service in 2008. The service is provided by one senior resident, 2 junior residents on rotational posting, supervised by two faculty members. The study was conducted by the CL Psychiatry Team using a questionnaire-based survey approach with a cross sectional design.

For the purposes of this study, a semi-structured 27 item questionnaire was developed by the authors. The contents of this questionnaire have been taken from literature review and clinical experience. The questionnaire was intended to be answered in a self-report fashion by the participants. The questionnaire collects non-identifying details such as subject of specialization, department of work, year of completing post-graduation and years of experience as faculty. The rest of the items in the questionnaire were intended to obtain data pertaining to the three broad areas of interest including the kind of psychiatric symptoms/illness seen by them in their clinical practice, reasons for seeking psychiatric referrals, their general awareness about psychiatric symptomatology, psychiatric medications and non-pharmacological treatments, and their awareness about CL psychiatry. The complete questionnaire can be obtained from the authors on request.

At the time of study, there were 239 faculty members working in various clinical disciplines excluding psychiatry. All clinical faculty members working currently at the institute (n=239) were sent mail giving background of the study with an online version of the questionnaire created using the free platform of Google Documents and Google Survey. The faculty members were requested participation in the study. Two reminders were sent after one and three months. The questionnaire took about 10 minutes to fill and submit. Every participant was informed of the nature of the study and informed consent was obtained.

The study was approved by the Institute Ethics Committee. Full confidentiality was maintained. Individual characteristics of the respondents were tabulated and a content analysis of the responses was conducted.

Results

Responses were received from only 46 faculty members. Average experience of the participant faculty members from different disciplines varied from 14-22 years as faculty. The participant characteristics are given in Table 1. Thirty five respondents were not aware that CL Psychiatry was a separate sub-speciality with a designated workforce. Most of the participants saw about 10 inpatients and 50-100 outpatients per week. All but two of them agreed that focussed training of residents in this area would be useful.

Content Analysis

Most of the participants felt that their residents needed training to intervene for common psychiatric disturbances occurring in the context of the primary physical illness. A. comparable number of the respondents also highlighted the need for training on communication skills and building a better rapport with the patient.

Most participants felt that 20-40% of their patients seemed to have some psychological/ psychiatric difficulties. Of those with such

Table 1: Characteristics of the participants (N= 46)

Department	Number of responses	Average experience as faculty in years
Gastroenterology/ GI Surgery	5	14.0
Paediatrics	5	22.0
Physical Medicine and Rehabilitation	4	17.5
Anaesthesia	4	19.0
Community Medicine	4	19.7
Deaddiction	3	14.3
Cardiology/ Cardiac Surgery	3	19.7
Otorhinolaryngology	3	18.0
Others	15	-

psychological/ psychiatric difficulties, majority felt that upto 20% had an already diagnosed/ diagnosable independent psychiatric illness and upto 40% may have problems seemingly secondary to the medical illness. An overwhelming majority also felt that upto one fifth of their patients may have a psychological problem masquerading as a physical problem. Anxiety was the most common symptom encountered, seen in almost half of their patients seen, while about a fifth of their patients had depressive symptoms. A smaller number of them reportedly may have had psychotic symptoms. About half of the participants thought that upto 20% of the patients had medically unexplained physical symptoms whereas about a third of the participants felt that the number may be as high as 40%. Substance use problems and confusional states were also seen in less than 20% of the patient population.

Almost all participants agreed that psychological factors can influence course and outcome of physical conditions and their modification can expedite improvement. The basic evaluation and management of psychological problems were to be done by the primary treating doctor and also that such an evaluation was not being conducted. Opinion was divided on whether psychiatrists/ psychologists must be readily available for providing emotional support to patients for health-related anxiety. While a little over half of the participants did feel so, one third of them were either undecided or did not agree to the same. The respondents were undecided on whether time constraint was the reason for inadequate psychological evaluation with responses spread across the agree-disagree continuum, with a slight skew towards agreement. A little over half seemed to suggest that lack of awareness was a major reason. An overwhelming majority felt that psychotherapeutic as well as biological interventions were of great value in this profile of patients as well. Almost all agreed that their patients have been benefitted by psychiatric consultation in the past. Over one third of the participants were unsure whether common mental disorders in their patients should be managed by non-psychiatrist doctors themselves.

For anxiety and somatic symptoms, majority of the clinicians considered treating with an Selective Serotonin Reuptake Inhibitor (SSRI), benzodiazepine or reassurance/ supportive counselling and only a minority considered referral. For suicidal thoughts, disorganized behaviour, hallucinations, almost all participants considered referral. For depressive symptoms without suicidal thoughts, half of them considered starting an antidepressant on their own while the rest

considered referral.

Three/fourth of the participants felt that despite referral, patients were reluctant and feared stigma. Two third felt that inclusion of a psychiatrist (like a senior resident) in the medical team would be of significant value.

Suggestions for the future included: training of all specialists in basic psychological evaluation, more manpower for CL services, combined clinics between psychiatry and other departments, better teamwork, and need for evaluation by senior/ more experienced psychiatrists.

Salient points of this content analysis are provided in Box 1.

Discussion

The present study attempted to understand not only the knowledge and attitudes of clinicians towards CL psychiatry services but also to understand barriers against adequate evaluation and referral, as well as, the felt needs of these clinicians. This is an improvement over many previous studies in India that have focussed only on knowledge and attitude. Contrary to findings from certain previous studies, as well as popular belief, clinicians acknowledged the importance of psychiatric services, felt there was inadequate attention paid by them towards this, and showed eagerness towards improving the same. This is in keeping with an earlier study (14) that suggested that over the years, there have been significant changes for the better in attitudes of clinicians towards psychiatric services. Also in keeping with the findings of the same study was the fact that stigma was one of the main barriers for referral to be successful (14). Unlike many studies that seemed to suggest inadequate time as an important barrier, the present study did not endorse it so strongly (11). Also in contradiction with previous studies, most participants acknowledged that they were aware of the significant co-morbid presence of psychiatric/ psychological problems in their patients (13). In keeping with the idea of minimal undergraduate

Box 1: Predominant themes emerging from content analysis

Theme	Predominant view
Training needed for	Evaluation/ intervention for anxiety/ depression Communication skills Good rapport
Most common symptoms and their nature	Anxiety, usually secondary to underlying medical illness
Reasons for failure of referral	Lack of awareness Stigma
Nature of problem in referred individuals	Suicidality Psychotic symptoms
Nature of problems treated by clinicians themselves	Somatic symptoms Anxiety Depression without suicidality
Suggestions for future	Combined clinics, better teamwork More of Liaison than just consultation Training for other departments More manpower and experienced personnel

training, the study did find that there was considerable interest in clinicians to undergo basic training for psychological evaluation and management. Need for more mental health specialists, a concern raised by previous studies were also echoed by the findings of the current study. The acknowledgement of the fact that treatment of psychological problems expedites improvement and discharge is a welcome change in the attitude of clinicians (14). In keeping with previous literature, CL psychiatry was preferred rather than just consultation-based ones (16). Besides the above, the current study also brought out several nuances of previously known facts. For instance, clinicians chose to treat anxiety and somatic symptoms on their own while choosing to refer patients with psychotic or suicidal ideas. It also demonstrated that the participants had a great deal of confidence in psychiatric interventions, both biological and non-biological. They also substantiated it citing prior good experience with referrals.

The present study had a small sample size. The response rate was very low and therefore it raises questions about the representativeness of the sample for the institute. Persons responding may be quite sensitive to psychological aspects creating a bias. The bias, if present, may explain the relatively more positive response pattern in comparison to previous studies. Additionally, the sample from a single centre may not be representative for the country. Future studies should include larger sample, multiple centres and more robust qualitative methodology like use of focus group discussions to get a more nuanced and deeper understanding of the situation.

Conclusion

The study found that clinicians were aware of the existence and significance of psychological problems in their patients, but could do with further increased levels of awareness and more specific training in evaluation and intervention. Stigma, lack of awareness of available services, and lack of detailed understanding regarding psychological problems were the important barriers to referral/ intervention. Better teamwork, training and more manpower were the specific suggestions for improvement in the future.

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Mechanisms of Action of Human Mesenchymal Stem Cells in Tissue Repair Regeneration and their Implications

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ABSTRACT

Cell replacement therapy holds a promising future in the treatment of degenerative diseases related to neuronal, cardiac and bone tissues. In such kind of diseases, there is a progressive loss of specific types of cells. Currently the most upcoming and trusted cell candidate is Mesenchymal Stem Cells (MSCs) as these cells are easy to isolate from the tissue, easy to maintain and expand and no ethical concerns are linked. MSCs can be obtained from a number of sources like bone marrow, umbilical cord blood, umbilical cord, dental pulp, adipose tissues, etc. MSCs help in tissue repair and regeneration by various mechanisms of action like cell differentiation, immunomodulation, paracrine effect, etc. The future of regenerative medicine lies in tissue engineering and exploiting various properties to yield maximum output. In the current review article, we have targeted the repair and regeneration mechanisms of MSCs in neurodegenerative diseases, cardiac diseases and those related to bones. Yet there is a lot to understand, discover and then understand again about the molecular mechanisms of MSCs and then applying this knowledge in developing the therapy to get maximum repair and regeneration of concerned tissue and in turn the recovery of the patient.

Keywords: Differentiation, immunomodulation, exosomes, tissue engineering.

Introduction

Mesenchymal Stem Cells (MSCs) hold enormous potential in the management of tissue degeneration related to neuronal or cardiac or bone tissues of the human body. Their tissue regenerative potential has been explored in detail by various research groups. Also several investigators have studied their mechanisms of action pertaining to their effect in tissue repair and regeneration.

The evidence for the presence of stem cell population in the bone marrow (BM) that has the capacity to produce non-hematopoietic progeny emerged in the mid-1960's, after the pioneering work of Friedenstein *et al* (1-3). This group characterized BM derived cells of mesenchymal origin (hence called Mesenchymal Stromal/ Stem Cells) which are the plastic adherent cells, immunologically naive and are capable of forming clonal fibroblast colony (CFU-f). These workers also described a fundamental technique of isolation of BM-MSCs by simply plating bone marrow with suitable medium onto culture dish and discarding supernatant (non-adherent

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hematopoietic cells). After 24 hrs, only adhered cells are left (3, 4). Along with these findings, several independent studies proved the multipotent characteristic of MSCs and their capacity to differentiate into cells of mesodermal lineage, including osteoblasts (5-9), chondroblasts (5, 10, 11), adipocytes (7, 12) and myoblasts (13). Therefore, to characterize MSCs, they need to have multipotent capability to differentiate into osteoblasts, chondroblasts and adipocytes and should bear a defined set of markers like CD105, CD90, CD73, CD29 and negative for HLA class II, CD34, CD45 (14).

MSCs are unique type of stem cells that have capability of differentiating into different cell types and can rescue or repair the injured or degenerating cells. The most unique feature of these cells are capability of expression of immunomodulatory and tropical factors. These factors can augment and modulate both the adaptive and innate immune responses as all of these pertain to the regenerative paradigm. The mechanisms of damaged tissue repair is associated with activation of inflammatory cells, including all adaptive and innate immune cells, i.e. T cells, B cells, which are further chemotaxis by damaged, necrotic, apoptotic cells and stroma. In response, phagocytes also secrete or mediate the response by tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), chemokines and leukotrienes. Thus, combination of inflammatory molecules/immune cells with endothelial cells and fibroblast, leads the changes in microenvironment which results in the mobilization and differentiation of MSCs in exchange of injured tissue cells (15-18).

Stem cells have also been studied in a number of studies and have shown immense potential in repair and regeneration. There have been studies where it was proposed that these MSCs are emerging as key players in regenerative medicine. Currently, there are 344 registered clinical trials in different phases across the world (19). However, recent research has shown that stem cells implanted in various studies have low and transient homing to the site of injury and this has given rise to paracrine effect where stem cells not only release soluble factors, but also extracellular vesicles like exosomes which elicit similar biological activity to the stem cells themselves (20).

Recent advances in stem cell research have appreciably influenced the background of regenerative medicine and tissue engineering. The success of stem cell-based technologies is due to its precise and reproducible control and its lineage differentiation and specification. Although stem cells have potential to regenerate tissues, current research scenario is shifted towards developing fully functional organs and various clinical uses including cell or tissue repair through three-dimensional printing methods.

Hence, this review article will provide a brief of the recent advances in the field of understanding mechanisms of action of MSCs in tissue regeneration as described above.

Mechanisms of Action of Stem Cells

a. Differentiation into Cell Types

Neuronal differentiation potential of human Mesenchymal Stem Cells (hMSCs)

Several research groups have explored the neuronal differentiation potential of MSCs. The reports of differentiating stromal cells into neural cells/ neurons were reported by Sanchez-Ramos *et al* and Woodbury *et al* (21, 22). After this, several research groups started exploring the differentiation potential of MSCs by using different strategies, viz. using chemicals like Dimethyl Sulphoxide (DMSO)/ Butylated Hydroxyanisole (BHA), 3-isobutyal-1-methylxanthine (IBMX)/dbcAMP, all-trans retinoic acid (ATRA), safrole oxide, etc. (23-26), growth factors like fibroblast growth factor 8 (FGF8), sonic hedgehog (SHH), nerve growth factor (NGF), along with epidermal growth factor (EGF) and FGF2, etc. (27-32),

conditioned media or co-culturing with brain cells (21, 33, 34), genetic engineering (35, 36), and recently by reprogramming cells and generating induced pluripotent stem cells (37, 38) and by using different kinds of scaffolds for mimicking the matrix (39, 40).

With the developing need to treat Parkinson's disease by a method other than conventional administration of L-DOPA, the cell replacement therapy emerged as a potential solution. The idea to differentiate stem cells into dopamine producing neuronal cells and then transplanting them into the patients came up as an upcoming field to explore. The idea emerged after successful transplantation of embryonic stem cells (ESC) into newborn rat, followed by a series of such studies later by various research groups (41-43). Starting from early 2007 and 2009, MSCs were differentiated using cocktails of cytokines or/and growth factors or/and chemical reagents, etc. (44-50). Various combinations of induction media cocktails using SHH, FGF8, FGF2, EGF, brain-derived neutrotrophic factor (BDNF), ATRA, IBMX, cyclic adenosine 3', 5'-monophosphate (cAMP) and forskolin have been applied for in vitro differentiation of stem cells.

Cardiac differentiation potential of hMSCs

Trans-differentiation of MSCs has been proposed as one of the major mechanisms which participate in damage repair of cardiac tissue caused by myocardial infarction (51). MSCs have been differentiated into cardiomyocytes in vitro using various inducers such as 5-Aza, Transforming growth factor-β1 (TGF-β1), DMSO, etc. or co-culture method. Among them, 5-Aza is the most studied inducer for cardiac differentiation of MSCs (52). However, their translational value is limited due to its demethylating properties. TGF-β1, oxytocin and other small molecules including Bone Morphogenetic Proteins (BMPs) have also been used which are devoid of notable side-effects. Upon in vitro differentiation, these cells show morphological similarities with cardiomyocytes, like flattening of cells, formation of intercalated discs, bi-nucleation or multi-nucleation and expression of cardiomyogenic markers like myosin light chain, myosin heavy chain, actinin, troponin I, etc. In spite of numerous available protocols, till date no induction protocol has resulted in generation of electro-physiological functionality in cardiomyocytes from adult MSCs (53, 54). Additionally, their poor survival and engraftment at the injury site, questions the translational efficacy of this method.

It has also been demonstrated that oxytocin or TGF-β1 treatment works as an efficient cardiomyogenic inducers (55, 56). In a previous study, our group established that TGF-β1 is a potent cardiogenic inducer in BM-MSCs. Upon 14 days treatment, they expressed similar levels of cardiac-specific marker as compared to those treated with 5-Aza for 30 days (56). Also, priming of BM-MSCs with conditioned media of cardiac biopsy tissue increases the level of cardiac-specific markers like myosin light chain and cardiac troponin I (57). Besides the use of exogenous inducers, co-culture with cardiac cells has also been studied for MSC cardiac differentiation (58, 59). Injured myocardium is known to recruit MSCs for tissue regeneration, but is not sufficient if the infarcted region is large. Therefore, in such cases exogenous MSCs are injected directly to the peri-infarct area. After intra-myocardial injection, they have been found to engraft and make contacts with native cardiomyocytes (60). Additionally, the expression of connexin 43 (junctional protein) in MSCs may help them in electro-mechanical coupling with host cardiomyocytes (61).

In spite of so much research, authentication of this phenomenon is incomplete and other mechanisms including paracrine factors, mitochondrial transfer and cell fusion have been proposed as important players in the regeneration ability of MSCs.

Osteogenic potential of hMSCs

The existence of osteogenic stem cells within the bone marrow stroma was first described over fifty years ago, when Petrakova *et al* (62) obtained an osseous tissue by implanting pieces of bone marrow under kidney capsule.

Various protein-based cytokines and growth factors such as bone morphogenetic proteins (BMP) (63, 64), TGF-β1 (65, 66), interleukin-6 (IL-6) (67), growth hormone (68), leptin (69), sortilin (70), and transglutaminase (71) have been suggested to be involved in regulating osteogenesis. Besides these, several synthetic chemical compounds such as dexamethasone (72), β-glycerophosphate (73), L-ascorbic acid (74), prostaglandin E2 (75, 76), 1,25-dihydroxyvitamin D3 (77), TAK-778 (78), and a family of compounds known as the statins (79, 80) have also been identified as key soluble factors which induce osteogenic differentiation of MSCs in vitro. In addition to supplements added to the basal medium, other techniques to optimize osteogenic induction have been investigated as well. In some studies, mechanical stress (81), pulsed electromagnetic field (82), and hydrostatic pressure (83) were added to the osteogenic factors, while in others these factors were used to stimulate osteogenic differentiation without osteogenic induction supplements. The process of osteoblasts differentiation can be subdivided into three stages of proliferation, extracellular matrix synthesis and maturation, mineralization.

Each stage is characterized by expression of distinguishing osteoblast markers. The most frequently used markers of osteoblast differentiation are alkaline phosphatase (ALP), collagen type 1 (Col 1), osteopontin (OPN), bone sialoprotein (BSP), osteocalcin (OCN) and PTH/PTHrp receptor (PTHR). In general, ALP, BSP and Col 1 are early markers for osteoblast differentiation, while PTHR and OCN appears late, parallel with mineralization (84).

Transcription factor, Runx2 is a master regulator of osteogenic differentiation. It regulates the differentiation of MSCs towards osteogenic lineage by two independent signalling pathways via TGF-β1 and BMP2 (85, 86). Along with Runx2, BMP2 and distal-less homeobox 5 (Dlx5) commit MSCs towards the osteogenic lineage. BMP2 induces the expression of osterix independent of Runx2 (87). Following commitment, MSCs are differentiated into pre-osteoblasts. These pre-osteoblasts express Runx2, D1x5, msh homeobox homologue 2 (Msx2), P2Y4 and P2Y14 (88, 89), and few markers of osteoblasts such as ALP, Col 1, and OPN, but their expression is weaker than that in immature osteoblasts. ALP is one of the early proteins that regulate bone mineralization. β-Catenin, Runx2, and osterix differentiate pre-osteoblasts into immature osteoblasts. These are spindle shaped cells and secrete bone matrix protein, bone sialoprotein, and OPN (90). At later stages, Runx2 inhibits the maturation of osteoblasts (91). Osterix causes the terminal maturation of osteoblasts and induces OCN expression (92). When osteoblasts are completely differentiated they become cuboidal and produce a self-mineralized organic matrix (93). The Golgi bodies and rough endoplasmic reticulum are well developed in mature osteoblasts as a result of increased need for protein production. The expression of OPN is reduced in mature osteoblasts; while the expression of other proteins such as P2X5 (89), ALP (94), Col 1 (94, 95), and OCN (95) is increased.

b. Immunomodulatory Effect of MSCs

MSCs are unique type of stem cells that have capability of differentiating into different cell types and can rescue or repair the injured or degenerating cells. The most unique features of these cells are capability of expression of immunomodulatory and tropical factors. These factors can augment and modulate both the adaptive and innate immune responses as it pertains to the regenerative paradigm (96). The mechanisms of damaged tissue repair is

associated with activation of inflammatory cells, including all adaptive and innate immune cells, i.e. T cells, B cells, which are further chemotaxis by damaged, necrotic, apoptotic cells and stroma. In response, phagocytes also secrete or mediate the response by TNF-α, IL-1β, chemokines and leukotrienes. Thus, combination of inflammatory molecules/immune cells with endothelial cells and fibroblast, leads the changes in microenvironment which results in the mobilization and differentiation of MSCs in exchange of injured tissue cells (97). The mobilized MSCs can be taken from bone marrow or from the nearby vicinity of the injured tissue. However, the concert mode of actions for homing and recruitment to the injury site are not known. In response to injured tissue microenvironment, MSCs secret many factors, including tropical and immunomodulatory factors such as EGF, FGF, platelet-derived growth factor (PDGF), TGF-β, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), IL-10, Indoleamine 2, 3-dioxygenase (IDO), chemokine ligand-5 (CCL-5) or regulated on activation, normal T cell expressed and secreted (RANTES), prostaglandin E2, and nitric oxide (NO), insulin growth factor-1 (IGF-1), angiopoietin-1 (Ang-1), keratinocyte growth factor (KGF) and stromal cell- derived factor-1 (SDF-1) (19, 20). In return, the growth factors help the development of fibroblast, endothelial cells and progenitor cells near the injured tissue area to carry out the tissue regeneration and repair. Another known mechanism for repair and regeneration by MSCs, includes the cell to cell contact. MSCs and immune cells interaction induces the secretion of anti-inflammatory factors such as IL-10 which inhibits the T cell proliferation and further in line upregulates the human leukocyte antigen-G5 (HLA-G5) secretion and in response it helps in diminishing the activated T cells and natural killer (NK) cell cytotoxicity (98-100).

There is another class of paracrine trophic factors like Ang-1, VEGF, HGF, EGF, PDGF, FGF, KGF and TGF-β, to affect the endothelial cells and initiating angiogenesis through their potential to promote endothelial cell proliferation and production of extracellular matrix, which helps in reduction of endothelial permeability and inhibit the interaction between leukocytes and endothelial cells (101).

Clinical status

A plethora of studies of animal model and translational studies have identified the capability of hMSCs to home to sites of injury and/or inflammation, thus adding to their use for therapeutic purposes. According to the available database at National Institute of Health (NIH) clinical trial registry (https://clinicaltrials.gov/), as of April 2016, there were over 500 MSC-related clinical trials registered. Surprisingly, while the immunomodulatory properties of MSCs have only more recently been identified, nearly half of all registered clinical trials-230 trials or 42 % of all registered trials-are being conducted for immune-/inflammation- mediated diseases. Different tissue sources may also play an important role in terms of different diseases, with the most explored and reported source being adult BM-MSCs (41.2%). However, other tissue and fetal source MSCs are also popular choices, with 16.3 % of trials using adipose-derived MSCs, and 21.1 % of trials using fetal-source MSCs which includes MSCs isolated from umbilical cord, umbilical cord blood, and placenta (102). While 32.5 % of all trials specify the use of autologous sources, over 50.9 % of trials appear to use allogeneic sources, i.e. trials which use fetal-source MSCs on adult patients. Unspecified donor sources account for approximately 16.7 % of trials.

hMSCs are promising as a means of augmenting brain repair by paracrine signalling. During the brain injury, microglia are the first type of cell in inflammatory cascade followed by cytokines rush in the injury area. The proinflammatory M1 phenotype of microglia is associated with tissue destruction, whereas the inflammatory M2 phenotype of microglia

facilitates repair and regeneration. MSC therapy may improve outcomes of ischemic stroke, neural trauma, and heatstroke by inhibiting the activity of M1 phenotype of microglia but augmenting the activity of M2 phenotype of microglia (103).

The positive results seen in preclinical animal studies have largely not yet translated into clinical efficacy. Clearly, there is still much to learn and optimize with regards to the in vivo interactions of MSCs in human pathological states. As we improve our understanding on the mechanistic properties of MSC immunomodulation, we also need to clarify patho-physiological details and subsets within disease entities to better tailor MSC therapy. One important aspect is to delineate tissue-specific functional different in MSCs from difference sources; the current International Stem Cell Therapy (ISCT) standardization does not include immune-related functional tests or more detailed molecular validation.

c. Paracrine Mechanism of Action of MSCs

Stem cells have been investigated in a number of studies and shown to have immense potential in repair and regeneration. There have been studies where it was proposed that these MSCs are emerging as key players in regenerative medicine (19). Currently, there are 344 registered clinical trials in different phases across the world (104). However, recent research has shown that stem cells implanted in various studies have low and transient homing to the site of injury and this has given rise to paracrine effect where stem cells not only release soluble factors, but also extracellular vesicles like exosomes which elicit similar biological activity to the stem cells themselves (20). These extracellular vesicles such as exosomes secreted by MSCs carry proteins and RNAs that help in rescuing and repairing of the damaged or diseased tissues. Amongst RNA, microRNA (miRNA) specifically have been shown to play central role in many diseases. Aberrant miRNA expression is an emerging theme for a wide variety of diseases, highlighting the fundamental role played by miRNAs in both physiological and pathological states. Therefore repairing of diseased tissues via exosome delivery (for miRNA) has inspired an alternative approach in regenerative medicine, i.e. translating the potential clinical applications based on exosomes secreted by the stem cells rather than the stem cells themselves . Initial studies using these MSCs were in cardiovascular diseases where it was first observed that condition media of these MSCs has paracrine effect and help in repair and regeneration. In 2010 it was first investigated in a mouse model of myocardial ischemia/reperfusion injury that the condition media contain extracellular vesicles called exosomes (105). Following this, there were a large number of studies where these exosomes were isolated, characterized and studied in different disease models. To mention a few are: liver fibrosis, neurodegenerative diseases, kidney diseases, etc. In all these studies, MSCs derived exosomes have shown to elicit similar biological repair activity as that of MSCs themselves. Exosomes content have also been extensively studied including proteomics and RNA sequencing. It was observed that there is specific sorting of these biological molecules into these vesicles. Most of these studies related to the exosome profiling can be found in database of exosomal proteins and miRNAs at ExoCarta (www.exocarta.org). All these studies have successfully identified exosomes derived from MSCs as alternative source for therapeutic potential. These exosome due to their small size have vast applications like can cross blood brain barrier and are being studied in various neurodegenerative diseases. One such study has shown that MSCs-derived exosomes carry neprilysin protein which have therapeutic relevance in Alzheimer's, diseases (106-107). With recent advancements in research and technology, these exosomes may be new beacon for cell free therapy, where the content of these exosomes can be modulated as per the requirement and used as drug delivery system. These exosomes can be used for off the shelf

therapeutic purposes. Although a lot has been known about these exosomes, but few basic questions and problem still needs to be tackle before these exosomes can be successfully taken up to therapeutic study (108-109). These include standardized protocol for their isolation and characterization. Mass production for wide scale study is the current challenge that needs to be answered so far.

d. Tissue Engineering and MSCs

Bone tissue engineering

With the huge plethora of orthopaedic deformation cases occurring everyday, tissue engineering and stem cell researchers have been on a quest to develop clinically relevant bone graft equivalents. While autologous/allogenic grafts are still the gold standard for a complex bone injury, their advantages are outweighed by limited supply and associated health risks. Therefore, to recapitulate the osteoinductive properties of bone grafts, current scaffold-based bone tissue engineering relies upon bioinspired approaches to accommodate the requirements of the cultured cells to guide adhesion, proliferation, migration, differentiation and tissue morphogenesis.

Standard tissue engineering strategy involves culturing osteoprogenitors onto 3D scaffolds with appropriate osteoinductive factors for promoting new bone synthesis. Osteogenic potential of various stem cells including BM-MSCs, adipose tissue-derived stem cells (ADSCs), ESCs, umbilical cord blood-derived mesenchymal stem cells (UCMSCs), dental pulp stem cells (DPSCs) and induced pluripotent stem cells (iPSCs) is being utilized (110). However, appropriate use of stem cells to engineer artificial bone grafts requires proper isolation and standardization protocols for controlled differentiation of cells into osteoblasts or terminal osteocytes. Wang *et al* (111) compared the osteogenic differentiation potential of hBMSCs, hiPSCs and hUCMSCs using 3D calcium phosphate cement (CPC). In vitro results demonstrated high cell viability and enhanced osteogenic expression (Runx2, Col 1, OCN) across all groups; however, de novo bone formation in rat cranial defects demonstrated highest hiPSCs and lowest for BMSCs. Moreover, cell-laden 3D constructs demonstrated increased vascularized bone over cell-free scaffolds after 12 weeks. While these results hold true for the CPC scaffolds, the response might vary depending upon different material compositions and topography, source of stem cells, their differentiation protocols employed, genetic modifications induced in cells, if any. Therefore, a standard consensus on the most optimal strategy for the application of stem cell technology in bone tissue engineering still remains elusive (112, 113).

Another critical aspect that can dictate the course of stem cell differentiation is the choice of scaffold used. Since bone is primarily composed of calcium phosphate, ceramics and ceramic-glasses like hydroxyapatite (HA) (114), tricalcium phosphate (115), bioactive glass (116) have been extensively used for treating bone injuries. Since ceramics on their own are brittle, combining HA with various scaffold formulations made of polymers (both natural and synthetic) such as HA-chitosan (117), HA-chitosan-polycaprolactone (118), HA-silk (119, 120) can fabricate biocomposites that possess hierarchical resemblance to native bone tissue. Also, recent applications in the field have also incorporated nanostructured ceramics and polymers with patterned topography for guided cell differentiation (121). Nanoparticles developed using this technology not only serves as mechanical strength inducers for scaffold fabrication (122), they have profound applications in stem cell tracking with promising applications in leukemia (123).

While most of the commercial bone grafting materials such as titanium plates, hydroxyapatite, bioactive glasses and polymers fail to cater to the needs of individual patients due to their standardized production. Therefore, the field of bone tissue engineering has advanced

towards 3D (124) and 4D (135) bioprinting of encapsulated stem cells to enable development of custom-made, on-demand, personalized bone grafts. With the powerful tool of reprogrammable stem cell technology combined with improved materials and 3D/4D fabrication strategies, regenerating complete functional limbs may even become possible.

Neuronal tissue engineering

Nerve diseases including acute injury caused by mechanical, thermal, chemical or ischemic factors such as peripheral nerve injury (PNI), spinal cord injury (SCI) and traumatic brain injury (TBI), and chronic disease like neurodegeneration disease can damage the nervous system and impair system functions like memory, cognition, language and voluntary movement (126).

Despite advances in microsurgical techniques and a progressive understanding of pathophysiological mechanisms, peripheral nerve repair continues to be a major clinical challenge.

The gold standard method for repairing damaged peripheral nerves is the nerve autograft. This is not an ideal method because of donor site morbidity, the requirement for additional surgery, and limited donor tissue availability. These limitations of autograft have led to the development of alternative therapies. The use of tissue engineering to construct artificial nerves that mimics the nerve autograft provides a potentially innovative solution for peripheral nerve repair (126).

Among the various forms of scaffolds highly porous electrospun nanofiber matrices are a logical choice because of the physical and structural similarities to the extracellular matrix (ECM) components such as collagen fibers and their high surface area.

Several studies have shown that MSCs, human (h) hASCs, nerve precursor cells (NPCs), neural stem cells or Schwann cells (SCs) in combination with electrospun nanofibrous scaffolds have the potential of neural tissue regeneration (126). SCs are the principal glial cells of the peripheral nervous system which are responsible for secretion of basement membrane ECM, neurotropic factors and cell surface adhesion molecule synthesis. Therefore, an ideal scaffold onto which SCs attach, proliferate, and migrate plays a key role in neural tissue engineering (127). Among the various physical structures that can impart to improve neural regeneration, nanofiber orientation has been shown to increase ECM production. Alignment of nanofiber has been reported to greatly influence cell growth and related functions in different cell sources such as neurons and human coronary artery smooth muscle cell (SMCs) (127). It has been reported in different studies that, unidirectional aligned nanofibers can provide better contact guidance effects towards neurite outgrowth and help in providing cues to enhance SCs extension and axon regeneration.

Cardiac tissue engineering (CTE)

Cardiovascular diseases (CVDs) are the leading cause of death in the developed world, and there is a soaring need for heart transplant as the ultimate treatment option left for many who suffer from end-stage heart failure. The common CVDs such as atherosclerosis, rheumatic fever, congenital malformations and thrombosis, they all cause damage to the heart muscle. Unfortunately, the damage is irreversible because the heart muscle cells, cardiomyocytes, are thought to be terminally differentiated and non-proliferative, which necessarily limits the regenerative potential of the heart (128).

CTE involves the growth of functional cardiac tissue in vitro on biomaterial scaffolds for regenerative medicine application in cardiac diseases (129). This strategy relies on the optimization of the complex relationship between cell networks and biomaterial properties.

Heart is a muscular hollow organ; its ECM morphology and elasticity regulate cell shape and coordinate myofibril assembly, thereby influencing tissue architecture and contractile strength (127). In CTE, biomaterials serve as scaffolds for tissue formation and vehicles for the delivery of stem cells or cardiomyocytes. Scaffolds for CTE require a number of criteria to be carefully considered to allow for optimal tissue function including: physical properties of the polymer (e.g. strength and elasticity), degradation rates, and host immune response. Natural polymers such as alginate and collagen are most commonly used as scaffolds for CTE due to their availability and biocompatibility. In synthetic materials, FDA approved polyesters such as polycaprolactone, poly-L-lactic and poly (lacticco-glycolic) acids are commonly used as they meet the most of the requirement of cardiac tissue (128). To satisfy the functional characteristics of heart, the ideal cardiac biomaterial should account for several design parameters. It should match the mechanical properties of the myocardium. A cardiac patch of rigid and inelastic biomaterial will impede heart contraction and a too soft cardiac scaffold cannot be used for mechanically reinforcing the myocardium in pathological cardiac dilation (129,130).

Recently, rat (130) and human (131) decellularized heart scaffolds have been shown to support the attachment, alignment and survival of rat neonatal cardiomyocytes and human mesenchymal bone marrow derived stem cells.

Future Prospects

hMSCs hold immense translational potential in the field of regenerative medicine. All the aspects described in this review are very crucial to establish the efficacy of hMSCs in treating degenerative and immunological diseases. In tissue engineering, directing the cells to differentiate at the appropriate time, in the appropriate place, and into the most appropriate phenotype, requires an optimum environment that governs cellular processes in vivo. Future directions in hMSCs and tissue engineering will involve elucidation of molecular mechanisms by which all types of external cues influence stem cells' behaviour, followed by translation of these findings to clinical applications. Further advances in controlling stem cell fate can be achieved by combining the above mentioned parameters in a more scalable and combinatorial manner to address the complexity of the natural stem cell niche.

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