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EDITORIAL

Physical Therapists in the Field of Physical Medicine and Rehabilitation

Asghar Khan

This is the Era of specialization and professionals in all areas are trying to improve their knowledge and skills for the betterment of their patients. Although some time this creates confusion in minds when there is a thin line to set the boundaries in the scope of practice in different specialties. The same is true for physical therapists and physiatrists in Pakistan. This profession was came into being in the US in 1921 with the combined efforts of Mary McMillan who founded the American Women Physiotherapy Association and few renowned physicians Dr.Frank Granger (Harvard's Graduate School of Medicine) Dr.Elliott Brackett and Dr.Joel Goldthwaite, chief surgeons in the Army's Orthopedic Military Corps.The title of physical therapist was proposed for the graduates of the profession but the physicians were in favor that this will be used for physicians only but later on with the efforts of Mary McMillan the non-physician graduates of the program were allowed by the court to use the same title . The Physicians then came up with the term physiatrist for physicians with such training. Since then the development of physical medicine and rehabilitation started on two tracks and different boards and association were established.[1]

The American Board of Physical Medicine and Rehabilitation defines Physiatrist; "A specialist in Physical Medicine and Rehabilitation evaluates and treats patients with physical and/or cognitive impairments and disabilities that result from musculoskeletal conditions, neurological conditions, or other medical conditions. Physiatrists have expertise in therapeutic exercise, medications, and injections for management of pain and spasticity; electro diagnosis; prostheses and other equipment to assist daily activities, and coordinate treatment to help patients improve their physical, psychological, social, and vocational function".[2]

The American Physical Therapy Association defines; "Physical therapists are health care professionals who help individuals maintain, restore, and improve movement, activity, and functioning, thereby enabling optimal performance and enhancing health, well-being, and quality of life. Their services prevent, minimize, or eliminate impairments of body functions and structures, activity limitations, and participation restrictions.[3] The Association also established a document that "Physical therapists shall establish a diagnosis for each patient/client. Prior to making a patient/client management decision, physical therapists shall utilize the diagnostic process in order to establish a diagnosis for the specific conditions in need of the physical therapist's attention".[4] The physical therapists also authorized for electro diagnostic procedure e.g. NCS and EMG with special training in that area. According to APTA limited prescription and nonprescription medication used for physical therapy interventions is also a component of patient/client management and thus within the scope of physical therapist practice.[4] In UK the physical therapists are allowed for intra articular injections and they also have been granted the full independent prescribing rights.[5] Based on these facts most of the time conflict arises among physical therapists and physiatrists .Although they have different scope of practice.

The word Physician Practitioner of PM&R and Non-Physician Practitioner of PM&R will clarify the difference.

If we look into the education level of physical therapist in Pakistan is equal to 17 years of schooling according to Higher Education Commission (HEC) criteria and the degree title Doctor of Physical Therapy (DPT) has been approved by HEC.Therefore with all these developments and innovations the scope of practice expanded too. Another edge of the physical therapists that they are well equipped with hands on techniques in the form of manual therapy (Mobilization and manipulation) for pain relief and movement dysfunctions. The neurologists, orthopedic surgeons and rheumatologists prefer to refer their patients to physical therapists directly for rehabilitation. If it comes to the application of physical agents, physical therapists have in depth knowledge of indications, contra indications and operation of all the physical agents including ultrasonic therapy, electrotherapy, hydro therapy

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and Cryotherapy. In other modality in physical medicine is the exercise therapy in which the physical therapists are being taught very much in detail and they are trained for specific exercise prescription including endurance, strength, conditioning and work hardening program.

Today's physical therapists have strong basic sciences knowledge including; anatomy, physiology, biochemistry, Microbiology, pharmacology and psychology. They have strong background of biomechanics and based on that they are the suitable clinicians to treat movement dysfunctions or musculoskeletal and neuromuscular disorders of mechanical nature.

Fortunately or unfortunately in Pakistan usually we follow either US or UK practices because we do not have our own defined scope of practice for deferent clinicians and the practice of physical therapists is one of those practices. The consequences of not being regulatory authority in the country to regulate the practice and educational standards of physical therapists, we have mushrooming and substandard institutes producing incompetent professionals which should be a serious concern for the country. The DPT in the country is emerging on the style of podiatric medicine (DPM) a complete degree program with further specialties. In the light of all those innovations and advancements they will be able to practice physical medicine and rehabilitation as independent non-physician practitioners in the future.

Currently in Pakistan most of the rehabilitation departments in the clinical setups as well as in the academics run by the physical therapists. They are emerging as the rehabilitation specialist with higher qualification and advance clinical skills. The curriculum has been design for the independent practice within the scope of practice. The needs of the society most of the times compel professionals to work together for the betterment of the people which some time create new discipline. The physiatrists and physical therapists are both very important members of the rehabilitation team should recognize and support each other to further develop this field in Pakistan.

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ORIGINAL ARTICLE

Endurance Training vs Strength Training in Improving Functional Status in Women with Chronic Neck Pain

Shafaq Altaf[1], Qurat ul Ain[2], Pashmina Fayyaz[3], Arshad Nawaz Malik[4]

ABSTRACT

Objective: The purpose of this study was to compare the effects of endurance vs strength training in improving functional status of women with chronic neck pain.

Study Design: Experimental, Randomized control study.

Place and Duration of Study: The study was conducted at Kulsoom International Hospital, Islamabad from February to July 2014.

Materials and Methods: Sample of 40 patients with chronic neck pain of age limit 20- 50 years were collected using purposive sampling technique and were randomly divided into two groups i.e. Endurance training and Strength training. Female patients having chronic neck pain (≥ 3 Months) were included in the study. Male patients, any pathology of neck and acute pain were excluded. Standardized treatment protocol including ultrasonic therapy, hot pack, shoulder dynamic exercises and postural education, was implemented to both groups. The improvement was measured by Shoulder Pain & Disability Index and Vernon Neck Disability Index. Data was analyzed on SPSS-21 and comparison among the two groups was analyzed using independent sample T-test.

Results: Mean age of the participants was 34.00 ± 5.67 years. 78 % females had pain for 5 months and 67% had radiating symptoms in to one or both upper limbs. Significant improvement was found in terms of reduced pain and disability in endurance training group for both tools (Disability Index and Vernon & Neck Disability Index) in comparison with strength training group.

Conclusion: It is concluded that Endurance training together is more effective than Strength Training in improving functional status in female patients of chronic neck pain of nonspecific nature.

Key Words: *Endurance Training, Range of Motion, Shoulder Pain and Disability Index, Strength Training, Vernon Neck Disability Index.*

Introduction

Cervical complaints are found to be one of the most emerging musculoskeletal complaints & requires attention of health professionals.[1] Neck pain is not only the most common musculoskeletal issue in West but it is also a recurrent source causing disability and badly effecting well-being.[2] Neck pain is categorized by ache, pain and soreness around the region of inferior surface of occipit to the first thoracic vertebrae. Chronic neck pain lasts more than 03 month of occurrence and affects the quality of life of persons with neck pain.[1] Non-specific neck pain refers to the pain which is postural or mechanical in nature.[3] There are different contributing factors for neck pain including poor posture; prolong sitting, computer users, work station and static posture. Young age and female gender are also risk factors for developing neck pain.[4] Neck pain has direct and indirect cost on society and puts great burden on economy of countries.[5]

It is the need of hour to find better treatment option for neck pain. There are numerous treatment options in literature for managing chronic neck pain. The conservative treatment includes active exercises, manual therapy, posture education, resisted exercises, and manipulative therapies for managing pain intensity and stabilization of neck. Recent advancement shows that conservative management is good and cost effective, with good exercises.[6,7] But there are certain limitations regarding long term effect and sustainability of muscles after treatment.[8,9] The excessive stress and static posture continually putting greater pressure

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on neck region and the treatment relives the temporary symptoms.[10]

Both endurance and strength training have impact on relieving neck pain. It is observed that long duration of work has reduced endurance of muscles around neck region and fatigue is common in people with neck pain. Endurance training has significant outcome regarding ranges and functional outcome.[10] Whereas general strengthening is good for relieving pain and functional status but yet it is controversial that pain reduction is either due to strengthening of muscles or reduction in fatigability of muscles.[11,12] So the current study is designed to further elaborate if strength training is more effective or endurance training for long term improvement in terms of functional status.

Materials and Methods

The study was randomized control trial and it was conducted at Kulsoom International Hospital, Islamabad from February to July 2014. Females with chronic neck pain for at least 3 months were included in the sample. Any patient with acute neck pain or any other pathology and males were excluded from the study. 40 patients with chronic neck pain were recruited through purposeful sampling technique. They were randomly allocated to each groups' i.e. Endurance Training Group (n=20) and Strength Training Group (n=20). Data was collected by physiotherapist. Written informed consent was taken.

Strength training group contained 20 patients who were treated with ultrasonic therapy followed by applying hot pack and intensive isometric neck strengthening exercises. Endurance training group contained 20 patients who were treated with ultrasonic therapy followed by applying hot pack and active neck exercises which included lifting the head up from supine and prone positions. Patients from both groups were given correct postural education and were made to do dynamic exercises for shoulder. Both groups were suggested to repeat the exercise plan once a day for 3 weeks. Their pain intensity was assessed by therapist through Visual Analogue Scale. Functional status was assessed through shoulder pain and disability index and Vernon neck disability index. Statistical analysis was done applying the statistical package for social sciences version 21 (SPSS - 21). Documented results were in the form of Mean ± Standard Deviation. Test of choice for comparison among the two groups was independent sample t - test based on normality test. P value of < 0.05 was considered significant for analyzing both groups.

Results

Data of 40 female participants with chronic neck pain was included in the study. Mean age of the participants was 34.00 ± 5.67. Almost 78 % females had pain for 5 months and 67% had radiating symptoms in to one or both upper limbs. Independent sample t-test of homogenous data with comparison of mean and standard deviation is shown in table Is.

Table I: Comparison of Mean ± Standard Deviation of Assessment Tools for Strength Training Group and Endurance Training Group

Variables	Strength Training Group Mean ± SD	Endurance Training Group Mean ± SD	P- VALUE
Shoulder Pain & Disability Index (Baseline)	42.34 ± 7.87	40.09 ± 8.67	0.152
Shoulder Pain & Disability Index (Post Intervention)	33.72 ± 9.35	26.49 ± 8.67	0.015*
Vernon Neck Disability Index (Baseline)	17.23 ± 4.56	18.02 ± 3.65	0.071
Vernon Neck Disability Index (Post Intervention)	13.60 ± 3.69	11.15 ± 2.96	0.026*

* = P < 0.05

Above table shows that after applying intervention significant improvement was seen in endurance training group with p value <0.05.

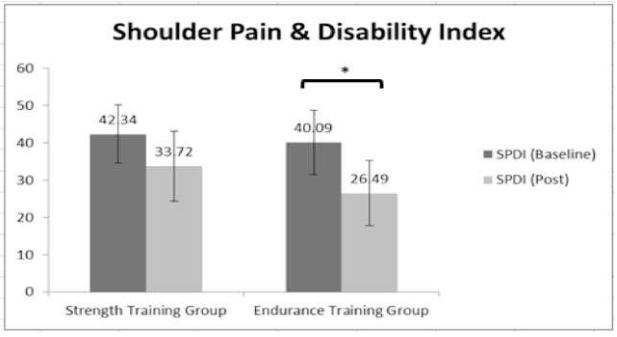

Fig 1: Comparison of Mean & Standard Deviation of Shoulder Pain & Disability Index after Treatment across Groups

Above figure shows mean and standard deviation on Shoulder pain and disability index for both groups. Significant P - value was found after intervention in endurance training group.

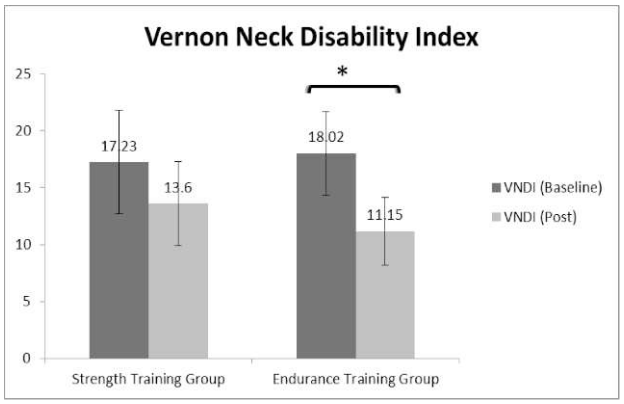

Fig 2: Comparison of Mean & Standard Deviation of Vernon Neck Disability Index after Treatment across Groups

The P value of Post-Total Vernon Neck Disability Index (NDI) is 0.026 ($P < 0.05$) showing that there is a significant improvement in endurance training group

Discussion

The study summarizes that the endurance training has significant improvement in functional status in females with chronic neck pain as compared to strength training in addition to conventional therapy. The endurance training also reduces the disability level as compare to strength training. A similar study done by Taimela also reported that active exercises and endurance exercises along with posture re-education in chronic neck pain gives better results in reducing the disability level.[13] Ylinen J and colleagues in their study also supported that the intensive endurance exercises for non-specific neck pain notable effects on pain intensity, ranges and functional level.[10] Another study was conducted to evaluate similar training in the treatment of chronic neck pain in 2007, in which individuals of both strength and endurance training groups showed marked reduction in neck pain and disability. Contradictory study was carried out for longer duration and both genders were part of it.[12]

In 2001 Bronfort G concluded that active neck exercises has good results in long term, regarding the functional and disability level of chronic neck patients.[14]

Falla D, Jull G, Hodges P & Vicenzino B conducted a study on effect of endurance-strength training regime in reducing myoelectric manifestations of cervical flexor muscle fatigue in females with chronic neck pain and also reveals results similar to current study that endurance training has capability to improve the muscle flexibility and reduces the fatigability for prolong postures and hence it is a good treatment option for patients with chronic neck pain.[11] Ninaknder reported that the energy expenditure have strong links with severity of symptoms in chronic neck pain, disability level and functional status in females. The specific exercises are beneficial and endurance exercise with specific frequency are beneficial for relieving the symptoms in female with chronic neck pain.[15] The study emphasizes that endurance training in females also reduces re-occurrence of neck pain. This type of training also improves the quality of life and improves functional status while working for prolong duration after episodes of neck pain.[16]

Peolsson conducted a similar study on females having non-specific neck pain and compared with healthy individual, he concluded that endurance exercise increases the muscular endurance and decrease the impairment after neck pain. The specific designed treatment is effective in improving the functional status of patients.[2]

Further studies should be conducted with larger sample size and longer duration.

Conclusion

Conclusion derived from the current study is that endurance training is more effective as compared to strength training in improving functional status in subjects of chronic neck pain of non-specific in nature. Endurance training yields significant additional benefits with regards to pain, function or disability. Endurance training should be considered as a therapy of choice for patients with chronic neck pain of nonspecific nature caused by poor postural habits due to muscular imbalance.

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ORIGINAL ARTICLE

The Effect of Virtual Rehabilitation and Constraint Induced Movement Therapy (CIMT) on Improving Upper Extremity Motor Activity Post-Stroke

Aiman Farogh Anjum, Humaira Fayyaz, Arif Siddiqui

ABSTRACT

Objective: This study aimed to assess the effect of Constraint Induced Movement Therapy (CIMT) augmented by Virtual Rehabilitation in improving upper extremity activity capacity and ability to perform Activities of Daily Living (ADL).

Study Design: Pre/Post Quasi Experimental Study.

Place and Duration of Study: It was carried out at the Physiotherapy department at Holy Family Hospital, Rawalpindi in collaboration with Islamic International Medical College, Riphah International University, Islamabad, Pakistan from March 2015 to March 2016.

Materials and Methods: The study included 20 stroke patients who were subjected to Constraint Induced Movement Therapy augmented by Virtual Rehabilitation using Nintendo WiiTM four times a week for four weeks. Upper extremity activity capacity and Activities of daily living (ADL) were measured pre- and post-intervention using Action Research Arm Test (ARAT) and Barthel Index respectively.

Results: The ARAT improved from a pre-intervention mean score of 16.20+3.942 to 48.30+5.768 post-intervention ($p<0.001$). Barthel index showed improvement from the pre-intervention mean score of 9.05+2.544 to a post-intervention mean score of 16.80+1.609 ($p<0.001$).

Conclusion: Virtual rehabilitation using Nintendo WiiTM has a positive impact on upper extremity motor recovery and subsequently the ability to carry out Activities of Daily Living, if used as an adjunct to Constraint Induced Movement Therapy (CIMT).

Key Words: *Constraint Induced Movement Therapy (CIMT), Nintendo WiiTM, Stroke, Upper Extremity Motor Capacity, Virtual Rehabilitation.*

Introduction

Stroke is the most common cause of disability worldwide[1] with 350,000 new cases per annum and a prevalence of 4.8% in Pakistan.[2] Thromboembolism of cerebral arteries and hemodynamic disturbances are the two main mechanisms involved in the pathophysiology of stroke. Ischemic stroke accounts for 87% of the total burden of stroke while hemorrhagic strokes (spontaneous intracerebral and subarachnoid hemorrhage) comprise of the remainder of all cases.[3]

Survivors of stroke suffer debilitating sequelae like impairment of motor function of the upper and/or lower limbs, speech difficulties, sensory deficits, impairment of genitourinary or bowel functions etcetera, that hinder activities of daily life and lower the chances of leading a normal, productive and fulfilling existence.[4] Upper limb motor function impairment after stroke (the primary focus of this study) is one such complication that neuro-physicians and physiotherapy specialists strive to improve as it allows the patients to, not only carry out daily life tasks with more ease and comfort but it has been shown to have a positive impact on the psychological well-being of stroke survivors as well.[5] Regaining upper limb motor function post stroke by means of physical therapy has a corresponding positive impact on the ability to carry out routine tasks like bathing, dressing, toilet use, feeding and climbing up and down the stairs (collectively termed as Activities of Daily Living (ADL)).[6] Constraint-induced movement therapy (CIMT) is based on forced use of the affected limb by restraining the uncompromised limb for 90% of the waking hours and "shaping", a form of behavioral conditioning in which certain task specific exercises are aimed at

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achieving a specific goal (such as movement of the impaired arm). The intensity of these tasks is increased in a step-ladder pattern according to the patient's improvement.[7]

Virtual Rehabilitation is a novel technology that relies on the mirror-neuron system to bring about restructuring of the neuronal network in regions of the brain concerned with memory, learning and motor function.[8] In this aspect, Nintendo WiiTM is an innovative, cost-effective and ubiquitous technology which makes use of an on-screen "avatar" in order to incite "mirror-neurons" through "audio-visual" input to bring about neuronal re-organization and subsequent motor improvement. It has an added benefit of modifiable skill and speed which can be tailored according to the user's needs.[9]

Numerous studies have been conducted to shed some light on the effectiveness of Virtual Rehabilitation on motor improvement in stroke survivors but they have mainly focused on regaining lower limb function. Furthermore, combination of VR with CIMT for the purpose of improving the motor function profile in stroke survivors is an area that has not been explored at length as yet.[10]As far as the role of CIMT augmented by motion capture consoles like Nintendo WiiTM in improving upper extremity motor function is concerned, a dearth of bonafide research still remains.[11]

Application of virtual reality based video games, Nintendo Wii in particular, for objectives other than stroke rehabilitation have shed some light on the effect of this console on improving hand-eye coordination. A study conducted at Arizona State University by Kullman, 2008, evaluating the efficacy of Nintendo Wii as a tool for improving hand-eye coordination in surgeons found that the use of Nintendo Wii among surgeons "improves hand-to-eye coordination, strength and dexterity as the motion-sensitive controller allows some games to require very precise hand movements, similar to those executed during surgery".[12] This effect might similarly manifest, albeit to a lesser extent, in the stroke survivor population undergoing "Wii-habilitation"[13] allowing them to regain some level of coordinated hand and arm motion as well as a greater degree of strength and skill.

Data pertaining to upper extremity rehabilitation employing Constraint Induced Movement Therapy based on exergaming technology is scarce. This study aimed to assess the effect of Constraint Induced Movement Therapy (CIMT) augmented by Virtual Rehabilitation in improving upper extremity activity capacity and ability to perform Activities of Daily Living (ADL).

Materials and Methods

This was a Pre/Post Quasi Experimental study with one year duration from March 2015 to March 2016. The study was conducted at Medical Unit I and II and the Physiotherapy department of Holy Family Hospital Rawalpindi in collaboration with Islamic International Medical College Rawalpindi after the approval of Ethical Review Committees at both institutions.

The inclusion criteria were; first event of stroke, age ranging from 30 to 60 years, impairment of motor function in one arm, 1-4 months since the event of stroke, an understanding of computer/video game technology and clinically defined stroke by CT-scan or MRI. Patients of stroke with other neurological diseases, cognitive deficits, diagnosed dementia or epilepsy, language difficulty that would affect the capacity to receive information about the training procedure, visual impairment, orthopedic injuries that could impair locomotion, and inability to carry out voluntary arm movement were not included in the study. A total of 20 stroke patients were recruited in the study by purposive sampling.

Written informed consent was taken from all the subjects. The subjects were asked to report at Physiotherapy Department at Holy Family Hospital Rawalpindi in the morning. Age, gender, blood pressure, hemisphere, type of stroke, and handedness of the subjects were recorded. "Activities of Daily Living (ADL)" were measured using the "10-item Modified Barthel Index" which has a score of 0-20. Upper extremity "activity capacity" was assessed using the "Action Research Arm Test (ARAT)", scored from 0 to 57.

The subjects were then subjected to Constraint Induced Movement Therapy/CIMT for the upper extremity (a physiotherapy regimen where the use of the un-affected limb is limited by putting a weighted mitt over it for majority of the waking hours) using interactive video gaming technology (Nintendo WiiTM) for four sessions a week for four weeks (total 16 sessions) of 20 minute duration. The subjects

were made to wear a weighted mitt on the unaffected arm during the course of the session. The speed and difficulty of each game was set according to each subject's level of comfort and the dexterity and mobility of the affected arm. The tasks each of the games demanded to be performed are listed in Table I.

Table I: Tasks/Movements Demanded by each of the Nintendo WiiTM Games Employed in the Study

Nintendo Wii™ Games	Tasks Performed by the affected upper limb
Wii Bowling	Gripping the Wiimote Swinging of the arm Wrist movement Supination and pronation of the forearm Flexion and extension of the wrist Flexion and extension of the elbow joints Flexion, extension, abduction and adduction of the shoulder joint
Cooking Mama for Nintendo Wii™	Gripping the Wiimote Slicing movements, Grasping, pinching, rolling of fingers

The gameplay was displayed on a projector screen. Since the intervention pertained to the upper extremity only, the patients performed the tasks demanded by the games while sitting upright on a stool. The researcher herself carried out the intervention sessions, providing guidance to the subjects throughout the duration of the gameplay as well as assisting the subjects in case they encountered instability in maintaining sitting position (a rare occurrence). The gameplay sessions were scheduled for four days a week. Ten minutes were designated to each of the two games mentioned. Patients initially found it difficult to grasp the Wii-mote optimally and were thus assisted by the Velcro strap provided along with the Wii-mote to cater for such difficulties.

Each subject's progress in terms of gameplay, speed and dexterity was charted along the course of the intervention phase. The level of difficulty of each game was progressively increased after the subjects showed improvement in performing the tasks demanded by the previous level of the videogames. The subjects were also asked to limit the use of the unaffected arm for the rest of the day and note the time for which they complied with this undertaking. They reported the time for which the unaffected was not used during the previous days before every session.

"Activities of Daily Living" and upper extremity "activity capacity" were assessed using the "Barthel index" and "ARAT" respectively.

Statistical Analysis was done using SPSS 21. Results were documented as mean + SD. Statistical significance was set at $p < 0.05$. Paired-samples t-test was used to check difference in pre- and post-intervention scores of ARAT and Barthel index.

Results

The demographic variables of the 20 subjects included in the study are given in Table II. Highly significant ($p<0.001$) improvement was seen between the pre- and post-intervention scores of ARAT. All four subtests of ARAT i.e, grasp, grip, pinch and gross movement also showed highly significant improvement between the pre- and post-intervention scores, given in Table III. The pre-intervention mean Barthel index score was 9.05 +2.544. Post-intervention Barthel index scores improved to a mean value of 16.80 +1.609. The difference was highly significant ($p<0.001$), given in Table IV. Figures I, II, III and IV depict the comparisons of the items pertaining mainly to upper extremity function, namely "grooming, toilet use, feeding and dressing" in the Barthel index, between the pre- and post-intervention scores of the subjects. The differences between the pre- and post-intervention scores in the "grooming" item was significant ($p<0.05$) while it was highly significant in the "feeding", "toilet use" and "dressing" ($p<0.001$).

Table II: Demographic Variables of the 20 Stroke Patients in terms of Mean ± SD of Age (years), Gender, Type of Stroke, Stroke Hemisphere, Handedness and Mean ± SD Time since Stroke (months)

Parameter	Values
Age (years)	51.85 + 6.107
Gender	Male = 14 Female = 6
Stroke type	Ischemic = 11 Hemorrhagic = 9
Stroke Hemisphere	Right = 11 Left = 9
Handedness	Right = 20 Left = nil
Time since stroke (months)	2.70 $\pm$1.174

Table III: Comparison Between the Mean ± SD Total Scores of ARAT, Grasp, Grip, Pinch and Gross Movement, Pre and Post Intervention

Parameter	Pre-intervention	Post-intervention
Total ARAT score (0-57)	16.20±3.942	48.30±5.768**
Grasp (0-18)	4.20±2.567	14.65±2.300**
Grip (0-12)	4.05± 1.395	10.25±1.293**
Pinch (0-18)	6.40±1.569	15.40+1.930**
Grosmt (0-9)	1.90±2.292	8.00±1.214**

Action Research Arm Test (ARAT), Gross movement (Grosmt) * = P<0.05 (pre-intervention vs post-intervention)
** = P<0.001 (pre-intervention vs post-intervention)

Table IV: Comparison Between the Mean ± SD Barthel Index Scores Between the Pre- and Post-Intervention Assessment

Parameter	Pre-intervention	Post-intervention
BI (0-20)	9.05±2.544	16.80±1.609**

Barthel Index (BI)
* = P<0.05 (pre-intervention vs post-intervention)
** = P<0.001 (pre-intervention vs post-intervention)

Discussion

The results showed that CIMT augmented with Virtual Rehabilitation has a positive effect on improving upper extremity activity capacity which lends credibility to similar results seen in the single subject design trial by Slijper et al. 2014, testing the effectiveness of computer game based upper extremity function showed marked improvement in ARAT scores in 11 of its 12 subjects after a five week intervention phase.[14] Other similar trials have also shown improvements in ARAT scores following interventions based on computer, video game consoles as well as virtual rehabilitation. A pilot study aimed at evaluating the safety and effectiveness of Nintendo WiiTM as a rehabilitative tool for stroke survivors (Christie et al., 2010) showed that although there was an overall improvement in ARAT scores in 6 out of the 9 total subjects after an intervention phase of 6 weeks, only the Grip subscale showed significant improvement (p<0.05) in the affected arm. The finger Pinch subscale also showed marked improvement but was not found to be statistically significant.[15] The improvement in ARAT scores in the current study could be attributed to two things. Firstly, the current study employed not only Nintendo WiiTM but also subjected the patients to a detailed regimen of CIMT while Christie et al., used Nintendo WiiTM as the sole tool for improving motor function. Secondly, the improved motor status of the upper extremity, is believed to be a consequence of long-term potentiation, as observed by Saposnik et al. 2010 in the EVREST trial, which results in the motor cortex retrieving old, and securing new patterns of motor function as a result of task repetition (one of the hallmarks of virtual rehabilitation).[16]

It has been observed that the scores of ARAT, although consistently improved, are not statistically increased in majority of the trials that employ Nintendo WiiTM as the sole vehicle for upper extremity rehabilitation. However, studies that have used Virtual rehabilitative tools as adjuncts to either conventional therapy or to CIMT[17] have shown significant improvements in ARAT scores post-intervention; findings supported by the current study's results.

The highly significant improvement seen in the study group Barthel index scores lend strength to a number of similar studies that employed virtual rehabilitation to achieve motor function improvement for the upper extremity. One such study was the 12 week trial by Cameirão et al. aimed at "using a multi-task adaptive VR system for upper limb rehabilitation in the acute phase of stroke" which showed significant improvement in Barthel index, Motricity and Fugl-Meyer scales for the upper extremity in the study group which underwent rehab therapy using a "Rehabilitation Gaming System (RGS)".[18] Trials conducted by Kyoung-Hee Lee 2015, Kwon JS et al. 2012 and Yoon J et al. 2015 were based on comparing the effectiveness of Virtual Rehabilitation as an adjunct to conventional therapy versus conventional therapy alone for upper extremity rehabilitation. All three trials exhibited significant improvement in the respective forms of Barthel indices used in each study in the study groups.[19-21] Evidence based on such data suggests a positive impact of Virtual rehabilitation on the improvement in upper limb function and subsequently on the quality of everyday life. The possible explanation for the overwhelming improvement in the ADL scale scores of the study groups in each of the trials mentioned as well as those of the current study could be the self-driven effort of the subjects in each study to adhere strictly to the therapy regimen, improvement in hand-eye

coordination and overall increased therapy time (CIMT plus time spent playing the virtual rehab games) in addition to the long term potentiation induced by repetitive tasks required to be performed during the gaming sessions.

The role of CIMT in bringing about significant improvement in motor function, as reflected by results of ARAT and Barthel index in the current study cannot be ignored. According to Langhorne et al., 2009 and van Peppen et al., 2004, CIMT has "a significant effect on increasing upper extremity (arm) function" and a "moderate effect on increasing performance of the activities of daily living immediately following treatment (Sirtori et al., 2009)."[22-25]

Conclusion

Virtual rehabilitation has a positive impact on upper extremity motor recovery if used as an adjunct to more robust models of therapy like Constraint Induced Movement Therapy (CIMT). It is an effective and safe means of engaging stroke survivors in rehabilitative practices by increasing self-directed therapy time and improving motor function. However, there is a need for trials to be carried out on a larger scale, involving a greater population of stroke patients.

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ORIGINAL ARTICLE

Effects of Iron Supplementation on the Height of the Zones of Growth Cartilage of Rat

Faiza Umbreen[1], Aamna Khalil[2], Saadia Rashid[3], khadija Qamar[4]

ABSTRACT

Objective: To study the effects of iron supplementation in pregnancy on the height of zones of epiphyseal growth plates of off springs.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: This study was conducted at Department of Anatomy, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from March 2016 to November 2016.

Materials and Methods: Five lactating rats with ten pups were selected for control group A1and experimental groups B1 and B2 each. Experimental groups were given iron supplementation daily throughout the pregnancy. Mothers of group B2 were given oral iron during pregnancy as well as during lactation. Control group was on normal diet throughout the pregnancy.

The infant rats were allowed to reach seven weeks of age till dissection. They were weighed before euthanasia. Right femur of each rat was removed for the epiphyseal growth plate analysis. Femurs were processed, embedded and stained with Hematoxylin & Eosin, Perl's stain, and toluidine stain for histological study. The heights of hypertrophy and proliferative zones were analyzed histologically and statistically.

Results: The height of hypertrophy zone and proliferative zone of epiphyseal plates of long bones were measured. Mean values of the heights of hypertrophy in group A1 276.19 ±43.61, B1 136.73±3.58and B2 205.00±12.76. Mean values of the heights of proliferative zones in group A1 377.29±50.73, B1 202.89±11.56 and B2 281.07±11.96 were taken. The heights in hypertrophy and proliferative zones were statistically decreased in group B1 and B2 as compared to group A1.

Conclusion: Indiscriminate iron supplementation during pregnancy and lactation can decrease the height of hypertrophy zone and proliferative zone of epiphyseal growth plates of long bones of the off spring.

Key Words: *Growth Plate, Hypertrophy Zone, Iron Supplementation, Proliferative Zone.*

Introduction

Iron is an essential nutrient required by each cell of the body. The deficiency as well as excess of iron in the body has clinically significant effects.[1] Iron deficiency can be due to either increased requirements of body as in pregnancy and period of rapid growth or inadequate iron supply. Iron deficiency is the most common cause of anemia.[1] Availability of iron in the diet, with the food fortification and the use of iron supplementation are methods which can correct iron deficiency anemia. Iron excess in body may be associated with excess dietary intake. Iron overload is associated with problems like ineffective erythropoiesis. Clinically, systemic iron overload can present as liver disease, diabetes mellitus, gonadal insufficiency and other endocrine disorders, cardiac dysfunction, arthropathy, and increased skin pigmentation.[2] There is a negative effect of excess iron on bone structure, which exposes patients to fractures.[3] There is a decrease in osteoblast activity due to iron . Iron supplementation is given during pregnancy as well as during lactation routinely even in nonanemics.[4] Excess iron intake during pregnancy is associated with reduced fetal growth. Fetuses of pregnant women with iron intake in the third trimester have a significantly lower biparietal diameter, abdominal circumference and femur length than the fetuses of mothers taking iron in the second trimester.[5] Excessive iron can lead to oxidative damage and decrease in the absorption of

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copper and zinc, which are the important micronutrients for fetal growth.[5] There is an association between elevated maternal hemoglobin and adverse birth outcome, including low birth weight, preterm birth, and small for gestational age at birth in addition to anemia.[6] Effects of iron supplementation on the growth plate need to be evaluated further . Growth plate is responsible for the elongation of bones.[7,8]

As iron supplementation is given as a routine during pregnancy and lactation so its effects on the height of hypertrophy and proliferative zones of epiphyseal growth plate of femur of new born will be determined with the help of present study.

Materials and Methods

This laboratory based randomized controlled trial was approved by Ethical Committee, of the Army Medical College Rawalpindi. It was conducted at the anatomy department of AMC Rawalpindi from March 2016 to September 2016 in collaboration with the National institute of health (NIH) Islamabad. Twenty adult Sprauge Dawley rats (sixteen female and four male) with average weight of 250gms and average age of seven weeks were selected for the study. The animals were kept at standard temperature21±2 ċ in a room maintained on 12 hour light/dark cycle.[9] A battery powered fan was used to maintain the temperature. They were fed on standard lab diet and water ad libitum.

They were kept on breeding.[10] Presence of vaginal plug was checked in the dames daily.[11] Its presence confirms mating and considering it the first day the pregnant rats of both experimental groups B1 and B2 were started with oral iron supplementation daily in water. Sytron syrup was given in a dose of 0.5ml once daily for each pregnant rat throughout pregnancy till the day rat delivers through spontaneous delivery. The pups of group B2 were separated with the mothers on iron supplementation throughout the lactation as well.[12] Ten pups were separated as control group which was group A1, ten pups in experimental group B1and ten pups in experimental group B2. Control group was kept on normal laboratory diet.

On completion of 7 weeks of age the rats of all the three groups were weighed and killed by euthanasia by ether inhalation. Right femurs were removed. Bones were fixed in 10% formalin decalcified in 2% EDTA solution. Lower ends of femurs were separated for evaluation of growth plate. Processed into 5-micron thick sections using rotary microtome. Staining with Hematoxylin & Eosin, Perl's stain[13] (for detection of iron deposition), and toluidine stain was done.[14] Slides were observed under the light microscope for histological analysis of the height of hypertrophy and proliferative zones and measured with image J.software.[15] The height of the hypertrophy and proliferative zones was measured at three different areas of epiphyseal plate (in the Centre and two extreme zones). Average of these three readings was recorded as final height. Firstly the boundary between reserve and proliferative zone and proliferative hypertrophic zone had to be identified first. Between the proliferative and hypertrophy zone it was identified by the first considerably enlarged chondrocytes proportional to the proliferative cells. The limit between the reserve and the proliferative zones was also recognized by the upper border of the proliferative columnar organization, till the beginning of hypertrophy zone.[16] Data was analyzed using SPSS version 21. Parameter was expressed as mean and standard deviation. The means were compared for significance among groups using one way analysis of variance (ANOVA) followed by post hoc Tukey test for comparison. P value less than 0.05 was considered significant.

Results

The mean ±SD of height of hypertrophy zone in groupA2 (figure-1) was 276.19 ±43.61, Mean± SD of group B1 (figure-3) was 136.76±3.58um (table-1). The mean ±SD of group B2 was 205.00±12.76um (table-I).

The p-value for the height of hypertrophy zone was < 0.005 (table-II) which is statistically significant. In proliferative zone Mean ±SD of group A2 was 377.29±50.73um (table-1), Mean± SD of group B1 (figure-2)

was 202.89± 11.56um and Mean ±SD of groupB2 (figure-2) was 281.07 ±11.96 (table-1), The p-value for height of proliferative zone was < 0.005(table-1) which is statistically significant.

Discussion

Supplementation of mothers with iron during pregnancy and lactation without checking the serum iron level can have damaging effect on the

Table I: Showing Comparison of Mean Value of Height of Hypertrophy and Proliferative Zones

	Group A1 Mean ±SD (n=10)	Group B 1 Mean ± SD (n = 10)	Group B 2 Mean ± SD (n = 10)	Group A1 vs. B1 p-value	Group A1 vs. B2 p-value	Group B1 vs. B2 p-value
Height of hypertrophy zone	276.19±43.61	136.73±3.58	205.00±12.76	< .001	< .001	< .001
Height of proliferative zone	377.29±50.73	202.89±11.56	281.07±11.96	< .001	< .001	< .001

P value <0.05 is statistically significant

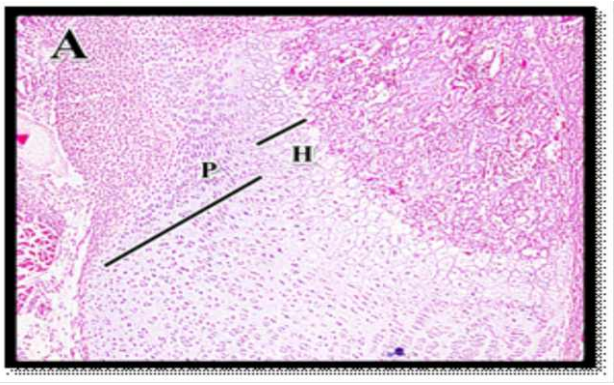

Fig 1: H&E Stain at 10X. Photomicrograph of Histological Section of Growth Plate of Control Group A1 Showing Height of Hypertrophy and Proliferative Zones

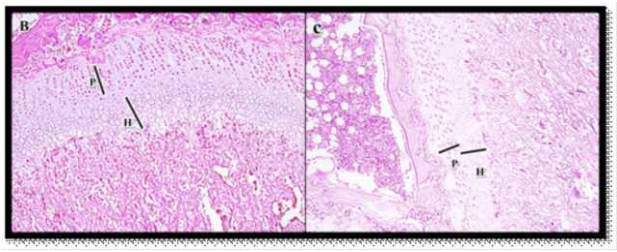

Fig 2: H & E at 10x. Photomicrograph of Histological Section of Growth Plate of Experimental Groups B1 (B) and Group B2(C) Showing Hypertrophy Zone(H) and Proliferative Zone (P)

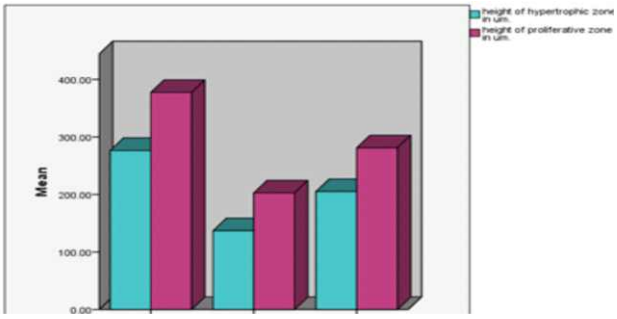

Fig 3: Cluster Bar Chart Showing Comparison of Mean Values of Height of Hypertrophy and Proliferative Zones in Micrometers (along Y-axis) among the Groups (along X-axis), Control Group A1 and Experimental Groups B1 and B2

longitudinal growth of the long bones of the offspring via its effects on epiphyseal growth plate. In the current study iron administration during pregnancy and lactation resulted in the reduction of height of the hypertrophy zone and proliferative zone of growth plate of long bones of the off spring as compared to the control group of rats. Effects on the height of hypertrophy and proliferative zones of growth plates of experimental groups B1 and B2 are significant statistically. The body has limited capacity to excrete iron, and iron in excess can lead to long-term damage to tissues.[17] Iron supplementation in non-anaemic pregnant women may not be beneficial. This may permit the consideration of a personalized approach for antenatal iron supplementation, especially in non-anaemic women.[18] Current study shows that injudicious iron supplementation of mothers during pregnancy and lactation significantly reduces. the height of hypertrophy and proliferative zones of epiphyseal growth cartilages of long bones of the off springs. It is in accordance with the previous researches showing the orally supplemented iron can easily cross the placental barrier and accumulates in the fetal tissues. A study shows that multiple doses of positively-charged nanoparticles given over several days resulted in significantly increased fetal deaths and accumulation of iron in the fetal liver and placenta.[19]

It is also in accordance with the previous studies showing the height of the hypertrophy zone is the main contributor of the growth plate height.[20] The growing cartilages are affected more as compare to the adult cartilage. Iron can be detected in isolated chondrocytes after a short culture period in the presence of either iron or haemoglobin, which negatively influences DNA content and proteoglycan synthesis rate. This inhibiting effect is reversible in the absence of a pro-inflammatory signal.[21] The decrease in the height of hypertrophy zone and the height of proliferative zone in the iron supplementation groups are comparable with the earlier study which shows the effects of iron on the bone.[22]

Conclusion

This study recommends that Indiscriminate iron supplementation of rats throughout pregnancy and lactation without checking serum iron levels can

affect the height of the hypertrophy and proliferative zones of epiphyseal growth plate of long bones of the off springs.

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ORIGINAL ARTICLE

Use of Alanine Aminotransferases Level and Platelet Count to Predict Dengue Fever Severity

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ABSTRACT

Objective: To determine the use of raised Alanine Aminotransferases and low platelet count as predictors of dengue fever severity.

Study Design: Cross sectional observational study.

Place and Duration of Study: Department of Medicine District Headquarter Hospital Rawalpindi, from 1st August 2014 to 31st December 2014.

Materials and Methods: Diagnosed cases of dengue fever based on history, examination and positive non structural protein 1(NS 1) antigen were included consecutively. Platelet count and Alanine Aminotransferases level were performed on admission. Patients were classified into different groups on the basis of Alanine Aminotransferases level and Platelet count. Disease severity and outcome was observed as; having dengue without complications, dengue hemorrhagic fever and dengue shock syndrome. The relationship of Aminotransferases levels and Platelet count were studied with the disease severity. The statistical analysis of data was done in SPSS version 20.

Results: Among 124 confirmed dengue fever cases, 66.9% were males and 33.1% females with mean age of 30.79+13.78 years. Mean duration of fever was 5.59+1.32 days and mean duration of hospital stay was 3.74+1.04 days. Elevated Alanine level was found in 89.5%. Thrombocytopenia was observed in 99.58% patients. Most of our patients were found to have dengue fever without complications, only 20.2% developed dengue hemorrhagic fever and 11.3% developed dengue shock syndrome.

Conclusion: Neither Alanine Aminotransferases, nor low platelet count can predict the severity of Dengue fever.

Key Words: *Alanine Aminotransferases, Dengue Fever, Platelet Count.*

Introduction

Dengue fever is an acute febrile illness of viral etiology, and currently it is the most common cause of arthropod borne viral disease globally.[1] Dengue virus belongs to the Flaviviridae family with 4 serotypes. The disease is endemic in over 100 countries, including Pakistan; around 2.5 billion people are at increased risk of infection worldwide.[2,3] About 390 million cases of Dengue fever occur annually and 96 million develop severe Dengue[4], causing 20,000 deaths every year in developing countries.[5] Mortality is high in patients developing dengue hemorrhagic fever or dengue shock syndrome, mortality is as high as 20% in severe dengue if untreated.[6] It is thought that development of severe dengue and evolution to death is related to some clinical and laboratory findings that are still not fully understood.[7] Its first outbreak in Pakistan was reported in 1994 and it has become a major health problem in Southeast Asia with 2-3 epidemics every year.[8]

Dengue virus is transmitted to humans by bites of infected female Aedes mosquito.[1] Infection with dengue virus can present after 5-7 days of mosquito bite with high grade fever, rash, severe headache.[9] Other symptoms include severe joint and muscular pain, nausea, vomiting, and eye pain. Illness ranges from a mild, non-specific febrile syndrome to classic dengue fever (DF), to the severe forms of the disease, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[10] In 2009 WHO classified dengue according to levels of severity based on clinical and laboratory parameters.[11] This classification may help in clinical management of patients but the parameters of the severity used in this classification

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may vary according to different epidemiological settings.[12]

The identification of some clinical as well as laboratory parameters that can serve as early predictors of severe Dengue is important to reduce the morbidity and mortality of Dengue fever. Many studies has been conducted to find the predictors of mortality in severe Dengue, gastrointestinal bleeding, hematuria, thrombocytopenia, dyspnea at rest, late presentation in hospital, age ≥ 50 years and high hematocrit were found as risk factors for increased mortality in severe Dengue.[13,14,15] Few studies has also been conducted to indentify the early predictors of severe Dengue, high Lactate Dehydrogenase, high Lactate[16] and Ferritin[17] levels were found as early predictors of severe Dengue at the time of hospital admission.

Raised Alanine Aminotransferases levels (ALT) and low platelet count are the most consistent laboratory findings in patients of Dengue fever.[18] This observation leads to the idea that raised Aminotransferases levels and thrombocytopenia may serve as early predictors of severity in the patients of dengue fever. The objective of this study was to evaluate Aminotransferases levels and platelet counts of the patients admitted with dengue fever as early predictors of dengue fever severity.

Materials and Methods

It was a cross sectional observational study conducted at Department of Medicine, District Head Quarter Hospital Rawalpindi, from 1st August 2014 to 31st December 2014. All diagnosed cases of Dengue fever, both males and females, from all age groups admitted in Dengue ward were included by convenient sampling. Patients with any of the following conditions were excluded: positive HBsAg or Anti-HCV antibodies, history of acute viral hepatitis in previous 3 months, history of idiopathic thrombocytopenia, those who had taken hepatotoxic drugs for any other illness in previous three months and DHF or DSS at the time of presentation. The diagnosis was suspected on the basis of two or more of the following symptoms: fever, headache, retro-orbital pain, myalgias, arthralgias, skin rash, nausea, vomiting, prostration and hemorrhagic manifestations, and confirmed by positive non-structural protein 1 (NS1) ELISA based antigen test as NS1 assay holds promise in early diagnosis of dengue infection.[19]

Patients were included consecutively, before enrollment; informed consent was taken from each patient. Ethical approval was obtained from Departmental Ethical Committee in this regard. Development of severe dengue (DHF, DSS) or discharge from the hospital without complications was the end point of the study.

Data was collected on a specially designed form. Platelet count and ALT levels were performed for all the patients on admission and were noted. Patients were classified into three groups on the basis of ALT level. Patients with normal ALT levels (6-39 IU/L) were included in Group-1, patients with level of ALT up to three times the normal value (40-127 IU/L) in Group-2 and those having ALT level above three times of normal (>127 IU/L) were classified as Group-3.On the basis of platelet count, patients were divided into four groups; those with platelet count more than 100,000/mm3 were classified as Group-A, count between 50,000 to 100,000/mm3 in Group-B, count between 20,000 to 50,000/mm3 in Group-C and count less than 20,000/mm3 were included in Group-D.

During hospital stay patients were monitored for the development of severe dengue. Severity was defined as presence of complications of dengue fever in the form of Dengue Hemorrhagic Fever or Dengue Shock Syndrome; both also known as Severe Dengue[11] Patients were later divided into three groups on the basis of disease severity and outcome observed; having dengue without complications; dengue hemorrhagic fever; and dengue shock syndrome. The relationship of ALT levels and platelet count were studied with the disease severity, development of complications and duration of hospital stay.

The statistical analysis of data was done in SPSS for Windows, version 20. Means and standard deviations were calculated for age, duration of fever at the time of presentation and duration of hospital stay. Frequency was used to calculate percentage for qualitative data like gender. Chi square test was used to compare categorical variables and to determine the relationship of ALT levels and platelet count with outcome of dengue fever. P values less than 0.05 were considered as significant.

Results

Total 124 confirmed dengue fever cases were

included in our study. Out of these 66.9% (n=83) were males and 33.1% (n=41) were females with mean age of 30.79 + 13.78 years (range 10 – 76 years). Fever was common presenting symptom in all the patients with mean duration of 5.59 + 1.32 days (range 2 – 11 days). The mean duration of hospital stay was 3.74 + 1.04 days (range 2 – 7 days).

Out of 124 patients only 20.2% (n=25) developed dengue hemorrhagic fever and 11.3%(n=14) developed dengue shock syndrome, with no significant correlation of severity of Dengue fever with Alanine Aminotransferase levels and Platelet count, shown in table I and II.

Table I: Correlation of ALT Levels with Severity of Dengue Fever

	ALT level (IU/L)			(p-value)
	7-39	40-127	>127	
Without complications	10	46	29	.733
Dengue Hemorrhagic Fever	3	14	8	
Dengue Shock Syndrome	0	8	6	
Total	13	68	43	

Table II: Correlation of Platelet Count with Severity of Dengue Fever

	Platelet count (cells/mm^3)				(p-value)
	> 100,000	50,000 – 100,000	20,000 – 50,000	<20,000	
Without complications	14	38	28	5	.513
Dengue Hemorrhagic Fever	2	10	10	3	
Dengue Shock Syndrome	1	4	8	1	
Total	17	52	46	9	

Discussion

Thrombocytopenia and raised ALT were two consistent findings in our study. But we did not find any significant relation between ALT levels at admission and development of DHF or DSS during hospital stay or with the duration of hospital stay. Also no such relation was found with platelet count.

Dengue is a common mosquito born infectious disease in many countries. In Asian countries dengue is more prevalent among males, many studies from Asian countries show male predominance.[20] Our results are in accordance with previous studies. Local studies also have almost same results.[17] But these results are in contrast with the studies from South America where male and females were equally affected.[20] This difference may be due to exposure difference among male and females in Pakistan and other Asian countries. Yew et al suggested that this may be due to difference in use of health facilities among two genders.[21]

Elevation of Aminotransferases and reactive hepatitis is a common complication of dengue infection[22], we found same. Our results are consistent with previous study by Kittitrakul et al who found raised AST and ALT levels in 88.2% and 69.3% of the patients, respectively.[23] Another study conducted in Vietnam showed raised ALT level in 97% patients also comparable with our results.[18] Though ALT levels were higher in majority of the cases in our study but raised ALT level was not found as an independent predictor of severity in our study. Same was concluded earlier by Villar-Centeno et al and Chhina et al.[24,25] Some other studies done by Khan et al and Ahmad A et al had different results. They showed that AST and ALT were statistically higher in patients with worse outcome thus can lead to early recognition of high risk cases.[8,26]

Thrombocytopenia is usually observed by 3rd or 4th day of the illness in dengue fever but is a constant feature and one of the diagnostic criteria of dengue hemorrhagic fever.[27]Thrombocytopenia was found in different national and international studies supporting our findings. Khan DM et al found thrombocytopenia in 71% patients in a study conducted in India.[28] The thrombocytopenia in Dengue may be due to decreased production of platelets due to bone marrow suppression.[27]

No significant correlation was found between degree of thrombocytopenia and severity of illness in our study. These results are contrary to the observation by Jayashree K, et al who stated that thrombocytopenia and platelet count is predictive as well as a recovery parameter of DF/DHF/DSS.[29] But another study conducted in Malaysia showed no relation between platelet count and hemorrhagic manifestations in dengue fever supporting our findings.[30]

The mean hospital stay reported in our study was 3.74 days. Various studies done at national and international level reported a mean stay of 3.4-6.2

days which are comparable to our results.[31] The duration of hospital stay had no significant correlation with severity of liver involvement or degree of thrombocytopenia in our study. Ahmad A et al had different results; they showed in their study that ALT level was significantly related with duration of hospital stay.[8]

Our study is subject to some limitations. First, our study included patients only at one hospital in Rawalpindi. Additionally, we enrolled patients with variable duration of fever at the time of presentation which may affect the laboratory investigations, however all the patients were enrolled before the development of complications. Furthermore, ALT level and platelet counts were not performed serially for every patient. Further studies should be conducted in other dengue endemic regions to establish the early predictors of dengue fever severity.

Conclusion

Neither Alanine Aminotransferase, nor low platelet count can predict the severity of Dengue fever. However more large scale multi-centre studies are required to confirm our findings.

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ORIGINAL ARTICLE

Frequency of Subclinical Hypothyroidism in Chronic Kidney Disease

Muhammad Saeed[1], Muhammad Ali Kashif[2], Asim Shehzad[3]

ABSTRACT

Objective: To determine the frequency of Subclinical hypothyroidism in patients with chronic kidney disease.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Medicine Military Hospital Rawalpindi, from 20th July 2015 to 05th January 2016.

Materials and Methods: In this study 250 patients were included, 50 patients for each stage of chronic kidney disease (CKD) starting from stage II to stage V and 50 peoples were from normal control group. Serum Creatinine, Thyroid Stimulating hormone (TSH) and Free T4 (thyroxine) were measured. Data was analyzed by SPSS version 17. The quantitative variables like age, free T4 and TSH was presented in mean and standard deviation for each group. Qualitative variables such as sex and presence or absence of subclinical hypothyroidism was reported as frequencies percentages for each group. Comparison of TSH level in four stages of CKD and control group was performed by using Kruskal Wallis ANOVA. Mann Whitening U test was used for Post Hoc analysis if required. Comparison of frequency of Subclinical hypothyroidism among groups was performed by using Chi-Square likelihood ratio. P value lower than 0.05 was taken statistically significant.

Results: Frequency of Subclinical hypothyroidism was 04 % in normal control group. Frequency of Subclinical hypothyroidism was 06%,10%,12% and 16% in stage II, III, IV and V respectively. Frequency of Subclinical hypothyroidism was increases with decrease of GFR. Frequency of Subclinical hypothyroidism was slightly increased preponderance in female as compared to male.

Conclusion: Frequency of subclinical hypothyroidism is higher in CKD patients than normal population, but it is statistically not significant.

Key Words: *Chronic Kidney Disease, Hypothyroidism, Subclinical Hypothyroidism, Subclinical Primary.*

Introduction

Progressive destruction of renal mass with irremediable and lasting sclerosis and loss of nephrons represent chronic kidney disease (CKD). It is characterized by decrease in GFR over months to years.[1,2] Numerous hematological, metabolic and other abnormalities like endocrine are likely to occur in CKD. [3,4]

Subclinical primary hypothyroidism, over last couple of decades has drawn unprecedented and unparalleled attention of researchers, capability to diagnose slight variation in thyroid is gradually upgraded.[5] Subclinical thyroid disease is defined as "serum freeT4 and free T3levels within their respective reference ranges in the presence of abnormally high serum TSH levels[6] It may be symptomatic or asymptomatic.[6,7] In routine clinical experience subclinical hypothyroidism have been seen in all age groups, however impact of subclinical hypothyroidism on population is under discussion.[8] There are no guidelines available to diagnose and screen subclinical hypothyroidism. Exact level of TSH to make diagnoses, is also under discussion. There is difference of expert view regarding complications of disease to develop, like Overt hypothyroidism, cardiovascular abnormalities and decreased GFR.[8,9] Subclinical primary hypothyroidism occurs in 5- 15% of the general population, and frequency of subclinical hypothyroidism is higher in females, old age group and among population with high iodine intake.[10] In addition, it has been suggested that primary hypothyroidism is more common in CKD population as compared to the general population.[9] However, little is known about the clinical features and implications of subclinical hypothyroidism in patients with CKD. In subclinical hypothyroidism

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cardiac abnormalities is the main cause of increased mortality and this further increases in CKD patients with subclinical hypothyroidism.[11] Cardiac complications of subclinical hypothyroidism are left ventricular systolic dysfunction, hypertrophy, and cardiomyopathy. Evidence of cardiomyopathy was confirmed by giving thyroxin therapy to these patients. There is evidence of improvement in cardiomyopathy with thyroxin therapy.[12] Left ventricular systolic dysfunction and LV hypertrophy are powerful predictors of death.[13]Various studies to diagnose the magnitude of problem have been done throughout the world both in pre dialysis CKD and ESRD patients and the estimated statistics ranges between 15-20% in different studies in different populations. This study was designed to measure the magnitude of problem in our population.

Materials and Methods

This cross sectional survey was performed in Department of Medicine Military Hospital Rawalpindi, from 20th July 2015 to 05th January 2016. CKD was defined as GFR below 90 ml/min/1.73 m2 body surface areas for more than 3 months. Stages of CKD is defined as a decrease in GFR as described in Table I.

Table I: Stages of CKD

Stage of CKD	GFR
Stage II CKD	60 to 89 ml/min/1.73 m^2
Stage III CKD	30 to 59 ml/min/1.73 m^2
Stage IV	15 to 29 ml/min/1.73 m^2
Stage V	Less than 15 ml/min/1.73 m^2

Subclinical hypothyroidism described as TSH more than5 mIU/L and T4 level is in normal range. Adult patients of CKD with either sex, age between 18-80 years, with different stages of pre dialysis CKD were included in the study. Healthy adults with GFR more than 100ml/min/m2 with no history of renal and thyroid disease were included as a control group. Patients of acute renal failure and CKD on dialysis were excluded from study. A sum of 250 patients, 50 for each stage II, III, IV, V and control group were selected by non-probability purposive sampling technique. Patients visiting Medical outpatient department, who fulfilled the inclusion criteria were included in the study after taking informed consent. Venous blood samples were taken from patients for serum TSH, serum creatinine and free T4 level estimation. An enzymatic method was used to measure serum creatinine on “Dimension” clinical chemistry system which employs a change of the kinetic Jaffe reaction given by Larsen. TSH and FT4 from a single lab were used in the diagnosis of subclinical primary hypothyroidism. ELISA kits from NETRIA UK were used to assess the thyroid status of the participants. Data for reporting was analyzed by Softmax Pro software using V Max from Nova Bio labs as plate reader. All information's obtained from patient were recorded on the predesigned performa. Data was analyzed by SPSS version 17. The quantitative variables like age, free T4 and TSH was presented in mean and standard deviation for each group. Qualitative variables such as sex and presence or absence of subclinical hypothyroidism was reported as frequencies percentages for each group. Comparison of TSH level in four stages of CKD and control group was be performed by using Kruskal Wallis ANOVA. Mann Whitening U test was used for Post Hoc analysis if required. Comparison of frequency of Subclinical hypothyroidism among groups were performed by using Chi-Square likelihood ratio. P value less than 0.05 was taken statistically significant.

Results

The study included 250 patients, 123 (49.2%) were male and 127(50.8%) were females. Group wise gender distribution is given in Table II. Mean age was 56.33+ 15.06. Data of TSH, GFR, and T4 in all groups are given in Table III with mean and slandered deviation. Frequency and percentage of subclinical hypothyroidism is given in table IV.

Comparison of TSH level to find out difference in frequency of subclinical hypothyroidism among CKD stages and normal population was performed by Kruskal Wallis. P value calculated was 0.587 and 1.000 which were not significant statistically. There was no significant change among each stage of CKD and normal population and among male and female groups P value 0.617. For post hoc analysis we performed Mann Whitney U test. Significant statistical difference was only seen in Stage IV of CKD among males and females. Rest of the stages of CKD and normal population did not have statistical difference.

Discussion

Our study showed that, frequency of subclinical hypothyroidism is higher in persons with decreased

Table II: Gender Distribution of Study Subjects (N= 250)

Gender	CKDII	CKDIII	CKDIV	CKDV	Normal Patients	Total
Female	28	23	24	29	23	127
Male	22	27	26	21	27	123
Total	50	50	50	50	50	250

Table III: Data of TSH, GFR, and T4 in all Groups (N=250)

Variables	N	Minimum	Maximum	Mean	Standard Deviation
Stage II GFR	50	60.00	89.00	74.0200	8.98636
Stage II TSH	50	0.02	12.10	2.2820	2.20674
Stage II Free T4	50	6.25	28.64	15.6756	4.49573
Stage III GFR	50	31.00	60.00	46.5200	9.52170
Stage III TSH	50	0.16	10.68	2.6614	2.48296
Stage III Free T4	50	8.71	23.80	16.4882	3.82532
Stage IVGFR	50	15.10	29.50	21.1106	4.17143
Stage IV TSH	50	0.45	9.61	2.5824	2.03005
Stage IV Free T4	50	10.23	23.01	16.2269	3.89478
Stage V GFR	50	10.05	15.00	12.6394	1.62898
Stage V TSH	50	0.14	14.58	2.9642	2.89625
Stage V Free T4	50	8.16	22.41	15.4818	3.88398
Normal Populatio n GFR	50	96.85	140.30	1.1638E2	11.43310
Normal Populatio n TSH	50	0.12	10.50	2.5347	1.92880
Normal Populatio n F T4	50	9.05	23.00	16.5592	3.92824

Table IV: Frequency Percentage of Subclinical Hypothyroid in Different Stages of CKD

Frequency of subclinical hypothyroidism in different stages of CKD and normal population		
Stages	**Frequency**	**Percentages**
Stage II	3	6
Stage III	5	10
Stage IV	6	12
Stage V	8	16
N.P	2	4

estimated GFR as compared to normal population but it was not statistically significant.

Previous studies have shown rising trend of frequency of hypothyroidism in ESRD requiring maintenance hemodialysis as well as peritoneal dialysis, and an increased prevalence of goiter. Only a few of the previous studies have examined the prevalence of hypothyroidism among patients with CKD not requiring dialysis. Bando et al in a small number study population with 32 diabetic and 31 no diabetic nephropathy (urinary protein excretion greater than 0.5 g/day), 24% of study subjects had overt or subclinical hypothyroidism, with a higher prevalence among patients with diabetes.[14] While in our study a large sample size was used. In our study we have compared normal population with diseased population. We also compared stage II, III, IV and V of CKD with each other. In another study, Lo et al. showed rising trend of frequency of subclinical and clinical primary hypothyroidism in population of deranged renal function in cohort of United States adults. In this study frequency of subclinical hypothyroidism was more than 20 % and in this study GFR was less than 60 ml/min.[15] In contrast, our study included only subclinical cases, those having clinical signs and symptoms of thyroid insufficiency were excluded from study. The criterion for CKD was same as in study. Our study did not adjust for the age, gender and ethnicity/race.

In a study by Chonchol et al subclinical primary hypothyroidism was found in 09 percent of study population and 09 percent of population were found with e GFR below 60 ml/mins. Frequency of subclinical hypothyroidism was 7 percent at GFR more than 90 ml/min and trend was on rising side with further decrease in GFR to 17.9 % ($P < 0.0001$ for trend).[5] This was a large study which used data base and analyzed the results. The study did not differentiate between those having abnormal thyroid function test with symptoms or those without symptoms. While our study excluded the patients with signs or symptoms of thyroid disease.

In our study it is shown that patients with chronic kidney disease have more percentage of subclinical hypothyroidism than normal population and frequency also increases with decline in GFR. Risk of cardiovascular events and atherosclerotic diseases and mortality is higher in those patients with CKD and subclinical hypothyroidism, so we should consider routine screening of subclinical hypothyroidism in CKD. It can lead to decrease mortality and improve patient's health.

Our study have certain limitations. The study did not adjust for the age gender, race, blood glucose levels, serum cholesterol and serum triglycerides levels.

The study was based on the estimation of GFR rather than more accurate methods to measure actual GFR. The study was cross sectional and limited in its ability to establish cause and chronological relationship between CKD and subclinical hypothyroidism.

In this study increased frequency of subclinical hypothyroidism was found in patients with reduced renal function not on dialysis as compare to normal population. Frequency of subclinical hypothyroidism also increases with decrease in GFR. Further studies are required to determine causes of decrease thyroid function in chronic kidney diseases. Studies are also needed to explore the potential benefits of screening CKD patients for subclinical hypothyroidism and possible role of the treatment to avoid cardiovascular risks.

Conclusion

Frequency of subclinical hypothyroidism is higher in CKD patients than normal population, but it is statistically not significant.

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ORIGINAL ARTICLE

Effects of Sleep Deprivation on the Area of the Prostatic Acini in Rats and the Protective Effects of Omega 3 Fatty Acids

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ABSTRACT

Objective: To study the protective role of omega 3 fatty acids on the histomorphological changes in the area of the prostatic acini in rats, induced by sleep deprivation.

Study Design: Lab based randomized control trial.

Place and Duration of Study: The study was conducted at Anatomy Department, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Rawalpindi for a duration of one year from 11th Nov 2014 to 11th Nov 2015.

Materials and Methods: Thirty male Sprague Dawley rats, 3-4 months of age with average weights of 200-300 grams (gm) were divided in three groups each having 10 rats .Group A served as control with standard lab diet and regular sleep -wake cycle. Group B was subjected to sleep deprivation of 16 hours followed by a sleep window of 8 hrs daily for 2 months and group C was administrated with omega 3 fatty acids and was sleep deprived as group B for 2 months. At the end of the experimental period rats were anesthetized and their blood sample was drawn for hormonal assay. They were dissected and the prostate gland was removed and fixed in 10 percent formalin. Five micrometer sections were obtained after tissue processing and stained with haematoxylin and eosin (H&E) for histological study.

Results: Microscopic examination revealed that the percentage area of the prostatic acini was decreased by 100% in group B and 30% in group C. This revealed that the area of the prostatic acini was decreased in the group B as compared to the control group A. Decrease in the acinar area in the experimental group C was not that marked as compared to experimental group B.

Conclusion: It is concluded that sleep deprivation has deleterious effects on the area of the prostatic acini and that omega 3 fatty acids has an ameliorative effect on the area of the prostatic acini.

Key Words: *Omega 3 Fatty Acids, Prostate, Rats, Sleep Deprivation.*

Introduction

Sleep deprivation has become one of the leading forms of stress causing detrimental effects to the mind and body. Prolonged sleep deprivation usually takes place in extreme situations or under experimental conditions.[1] The most common causes of sleep deprivation are those related to contemporary lifestyle and work-related factors; thus the condition affects a considerable number of people. Sleep deficiency (insomnia) accompanies certain pathological states and may require treatment[2] It has become one of the greatest health risk factor that contributes to several disease processes. It leads to biochemical, hormonal, behavioral and neurological alterations. Therefore, it is imperative to apprehend the impact of sleep deprivation on the body. Several experiments that have been conducted on rat models have established the physical effects of sleep deprivation such as dermatological findings, weight loss (in spite of regular food intake), decreased immunity followed by death in a couple of weeks proving that sleep is a basic biotic need that has impact on the operation of many organ systems.[3] As sleep deprivation has been proven to be a form of stress it exerts allostatic load i.e wear and tear of the systems in the body, circadian disruption including increase in the cortisol levels.[4] The increase in cortisol levels due to stress is associated with decrease in testosterone levels as sleep deprivation results in many alterations in the morphology of various organs and hormonal abnormalities.[5] It is strongly associated with alteration in the male sex hormone levels causing detrimental effects on the prostate gland.[6] Testosterone plays a pivotal role in the development

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and functioning of the prostate gland.[7] According to a latest study men with sleep problems have been linked to the development of prostate cancers.[8] Sleep deprivation is strongly concomitant with noticeable drop in the levels of androgens.[9] This shows reduced steroidogenesis or lessened expression of androgens in the target glands. These effects can be counteracted by the addition of healthy fats like omega 3 fatty acids. These lessen the action of 5 alpha- reductase enzyme and have a protective role on the prostate gland.

Omega 3 fatty acids (Omg 3 FA), an assembly of polyunsaturated essential fatty acids that are compulsory for human health but cannot be made de novo, so, they have to be attained from exogenous sources, found in enormous quantities in fish oil . The consistent use of omega 3 fatty acids greatly diminishes the risk of developing prostate cancer.[10] Eicosapentaenoic acid (EPA) a form of omega 3 FAs is linked with reduction in the progression of prostate cancer.[11] It also results in the decline of oxidative stress and cell apoptosis.[12] Chavarro proved that augmented blood levels of omega 3 FAs were concomitant with a decreased likelihood of development of prostate cancer.[13] Stress is known to cause inflammation which can impair tissues and organs if not controlled. Exploring the positive effects of omega 3 fatty acids on the histomorphology of sleep deprived rat prostates may be of great help. The modus operandi of current study was to ascertain the effects of sleep deprivation on the area of acini in the prostate gland of rats and to establish the beneficial effects of omega 3 fatty acids.

The effects of sleep deprivation on the secondary sex glands especially in males e.g prostate gland, have not been focused upon. Therefore, this study was aiming at the histological effects of sleep deprivation on the prostate gland in rats and the protective effects that omega 3 fatty acids brought about to minimize these detrimental changes.

Materials and Methods

The study was a laboratory based randomized control trial carried out in the Department of Anatomy, Army Medical College Rawalpindi, in alliance with National Institute of Health (NIH) Islamabad and Armed Force Institute of Pathology (AFIP), Rawalpindi. It was spanned from 11th November 2014 to 11th November 2015 with the approval of ethical committee on animal experiments, of the Army Medical College, Rawalpindi. A total of thirty rats were used by random number table method, selected by non-probability convenient sampling. The rats were 3-4 months of age and weighing 200-300 grams (gm). They were kept in a well ventilated room and under a temperature range of 22-26°C. Rats were given NIH laboratory diet for two months. Water was provided ad libitum. Rats were indiscriminately divided into three groups (10 animals in each group). The rats of group A served as controls, they were fed with standard lab diet and subjected to normal sleep wake cycle. The rats in group B were fed with regular lab diet and subjected to sleep deprivation for a period of 16 hours daily followed by a sleep window of 8 hours daily for 8 weeks. Rats in group C were also subjected to sleep deprivation as group B and were administrated with Omg 3 FA at a dose of 260 milligram/kilogram/day (mg/kg/day), through oral gavage in addition to the regular lab diet. The dose of Omega 3 fatty acids was established based on prior studies [14] and it was obtained from Good`N`Natural, imported by Route 2 Health Pvt Ltd. The sleep deprivation apparatus was based on a modified pendulum technique and it consisted of a cage partitioned into 2 for each of group B and group C. It was fitted with an electrical device that caused to and fro jerky movements every 2 minutes set by a timer. This brought unrest in the rats causing sleep deprivation.[15]

At the completion of 8 weeks, 5 milliliter (ml) blood was drawn from each rat via intracardiac puncture, for evaluating serum testosterone then rats were dissected under chloroform anesthesia. The prostate glands were excised and fixed in 10% formalin and processed in automatic tissue processor. The tissue was infiltrated and embedded with paraffin wax. Cross sections of 5 micrometer (µm) thickness were obtained from the tissue blocks. All processing and staining procedures were done in histopathology lab at AFIP, Rawalpindi. H&E stains were used for routine histological study. Area of the acinar lumen was calculated by using a morphometric computer software “Motic Image Plus 2.0” a software for calculating area and other user defined morphometric parameters.[16] Images of three

selected fields were taken from each slide with the help of Olympus digital camera (10 mega pixel), Stylus 1010 were used through the ocular of the Olympus DP21 light microscope. The images were then transferred in the computer. Each image was opened in morphometric computer software "Motic Image plus 2.0". A scale was set to measure the area in micrometer square at 10X. Measurement tool for irregular shapes was selected and the area to be measured was outlined, the measurement was then analyzed and recorded. The final reading was recorded as the mean of area of 3 acini per three fields/slide per specimen.

IBM-SPSS version 21 was used for data analysis. ANOVA test was applied followed by Post Hoc Tukey`s test, for intergroup comparison of quantitative variables which was taken as means and standard deviations (mean +SD). A p value <0.05 was considered significant.

Results

Thirty Sprague dawley rats with an average age of 3-4 months and a mean weight of 220.16±10.80 grams were used in the experiment. After dissection and tissue processing, histological examination of the prostatic acini in group A showed that the mean area of the acini was 471.48±101.66 µm.² The mean area of the prostatic acini in group B was markedly decreased as compared to control group A, it was measured to be 246.69±17.08 µm.² The area of the prostatic acini in group C was observed to be 341.70±68.60 µm.² Intergroup comparison of the area of the prostatic acini, after the application of Post Hoc Tukey`s test, revealed a p value of <0.001, when group A was compared to group B which was statistically very significant. On comparison of groups A and C p value was found to be 0.001. When group B was compared to group C the p value= <0.016 which was also statistically highly significant. Intergroup comparison of serum testosterone levels revealed a p value of 0.000 when group A was compared to group B and when group B was compared to group C, which was statistically significant. However, on comparison of group A with group C, p value= 0.526 which was statistically insignificant.

Discussion

The difference in area among all the three groups was statistically significant (p<0.001). the group that was exposed to sleep deprivation showed marked changes in the histomorphology when the area of the prostatic acini was studied. This is because it has been found that the prostate is an androgen dependent organ. Sleep deprivation results in decreased androgen levels in the blood which also effects the growth of prostate and hence, affects the prostatic acini. The decrease in area of the prostatic acini is a result of decrease in the testosterone levels in blood due to sleep deprivation. Sleep deprivation is a form of stress and its effect on the sex hormones of rats was also proved in a study conducted where different stress modalities including sleep deprivation were inflicted on rats with resultant decrease in the testosterone levels. Sleep deprivation causes alterations in hypothalamo-hypophyseal axis leading to decreased circulating androgens in healthy individuals.[17] Omega 3 fatty acids are known to be responsible for the upsurge in luteinizing hormone (LH) formation, especially in animals ,this leads to the production of testosterone inside the leydig cells .This is one of the basic reasons why the area of the prostatic acini is not markedly decreased in rats of omega 3 administrated group C as compared to those of group B ,hence, proving the objective of the study.[18] Sleep deprivation causes low levels of androgens and results in activation of apoptosis, hence, atrophy or decrease in size of the acini due to cell death.[19] The male sex hormone levels i.e testosterone levels were estimated which specified a noteworthy diminution in the hormonal level of the rats in the experimental group B when it was related with the control group A (p <0.001). Nevertheless, the variance in the serum testosterone level in the experimental group C was not statistically significant when it was equated with the control group A. This is in agreement with the previous study which proved that the lack of sleep is associated with decrease in testosterone levels in rats. Omg 3 FAs are known to cause rise in luteinizing hormone (LH), especially in animals. This leads to the making of testosterone inside the Leydig cells .This establishes why the levels of testosterone were increased in rats that were given Omg 3 FAs i.e group C as compared to those of group B.[20]

Conclusion

It is concluded that sleep deprivation has harmful effects on the prostatic acini and that omega 3 fatty

Table I: Showing Mean Area of the Prostatic Acini (µm2) among the Control Group A and Experimental Groups (b) And (c)

	Control group A (n = 10)	Experimental group B (n = 10)	Experimental group C (n = 10)	*p*-value
Area of the prostatic acini (µm²)	471.48±101.66	246.69±17.08	341.70±68.60	<0.001*
Serum testosterone levels (ng/ml)	1.32±0.25	0.53±0.16	1.18±0.41	<0.001*

p value ≤ 0.05 is statistically significant
*= highly significant

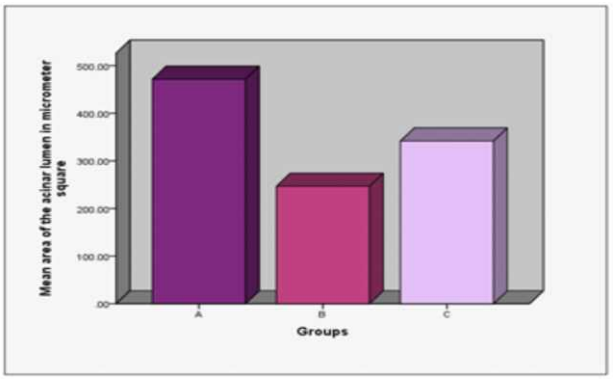
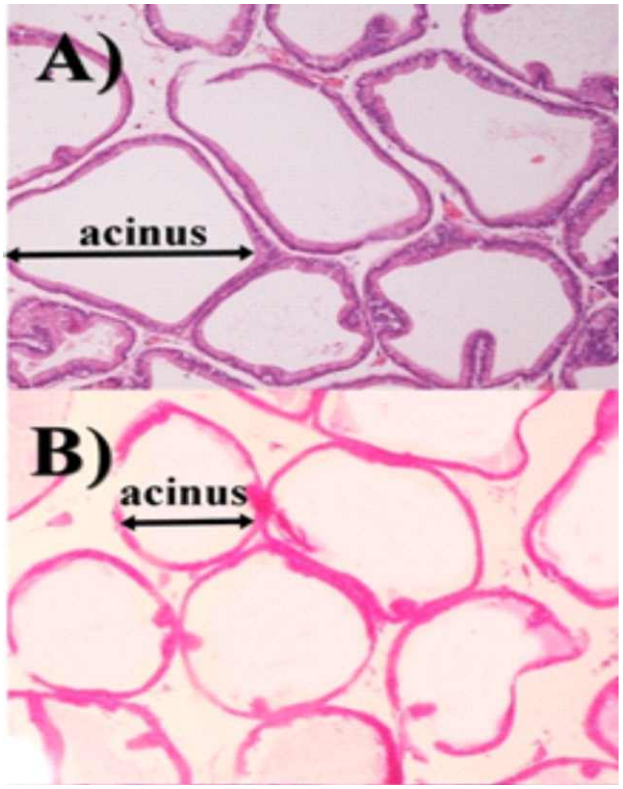


Fig 1: Bar Chart Showing Comparison of Mean Values of Area of the Prostatic Acini among the Control Group A, Experimental Group B and Experimental Group C



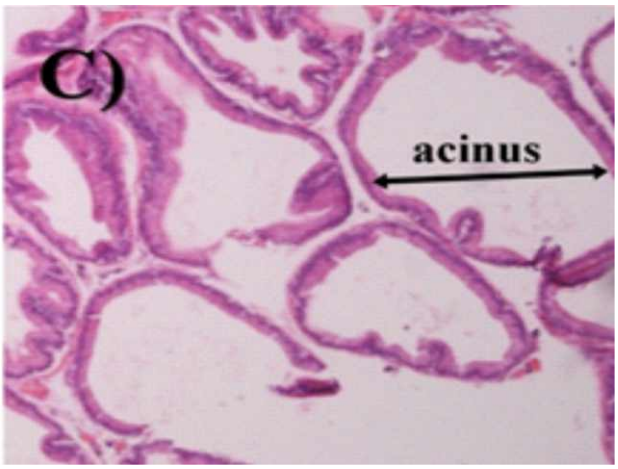


Fig 2: Photomicrograph Showing Comparison of the Area of the Acinar Lumen in Control Group (A), Experimental Group (B) and Experimental Group (C).

acids had a protective effect on the prostate gland in rats.

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ORIGINAL ARTICLE

Diagnostic Accuracy of Fine Needle Aspiration Cytology in Palpable Breast Lump; Our Local Experience

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ABSTRACT

Objective: To Determine the Diagnostic Accuracy of Fine Needle Aspiration Cytology In Palpable Breast Lump.

Study Design: Descriptive Cross Sectional Study.

Place and Duration of Study: The study was carried out at a Private laboratory Abbottabad from 1st January 2014 to 31st December 2016.

Materials and Methods: This study was carried out at a private laboratory Abbottabad from 1st January 2014 to31st December 2016. A total of 92 patients with palpable breast lump were included in the study. Fine Needle Aspiration Cytology of breast lump done and then histological examination was conducted on excised lumps after operation. The diagnostic sensitivity, specificity, diagnostic accuracy, positive predictive values, negative predictive value of Fine Needle Aspiration Cytology was determined by keeping the post operative histological results as gold standard.

Results: Out of 92, 4 cases (4.3%) C1, 06 (6.52%) C3, 07(7.60%) C4 were not included in study. Thus 75 cases with 41 (44.56%) C2 and34 (36.95%) C5 were included. It was calculated that Fine Needle Aspiration cytology for the diagnosis of breast lump has a diagnostic sensitivity of 97%, specificity of 100% with a diagnostic accuracy of 98.6%. Positive predictive and negative predictive values were 100% and 97% respectively.

Conclusion: Diagnostic accuracy of Fine Needle Aspiration Cytology is high, thus confirming that Fine Needle Aspiration Cytology of palpable breast lumps is a reliable method for early diagnosis and management of breast lump.

Key Words: *Breast Carcinoma, Fine Needle Aspiration Cytology, Palpable Breast Lump.*

Introduction

Breast lump is defined as any swelling present in the breast.[1]Different breast conditions ranging from benign to malignant can manifest themselves as breast lump.[2] A palpable breast lump is a matter of concern both for the patients and the clinicians because the rate of breast carcinoma has been increasing worldwide and in Pakistan the prevalence is also high.[3]

Breast carcinoma is the commonest malignancy in females all over the world and the second leading cause of death in females.[4] Studies reveal that the incidence of breast carcinomas has significantly decreased amongst females in USA.[5] In Asia, Pakistan has the highest rate of breast cancer and approximately 1 in every 9 Pakistani women is likely to have breast cancer.[6,7]

Due to this reason all the efforts are made to make correct diagnosis of the palpable breast lumps. A complete history and examination is the key to reach the diagnosis. Investigations like ultrasound and mammogram are important imaging tools used for diagnosis as well as screening purpose.[8]

Histological diagnosis of the breast pathology is made either via trucut biopsy or FNAC. FNAC has been considered reliable, rapid and economical method when combined with clinical impression and radiological findings (mammogram/ultrasound) to accurately diagnose palpable breast masses.[8]This procedure is easy, quick to perform and virtually painless, hence it has been considered a standard and first line tool for diagnosis in breast lump.[9]

The present study was done to determine the diagnostic cytological accuracy of FNA for palpable breast lump taking post surgical histological findings as gold standard.

Materials and Methods

This descriptive study was performed at a private laboratory of Abbottabad over a period of 03 years

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from 1st January 2014 to 31st December 2016. The study included 92 female patients presenting with lump/lumps in one or both breasts. Patients were included by non probability consecutive sampling. All the male patients presented with breast lump were excluded from the study. Demographic features of patient i.e. name, age and address were recorded on the proforma. FNAC was performed using a 22-guage needle attached to a 10ml syringe after explaining procedure to the patients and obtaining their informedconsent.[10] The area to be aspirated was cleaned with spirit before aspiration and multiple hits were made within the lesion, with sufficient negative pressure; the needle was removed and the pressure was applied to the area of aspiration to ovoid bleeding or hematoma formation. The aspirated material was smeared on glass slide and stained.[11]

Atleast two smears were fixed with alcohol and two air dried. Alcohol fixed smears were stained by Papanicolaou method while the air dried smears were stained by May-Grunwald-Giemsa technique. All the smears were screened and reported by pathologists. The diagnosis was given along with categorization of the lesion using an internationally recommended NHSBSP guidelines supplemented by a descriptive report.[12] There are five categories according to this format. C1 represents a non diagnostic or inadequate aspirate. C2 is benign. C3 isatypia probably benign and C4 is suspicious for malignancy. C5 is mentioned in the report when there is definite evidence of malignancy.

The diagnosis given on FNAC was then recorded along with the diagnosis given on histopathology when the resected specimen was received later for each patient. Sensitivity, specificity and diagnostic accuracy were calculated using standard statistical formulas.[13]Those cases having C1, C3 and C4categorization on FNAC were excluded from calculation. Those cases which were found to be malignant by cytology as well as by histology were labeled as True Positive(TP).False positive (FP) were those diagnosed as malignant on cytology and turned to be benign on histology. True negative (TN) were benign on both cytology and histology. False negative (FN) were negative on cytology but positive for malignancy on histology. The diagnostic accuracy was calculated as (TP+TN)/ (TP+FP+TN+FN).[13]

Results

In this study 92 patients who presented with breast lump underwent FNA. All were females. Age of population studied ranged between 18-80 years with a median of 37 years.

In majority (81.56%) of cases, a definite diagnosis of either a benign or a malignant lesion was given. Total 41(44%) cases were reported as benign (C-2 category) and 34 (37%) cases as malignant (C-5 category), shown in table I.

Table I: Diagnostic Categories of FNAC

Diagnostic Category	No (%)
C-1, Non diagnostic	4 (4.34%)
C-2, Benign	41(44.56%)
C-3,Atypia, probably benign	6 (6.52%)
C-4, Suspicious for malignancy	7 (7.60%)
C-5, Malignant neoplasm	34 (36.95%)
Total	92

The patients with malignant breast lump had age more than 29 years, with the median age of 47 years. The cases with inadequate result on FNAC i.e. (C1) were 4 out of 92, so excluded from final evaluation. Cases with FNAC report of C3 and C4 were also excluded from the final evaluation.

There were no false positive cases while 1 false negative case was reported. This case was reported as benign inflammatory lesion on FNAC, later on lumpectomy was performed and histopathological examination revealed invasive ductal carcinoma. Forty patients had benign cytological findings by FNA cytology;these 40 were also confirmed as benign by histopathology examination which represents (TN cases). Among benign cases maximum there were fibrocystic (62.5%) followed by fibroadenoma disease (32.5%), chronic mastitis (2.5%), lactating adenoma (2.5%).

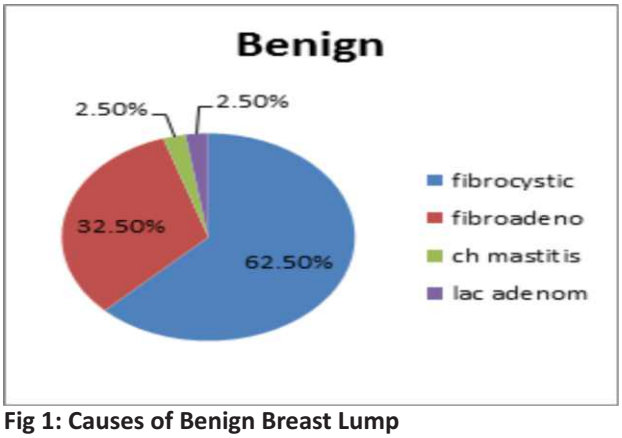

Fig 1: Causes of Benign Breast Lump

Thirty four cases that were diagnosed as malignant on FNA cytology as well as histopathology, thus considered (TP cases).Among malignant cases, most were invasive ductal carcinoma (94.11%), followed by lobular(2.94%) and mucinous carcinoma(2.94%).

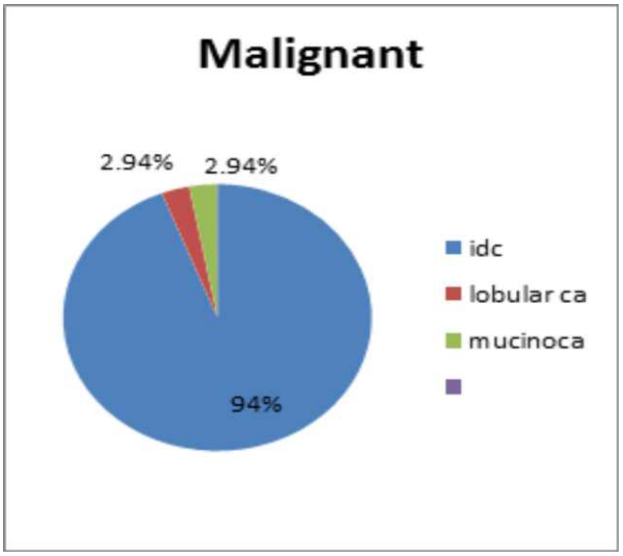

Fig 2: Causes of Malignant Breast Lump

Table II: Cytological and Histopathological Diagnosis

FNA cytology	Histopathology	
	benign	Malignant
benign	40(53.33%)(TN)	1(1.33%)(FN)
malignant	0 (0%) (FP)	34 (45.33%) (TP)

Sensitivity rate for breast FNA was 97% and specificity rate was 100%. Positive predictive value was 100% and negative predictive value was 97%. Diagnostic accuracy of FNA was calculated as 98.6%.

Discussion

Breast lump is the the most frequent clinical symptom which which the patients present in surgical department all over the world.[14] Most of the females with breast lumps have 80-85% benign lesions.[15]Still breast cancer is the leading cause of deaths worldwide and specially in developing Asian countries, it is emerging as the commonest malignanacy in females, which necessitates the use of an ideal i.e. quick, lesser invasive and accurate diagnostic tool.[16]FNAC has been considered an ideal initial diagnostic modality in breast lumps because it is less invasive, does not require anesthesia, thus comfortable to the patient and lacks false positive results.[17]The use of FNAC to find out the presence of malignancy before surgery has been well documented.[2]

For breast FNA cytology to be clinically useful, a satisfactory sample must be obtained. Smears from breast aspirates are considered satisfactory when the material is representative of the lesion, adequate in quantity and to cytopreparation is excellent. Our inadequate (C1) rate was 4.3%. In this study, we had a sensitivity rate of 97% and specificity rate of 100%, which gave diagnostic accuracy of 98.6%. Our false negative rate was 1.33% and there was no false positive result in our study. Positive predictive value and negative predictive value in this study was 100% and 97%.which is in comparable with previous

A study conducted by Ahmad F et al[18], found inadequate breast FNA smear(C1) reported to be 2.86% which is comparable to our study.

Our results are comparable with the study conducted by Bugti S et al on ; a comparative study of pre-operative FNAC with post operative histopathology in the diagnosis of breast lump in 2015, and obtained a sensitivity rate of 95%, specificity rate of 100%, which gives a diagnostic accuracy of 95%.our study supported this study.[2]

A study was published by Patel PJ et al in 2015[13] to evaluate sensitivity, specificity and accuracy of fine needle aspiration cytology of breast lump, calculated sensitivity 71.4%, specificity 100% and diagnostic accuracy of 94.6%.these results are similar to our study. A study was published by Ahmed S et al in 2010, they evaluate the accuracy of FNAC in palpable breast lumps at breast clinic of Abbasi Shaheed hospital Karachi, and they found sensitivity of 91.3%, specificity of 100%, with overall accuracy of 96.5%.[19]

Another study conducted by Gupta R et al in 2017 analyzed utility of fine needle aspiration cytology as a screening tool in diagnosis of breast lumps. They found 85% sensitivity, 95.8% specificity and diagnostic accuracy of 93%.20 Our results are comparable with these studies.

A study conducted by Hamdani NR et al worked on fine needle aspiration cytology breast lesions-its concordance with histopathological examination of excised lesion in 2015, found sensitivity of 89%, specificity 100%, and diagnostic accuracy was found to be 97%.[12] The results of this study is in favor of our study.

A study done in 2011 by Tasneem S et al, and Hamdani NR et in 2015 also found false negative rate to be 1.92% and 2.7% respectively,[21,12] which shows

similarity with our results.

There was no false positive result in our study equivalent with study conducted by Hamdani NRet al.[12]Positive predictive value, and negative predictive value were found to be comparable with a previous study conducted by B Saira et al,[2] who found values 100% and 93.3% respectively.[2]

Conclusion

Diagnostic accuracy of FNAC is high, thus confirming that FNAC of palpable breast lumps is a reliable method for early diagnosis and management of breast lump. FNAC should be used as a routine diagnostic tool for breast lumps due to its cost effectiveness, quick results and high accuracy.

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CASE REPORT

Effects of Autologous Bone Marrow Derived Stem Cell Transplant and Exercise Training Program on Rheumatoid Arthritis

Syed Salman Naeem Gilani[1], Muhammad Naeem[2], Tauseef Bukhari[3], Muhammad Asad Ullah[4]

ABSTRACT

A 65 year old female patient suffering from Rheumatoid Arthritis reported with multiple joint pains, malaise, fatigue, difficulty in breathing, difficulty in opening Jaw, difficulty in holding objects and menopausal symptoms. She had been taking multiple medications including Indomethacin, Aspirin, Prednisolone, Methotrexate etc. Treatment at Al-Sayed Hospital Rawalpindi included autologous bone marrow derived stem cell transplantation and exercise training program. Specific exercises were given four weeks before and eight weeks after stem cell transplant at our center. After one year of treatment, the patient had improvement in all of her signs and symptoms, which enabled her to discontinue several medications. There was marked improvement in her joint pain, range of motion, muscle power, grip function, functional activities and Activities of Daily Living (ADLs).

Key Words: *Bone Marrow Derived Stem Cells, Exercise Training, Rheumatoid Arthritis.*

Introduction

"Rheumatoid Arthritis is a chronic progressive inflammatory disease of the joints resulting in painful deformities and immobility, especially in the small joints". It decreases red blood cell count and causes inflammation around lungs and heart. It also presents with fever and low energy.[1] Middle age women are affected 2.5 times more than men. The diagnosis is made mostly on the basis of a patient's signs and symptoms.[2,3]

The main objective of the treatment is to reduce pain and inflammation, and improve a person's overall daily activities. This may be assisted by ensuring proper balance between rest and exercise, by using splints and assistive devices. The progression of the disease may be delayed by the use of a class of medicines known as disease-modifying anti-rheumatic drugs (DMARDs). In certain cases surgery to repair, replace, or fuse joints is opted for and proves useful.[4]Alternative medicine and related treatments are not yet supported by evidence. Before the year 1999 biological treatments were not available for public use. It was only then that these pioneering endeavors saw their first introduction, thus dramatically changing the lives of Rheumatoid Arthritis patients with highly selective immunotherapy.[5] In a vast majority of patients, who were given Anti-Tumor Necrosis Factor (TNF) therapy, significant improvement was noted clinically with minimal adverse effects right after the treatment. Of late, the first anti- Inter Leukin1 (IL-1) therapy has been approved for use clinically as an IL-1 receptor antagonist (anakinra).[6]

Bone Marrow derived Stem cell transplant is a promising treatment choice for patients suffering from RA. It is suggested that hematopoietic stem cell transplantation (HSCT) is a useful therapy for severe RA on the basis of animal models and case reports of patients undergoing the procedure for other indications.

Case Report

A Sixty Five year old female came to us with the history of multiple joint pains since she was 19 years old and was diagnosed with Rheumatoid Arthritis 01 year later. She had taken multiple medicines including Indomethacin, Aspirin, Prednisone, Methotrexate, and Gold. She was complaining of severe pain, decreased range of motion (ROM) decreased mostly in lower limbs, muscle tightness, deformities, functional limitations, unable to change posture from lying to sitting and sitting to standing, muscle wasting, pulmonary problems, difficulty in opening jaw, difficulty in holding objects. Muscle

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Power is given in Table I.

Lab investigations showed: Hemoglobin: 11.1 g/dl, ESR: 84, RA Factor and Anti-CCP (cyclic citrullinated peptide) antibodies: Positive, HbA1C: 9.2.

Exercise training program included active and passive range of motion, stretching of tight musculoskeletal structures, isometrics, deep breathing exercises, cycle ergometry for upper and lower limbs to improve endurance and aerobics was given at our hospital for four weeks before and eight weeks after stem cell transplant. This session was given twice a day with twenty to thirty repetitions of each activity. Patient was then transitioned to home exercise management and followed up at three, six and twelve months after the procedure.

Stem Cell Extraction and Transplant Procedure: Patient was given Injection Filgrastim (Granulocyte colony stimulating factor analog) 10 µg per kg body weight subcutaneously without methyl prednisolone or cyclophosphamide and baseline CBC with HPC (hematopoietic progenitor cells) was performed. Two days later CBC with HPC was repeated and patient's Bone marrow stem cells were harvested by apheresis. A total of 100cc of Autologous Bone Marrow Stem Cells were harvested. CBC with HPC was repeated at the end of apheresis to confirm the total number of hematopoietic stem cells. Harvested Stem cells were then injected into her Knee, Shoulder, Metacarpophalyngeal and Inter-phalyngeal joints bilaterally in the procedure room under aseptic technique. Afterwards stem cells were transfused to the patient intravenously.

Outcome Measurements

Patient showed marked improvement in muscle power one year after the stem cell transplant. A comparison of muscle power before starting the treatment at our center and one year after stem cell transplant is given in Table I.

Patient's pain was reduced from severe to pain free. Range of motion (ROM) significantly increased. Range of motion (ROM) was measured by Goniometry. Grip function was measured by the "Sollerman test". The results of Sollerman test are given in Table II.

Modified HSS (Hospital for Special Surgery) Score for both knees were measured that improved from 57 to 90 for her left knee and 57 to 87 for her right knee.

Table I: Muscle Power Before and One Year after Bone Marrow Derived Stem Cell Transplant

Region	Right Side		Left Side	
	Before Rx	1yr After Rx	Before Rx	1yr After Rx
Shoulder Girdle	-2/5	-4/5	3/5	4/5
Elbow (Flexor/Extensor)	2/5	4/5	-2/5	4/5
Wrist (Flexor/Extensor)	-2/5	4/5	2/5	4/5
Finger (Flexor/Extensor)	-2/5	+3/5	2/5	4/5
Hip Girdle	-2/5	+3/5	2/5	+3/5
Knee (Flexor/Extensor)	+2/5	+4/5	2/5	+4/5
Ankle(DorsiFlexor/Plantar flexor)	-3/5	+4/5	-3/5	+4/5

Table II. "Sollerman Test" Result. Performance is Graded from 4 (best) to 0 (worst). Grade 04 is Used for Correct Grip and Performance of the Activity within 20 Seconds. Grip Strength was Measured in Newton's by Means of an Electronic Hand Dynamometer

Type of Grip	Right Side		Left Side	
	Before Rx	1yr After Rx	Before Rx	1yr After Rx
Pulp pinch	1	3	0	3
Lateral pinch	1	3	0	3
Tripod pinch	1	2	1	2
Five finger pinch	2	3	1	3
Diagonal volar grip	2	4	1	3
Transverse volar grip	2	4	2	3
Spherical volar grip	2	4	2	4
Extension grip	1	3	1	2

Discussion

Bone marrow contains hematopoietic and non-hematopoietic (mesenchymal) stem cells, the latter being with immunoregulator properties. It is suggested that adult Mesenchymal Stem Cells (MSC) are useful as cellular therapy in several inflammatory diseases, including RA.[7] As MSC may transfer to sites of injury in vivo, it is suggested that inflamed joints are targeted by cells which might have a therapeutic effect on arthritis through MSC-mediated immunosuppression.[8] Leeds group presented a study in 1997 in which G-CSF was used at a dose of 5µg/kg/day to mobilize stem cells in five patients. In peripheral blood CD34+ count was checked to establish good efficacy. Patients remained stable after the treatment but we observed that administering methylprednisolone (median 80mg,

range 40–120mg) intramuscular or intra-articular diminished any pro-inflammatory effects of filgrastim. A phase I placebo controlled 1110 study was conducted in Australia to investigate the efficacy of G-CSF in patients suffering from active form of RA for the use of stem cell collection.[9] In Paris, four patients had their stem cells mobilized with cyclophosphamide 4g/m2 followed by G-CSF 5µg/kg/day. Probably, in three of the patients CD34+ cell yields were higher with G-CSF along with cyclophosphamide than with G-CSF alone. Arthritis and extra-articular manifestations improved markedly in these patients. To some extent the disease activity persisted in three patients, although it never went back to the same level even two years after the procedure.[10] In this case, the patient presented with inflammatory arthritis of almost forty five years' duration. She described the pain in her hands, wrists, elbows, shoulders and knees as frequently unbearable. She had significant muscle wasting, decreased muscle power, deformities, and weak grip strength. One year after autologous bone marrow stem cell transplant, the patient felt significant decrease in all subjective symptoms and discontinued her medications. ESR had normalized, indicative of clinical reduction of arthritis. She also felt improvement in ADLs. In our study Autologous Bone marrow derived Stem cells combined with exercises showed that it is a safe and effective treatment option for the patient suffering from Rheumatoid Arthritis and warrants a larger scale Phase I / II clinical study.

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COMMUNITY REPORT

Reorienting Primary Oral Healthcare – Pakistan Dental Mission 2017, An IMANA-Riphah Collaboration

Hajra Mustasim, Muhammad Humza Bin Saeed

ABSTRACT

"More than 90 percent of all systemic diseases have oral manifestations, meaning that your dentist could be the first health care provider to diagnose a health problem" (Raymond Martin, Academy of general dentistry). [1]

Oral health is widely recognized as a significant determinant of general health.[2] The burden of poor oral health has crucial social and economic implications, especially in the developing world.[3] Low BMI, poor pregnancy outcomes, low birth weight, diabetes mellitus and cardiovascular diseases are some of the conditions associated with compromised oral health.[4] In third world countries, other risk factors such as poor socioeconomic status, lack of dental health education and awareness and inappropriate health policies further contributes to the oral ill health of the general population.[5]

Islamic Republic of Pakistan ever since her embodiment has been paving roads to become a welfare state. On one hand it is the only Muslim nuclear power, while on other hand the country has been a victim of political instability and inaptness of beneficial policies. Pakistan has a total population of 180 million, majority of which resides in rural areas.[6] Pakistan ranks at 146 out of 187 countries in human development index and spends only 0.5% of GDP on the health sector which includes oral health services.[7] The challenges to Oral Health care system in Pakistan are substantial. With scarce resources it is difficult to contend with procurement of dental treatment; the prevention of oral diseases and the promotion of oral health. There lies a paucity of dental workforce in Pakistan, especially in rural areas. According to WHO recommendations, dentist to population ratio in developing countries should be 1:7500.[8] As of December 2016, the total number of registered dentists by PM&DC is 17,125.[9] This makes the dentist to population ratio of 1: 130,581 – this ratio drops to more than 1: 200,000 in rural areas of the country.

Oral health is still considered an amenity rather than a need among the masses in Pakistan. The population is burdened with many oral diseases because of the combination of limited resources and mismanagement of the available resources

To address the oral health situation in Pakistan, Riphah International University, in collaboration with the Islamic Medical Association of North America (IMANA), started a relief project focusing on the oral health needs of the socioeconomically deprived population in the rural areas of Northern Punjab. IMANA, founded in 1967, is the largest Muslim medical organization in North America carrying out medical relief programs all over the world. [10]

This outreach project named 'Pakistan Dental Mission 2017' (PDM 17) was jointly planned by the team of experts from IMANA and Riphah. The project was set up as a three step process. The first step of the project was to set up fully operational dental camps, providing free oral health services to the population who could not afford the dental treatment. Secondly, the mission set out to educate the masses with respect to oral health hygiene and its maintenance. Thirdly, an important objective with abiding aftermath was to conduct an oral health needs assessment of this population. These assessments, would in turn serve as a guideline for similar field projects directing towards the areas to be focused in the future. Also, this would be beneficial with respect to informing local health bodies to help build better oral health care policies.

After eight months of detailed planning, the first leg of the PDM 17 was initiated in August, 17. In this mission IMANA's 16-member team of dentists, dental hygienists and dental students from Howard University, Washington D.C, headed by **Dr. Sultan Chauhdary** took part along with a 26-member team from Riphah, Islamic International Dental College, inclusive of dentists, dental hygienists and dental

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assistants. Riphah's team was led by **Dr. Muhammad Humza Bin Saeed**.

This field mission lasted a week starting from 7th Aug till 11th Aug, 2017. The teams visited the districts of Doltala, village Missa Kaswal, Dharyala Kahun KalarKahar, Khora Khel Attock and lastly Pakistan Sweet Homes Orphanage, Islamabad.

Every day the camps were setup into mini dental hospitals around 9:00 am and operated unfalteringly till the treatment of last person. The camp treated patients with all kinds of dental problems, many counseled and medicated with free medicines. Respecting the cultural limitations and to provide comfort to the locals, male and female patients were treated separately. A total of about 1500 patients were treated over the period of these camps. The data collected during these dental camps suggested that the prevalence of dental caries among this population was quite high.

Traditional treatment of oral disease is extremely costly, the fourth most expensive disease to treat in most industrialized countries. The camp was equipped enough to manage all kinds of basic dental treatments and provide immediate relief to patients.

At the end of the camps, Dr Sultan commented that this was his first experience of working in Pakistan and most definitely, a memorable one. On the behalf of his team, he commended the efforts of the Riphah dental team and local authorities involved in arranging these camps. A particular observation that Dr Sultan was found to be highly impressive was the large number of female dentists who volunteered to participate in this camp.

When this project was being planned, it was expected that the total number of patients to be treated, would be around 4000. However, during the dental camps the patients' attendance was around 1500. Even though extensive efforts had been made by the organizers to spread the information regarding the dental cams, a relatively low attendance was observed. The low turnout proposes the possibility that the local population does not value their oral health as much as general health. This observation was supported by the clinical evidence, as many of the patients who came for the treatment had severely progressed caries and the only treatment that could be offered to them was extraction.

This alarming lack of concern for oral health must be tackled with an upstream approach by focusing on increasing oral health education among the masses. Engaging the lady health workers (LHWs) in the different Basic Health Units for oral health promotion would be a good place to start. This would require lobbying and advocacy at the government level to approve oral health education programs for the LHWs and mothers in rural areas. Dental camps supporting the effort of the oral health educators would prove to be highly effective adjuncts to the whole process. This education must reach the commoners. A healthy community will not only be able to perform better but will also impress less financial burden on the society.

This IMANA-Riphah camp is a step forward to public welfare of the country but the journey must not end here. This great initiative must continue so that the general public can benefit and be more active members of the society, each individual contributing in their own capacity. This would have an impact on lowering poverty and increasing the overall health of the society. Furthermore, it will improve maternal and child health and reduce geriatric health issues.

At the government level, acceptable and practical time-defined health related goals and standards of oral health should be decided and documented. Barriers to oral health promotion need to be overcome through co-operation between various sectors. Local and federal policies must focus on reducing such health inequalities. Advocacy of oral health education initiatives through collaborations such as The IMANA-Riphah PDM project must be supported and promoted equally by local health bodies and the government. Together a difference could be made, lives could be changed and the quality of life could in turn be improved.

"Only through improved dental awareness and education can we make an impact on dental health and ultimately the cost of providing care" (Sharon Zelkind, Vice president, Healthplx).[11]

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ABSTRACT

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Study design and sampling methods should be

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RESULTS

These should be presented in logical sequence in the text, tables and illustrations. All the data in the tables or illustrations should not be repeated in the text; only important observations should be emphasized or summarized.

DISCUSSION

The author's comment on the results supported with contemporary references, including arguments and analysis of identical work done by other workers. A summary is not required Brief acknowledgement may be made at the end.

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