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EDITORIAL

Early Diagnoses of Rheumatoid Arthritis is Important. It should be Clinical not Lab Dependent

Asim Zulfiqar

One of the most difficult task in clinical medicine is to evaluate a patient who presents with joint pain. If you were to open the index of a rheumatology text you would find a list of over hundred different types of arthritides (Table I). Fortunately the more commonly seen musculoskeletal conditions can be divided into five different groups. If approached logically a working diagnosis can usually be easily obtained.

Table I: Simple classification of Arthritic and Rheumatic Disorders[1]

Disorders	Examples
Inflammatory polyarthritis	RheumatoidArthritis, spondyloarthropathies
Degenerative arthritis	Osteoarthritis, spondylosis
Soft tissue rheumatism	Tennis elbow, bursitis
Acute monoarthritis or oligoarthritis	Crystal arthropathy, infectious arthritis
Diffuse connective tissue disease	SLE, scleroderma

One of the most common and devastating disease that has been encountered in clinical medicine practice is rheumatoid arthritis (RA). RA is a chronic inflammatory disease characterized by joint swelling, joint tenderness and destruction of synovial joints leading to severe disability and premature mortality.[2] RA affects between 0.5-1% of the general population, mainly during their working age affecting thus the functional capacity, with great economic burden to the individual and the society. In the last decades there was a clear evolution in knowledge about pathophysiology of the disease resulting in its approach and treatment. The association between symptom duration and RA persistence is not linear suggesting the presence of a confined period in which RA is most susceptible to treatment. Early RA (ERA) is defined as the diagnosis given in the first weeks or months of joint symptoms or signs. The concept of ERA and existence of a window of therapeutic opportunity (a time span in which the institution of effective therapeutic strategy in the form of DMARDs and biologicals) is important to modify the course of disease significantly, decelerating the progression of disease and minimizing joint damage and disability. Early diagnosis of RA is important as early therapeutic intervention reduces the accrual of joint damage and disability.[3]

In the first decade of current century the classification criteria set that was in widespread international use to define RA were the 1987 ACR (American college of rheumatology) criteria.[4] Those criteria gave emphasis to serological tests, rheumatoid nodules and joint erosions which are actually late features of disease. In fact these late features of disease which are pathognomonic for the diagnosis of RA can be prevented if effective therapy is given in early phase of the disease. Keeping in view the problems in diagnosing ERA the working group developed ACR/EULAR classification criteria for RA in 2010.[5] These classification criteria were introduced to select amongst the newly presenting patients with undifferentiated inflammatory synovitis, the subset of patients who are at sufficiently high risk of persistent and/or erosive disease (this being the appropriate current paradigm underlying the disease construct RA).

These classification criteria can be applied to any patient or otherwise healthy individual as long as two mandatory requirements are met. First there must be evidence of currently active synovitis in at least one joint. Secondly the criteria must be applied to those patients in whom the observed synovitis is not better explained by another diagnosis. Four additional criteria (Table II) can then be applied to eligible patients to identify definite RA. Application of these criteria provides a score from 0 to 10 with score of 6 or >6 being indicative of RA. A patient with a score<6 cannot be classified as having definitive RA at the moment but might fulfill the criteria at a later time point. To classify a patient as having definite RA or not a history of symptom duration, a thorough joint evaluation of both small and large joints and at least one serological test (RF or ACPA) and one acute phase response measure (ESR/CRP) must be obtained. It is acknowledged that an individual

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patient may meet the definition of RA without requiring lab test or even if the serological tests are negative (seronegative RA) e.g. patients with a sufficient number of joints and longer duration (> 6 weeks) of symptoms will achieve 6 points regardless of their serological or acute phase response status.

In conclusion RA is entirely a clinical diagnosis. Presence of positive serology (RF/ACPA) may potentiate your clinical diagnosis of RA but the presence of characteristic pattern of joint involvement of greater than six weeks duration almost makes certain the diagnosis of ERA. A due consideration of early aggressive treatment should be made in such patients to arrest the progressive disease at an earlier stage.

Criteria	Score
Joints Affected	
1 large joint	0
2 to 10 large joints	1
1 to 3 small joint	2
4 to 10 small joints	5
Serology	
Negative RF and APCA	0
Low positive RF or ACPA	2
High positive RF or ACPA	3
Duration of Symptoms	
< 6 weeks	0
>6 weeks	1
Acute Phase Reactants	
Normal CRP/ESR	0
Abnormal CRP/ESR	1

REFERENCES

1. Alderdice C. Approach to the patient with polyarthritis. Can Fam Physician 1990; 36: 553-4.
2. Pincus T, Callahan LF, Sale WG, Brooks AL, Payne LE, Vaughn WK, et al. Severe functional decline, work disability and increased mortality in RA patients. Arthritis Rheum 1984; 27: 864-72.
3. Van Nies J A B, Tsonaka R, Gaujoux-Viala C, Fautrel B, Van der Helm-van Mil A H M. Evaluating relationship between symptom duration and persistence of RA. Does a window of opportunity exists. Ann Rheum Dis 2015; 74: 806-812
4. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American rheumatism association 1987 criteria for the classification of RA. Arthritis rheum 1988; 31: 315-24.
5. Neogi T, Aletaha D, Silman AJ, Naden RL, Felson DT, Aggarwal R, et al. The American college of rheumatology/ European league against rheumatism classification criteria for RA. Arthritis Rheum 2010; 62: 2582-91.

ORIGINAL ARTICLE

Diagnostic Usefulness of Anti-Cyclic Citrullinated Peptide and Anti-Mutated Citrullinated Vimentin Antibodies in the Diagnosis of Seronegative Rheumatoid Arthritis Patients

Bushra Gohar Shah[1], Asma Saeed[2], Mohammad Faisal Khan[3], Hamid Javaid Quershi[4]

ABSTRACT

Objective: To determine the usefulness of anti-Cyclic Citrullinated Peptide and anti-Mutated Citrullinated Vimentin antibody in the diagnosis of seronegative rheumatoid arthritis.

Study Design: Descriptive Cross-sectional.

Place and Duration of Study: This study was conducted over a period of one year from January, 2010 to December, 2010. Subjects were recruited from Fatima Memorial Hospital, Rheumatology Outpatient Department, Lahore. The research work was conducted at the Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

Materials and Methods: A total of 58 known patients of rheumatoid arthritis fulfilling the American College of Rheumatology (ACR) Criteria were included in the study. After selection of subjects, written informed consent was obtained. The venous blood sample was taken and secured in vacutainers. Serum was extracted by centrifugation and stored at -20°C till analysis. Sera of all study subjects were tested by ELISA for presence of rheumatoid factor, anti-MCV and anti-CCP antibodies. The data obtained was analyzed by using SPSS version 17. The diagnostic significance of anti-CCP and anti-MCV antibody for the diagnosis of sero-negative rheumatoid arthritis patients was determined.

Results: Serum aCCP antibody was positive in 9 out of 28 RF-ive patients. So the sensitivity of serum aCCP in RF-ive group (n=28) was 32.1%. Serum aMCV antibody was present in 11 out of 28 RF-ive patients. The sensitivity of serum aMCV in RF-ive group was 39.2%.

Conclusion: Anti-CCP and Anti-MCV had a higher sensitivity for the diagnosis of seronegative RA.

KeyWords: *Rheumatoid Arthritis (RA), Rheumatoid Factor (RF), Anti-Mutated CitrullinatedVimentin Antibody (anti-MCV), Anti-Cyclic Citrullinated Peptide Antibody (anti-CCP).*

Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease of multifactorial etiology characterized by chronic inflammation of synovial joints that often leads to joint destruction. Rheumatoid arthritis typically produces symmetrical swelling of peripheral joints of hand and feet, but may affect the large joints as well. Rheumatoid arthritis has a worldwide prevalence of 0.5-3%, being 2-3 times more in women than in men, most frequent during fourth and fifth decade of life. Once established, rheumatoid arthritis is a lifelong progressive disease that produces significant morbidity and premature mortality in many patients.[1]

Many studies have shown that the disease progresses rapidly within first two years of onset and can lead to irreversible erosive joint destruction.[2] The diagnosis of RA depends primarily on history and clinical findings. The gold standard for the classification of RA is the American College of Rheumatology criteria (Arnett, et al., 1988).[3] This criterion was not designed for diagnosing RA, but rather to harmonize research in population and family studies for epidemiologic purposes. But they are ubiquitously used as a diagnostic aid. Patient must satisfy four out of seven criteria to be classified as RA. ACR criteria includes: 1)Morning stiffness of more than one hour for at least six weeks 2)Arthritis and soft tissue swelling of more than 3 of 14 joints/ joint groups,present for at least six weeks. 3) Arthritis of hand joints and wrist, present for at least 6 weeks. 4) Symmetric arthritis, present for atleast 6 weeks. 5) Subcutaneous nodules 6) Rheumatoid factor at a

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level above the 95th percentile. 7) Radiological changes suggestive of joint erosion/ and or periarticular osteopenia.

By the time clinical diagnosis of RA is made, irreversible joint erosions usually have occurred. Ongoing research has shown that early therapeutic intervention results in earlier disease control and consequently less joint damage.[2]

There is no single test or finding that can diagnose rheumatoid arthritis. Rheumatoid factor is the only serological test included in the ACR criteria. However, this auto-antibody lacks specificity. It may be found in patients with other autoimmune diseases and infectious disorders. It may also be present in sera of apparently healthy elderly individuals. Upto 25% of patients with rheumatoid arthritis have negative rheumatoid factor test (seronegative).[4]Therefore, disease-specific auto antibodies are needed for early diagnosis.

Currently available data suggest that the diagnosis of RA can be made by testing antibodies to citrulline-containing peptides such as anti-perinuclear factor(APF), anti-keratin antibody (AKA), anti-fillagrin antibody and anti-cyclic citrullinated peptides (anti-CCP) antibody. These all belong to the family of anti-citrullinated protein/ peptide antibody (ACPA).[5] All these antibodies recognize the antigenic epitope containing citrulline,[6] which is generated by post-translational modification of naturally occurring amino acid arginine by the activity of enzyme peptidyl arginine deiminase (PAD).[7] Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays.[6] Several assays for detecting anti-citrullinated peptide antibodies (ACPA´s) have been developed employing filaggrin derived peptides (CCP-assay), viral citrullinated peptides (VCP-assay), mutated citrullinatedvimentin (MCV-assay).[8] The Anti-MCV assay (ELISA) for the detection of antibodies against citrullinatedvimentin uses an antigen with a genetically modified sequence, which is most abundant in patients with rheumatoid arthritis.[8]

Positivity of these markers in the rheumatoid factor negative RA patients would suggest their additional benefit in the early diagnosis of this subgroup of patients, which are often diagnosed and treated late. It is expected that the results of the present study will help the clinicians in early diagnosis and timely management of this debilitating disease.

The aim of this study was to investigate the diagnostic value of antibodies against mutated citrullinatedvimentin (anti-MCV) and antibodies to cyclic citrullinated peptides (anti-CCP) in the diagnosis of seronegative rheumatoid arthritis patients.

Materials and Methods

This descriptive cross sectional study was conducted over a period of one year from January, 2010 to December, 2010. Fifty-eight subjects were recruited from Fatima Memorial Hospital, Rheumatology Outpatient Department, Lahore, by convenient sampling technique. The research work was conducted at the Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

A total of 58 patients attending the Rheumatology outpatient department of Fatima Memorial Hospital, Lahore were recruited in the study. All the patients fulfilled the American College of Rheumatology criteria for RA and were diagnosed by the rheumatologist. The study was approved by the Ethical and Review Committee of University of Health Sciences, Lahore. Informed written consent was taken from each study participant. A purposefully designed proforma was used to record data of the subjects including age, gender, disease duration, clinical characteristics and medication used. The venous blood samples were taken and secured in vacutainers. Serum was extracted by centrifugation and stored at -20°C till titer of anti-CCP and anti-MCV antibodies.

Rheumatoid factor titers were determined by ELISA (Highton, et al., 1986) using commercially available ImmuLisa anti-RF antibody IgM ELISA kit (Immco Diagnostics, USA), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA].Results were interpreted as follows:RF-IgM value of less than 7 IU/ml was considered as negative. RF-IgM value of 7-9 IU/ml was considered as borderline. RF-IgM value of more than 9 IU/ml was considered as positive.

Serum anti-MCV antibody levels were determined by ELISA[9] using ELISA kit (Cusabio Biotech Co., Ltd, China), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA]. Serum anti-CCP antibody levels were determined by ELISA[10] using commercially available ELISA kit (Immco

Diagnostics, USA), with an automated EIA analyzer [Coda, Bio-Rad Laboratories, Hercules, CA, USA]. 25U/ml was taken as cut-off value for anti-CCP antibodies.

The data was entered into SPSS version 17. Diagnostic sensitivity of anti-CCP and anti-MCV antibodies for the diagnosis of Rheumatoid arthritis in sero-negative patients were calculated by table of 2 x 2. Statistical analysis was done to determine the usefulness of the diagnostic sensitivities of anti-CCP and anti-MCV antibodies. P value of < 0.05 was considered to to be statistically significant.

Results

The study population (n=58), comprised of 58 rheumatoid arthritis patients, out of which 38 were females and 20 were males. Mean ±SEM age of the RA patients was 44±1.2 years. Median (IQR) disease duration was 5(4-8) years. Median (IQR) anti-CCP antibodies titer (IU/ml) was 10.8(0.00-340.5). Median (IQR) anti-MCV antibodies titer (IU/ml) was 19.7(14.2-30.06). (Table I)

Sub-grouping of RA group was done on the basis of presence or absence of RF, aCCPAb, or aMCV Ab in the sera. Subgroups were named RF+ive group (gp), RF-ivegp, aCCP+ivegp, aCCP-ivegp, aMCV+ivegp, aMCV-ivegp.

RF testing by ELISA technique, was done in a total of 58 RA patients. Out of the RA patients (n=58), 30 (52%) were RF+ive and 28 (48%) were RF-negative.

In the RA group (n=58), 34 (58%) were aCCP+ ive and 24(41.4%) were aCCP –ive. The sensitivity of serum aCCP antibodies for RA was calculated to be 58.6%. Serum aCCP antibody was positive in 9 out of 28 RF-ive patients. So the sensitivity of serum aCCP in RF-ive group (n=28) was 32.1%.

In the RA group (n=58), 20(34.5%) patients were aMCV+ and 38(65.5%) were aMCV –ive, at cutoff value of 25U/L (Table I). The sensitivity of serum aMCV antibodies for RA was calculated to be 34.5% at the manufacturer's cutoff value of 25U/L. Serum aMCV antibody was present in 11 out of 28 RF-ive patients. The sensitivity of serum aMCV in RF-ive group was 39.2%.

TableI: Serum RF, aCCP and aMCV status in the RA group (n=58)

Serum RF+ive	Serum RF-ive	Serum RF titer (IU/ml)
30 (52%)	28 (48%)	27.76 (2.51-32.9)
Serum aCCP +ive	Serum aCCP –ive	Serum aCCP titer (IU/ml)
34 (58.5%)	24 (41.4%)	10.8 (0.00-340.5)
Sensitivity of aCCP in RF-ivegp 32.1%		
Serum aMCV +ive	Serum aMCV– ive	Serum aMCV titer(IU/ml)
20 (34.5%)	38 (65.5%)	19.7 (14.2-30.06)
Sensitivity of aMCV in RF-ivegp 39.2%		

Discussion

A close relationship exists between autoimmunity and antibodies; despite this, some patients are persistently negative for disease specific autoantibodies. These conditions have been defined as seronegative autoimmune diseases. Although the prevalence of seronegative autoimmune diseases is low, they may represent a practical problem because they are often difficult and challenging cases for the clinicians/rheumatologist.[11] About 80% of the patients affected by RA are positive for RF, the rest 20-25% being seronegative. A more disease specific marker for RA may help in diagnosing early disease and seronegative RA patients resulting in reduced joint damage. It is therefore important to differentiate between RA and other forms of arthritis early after the onset of symptoms. Therefore, a specific and sensitive serological marker, which is present very early in the disease, is needed so that the rheumatologist are able to target the use of potentially toxic and expensive drugs to those patients, where the benefits clearly outweighs the risk. Keeping in view the need of a more sensitive marker, especially in the seronegative cases, the present study aimed to evaluate the sensitivity of anti-CCP and anti-MCV antibodies in local Pakistani seronegative RA subjects.

In seronegative cases of arthritis, the differential diagnosis is not easily established in the early disease course. Especially seronegative patients need the determination of an additional marker for RA besides rheumatoid factor to confirm diagnosis. The high specificity of anti-CCP antibodies has been reported in RF-neative RA patients. In the present study, 9 out of 28 seronegative patients were positive for anti-CCP antibodies. So, the sensitivity of anti-CCP antibodies in the seronegative sub-group was 32.1%.

In a study, conducted by Alexiou, et al., sensitivity in seronegative group was reported to be 34.9%, which is almost comparable to our results.[12] Similarly, Mobini, et al.,[13] found sensitivity in seronegative group to be 33.3%, whereas Vanichapuntu, et al.,[14] found sensitivity value of 20% in the seronegative sub-group whereas Serdaroglu, et al.,[15] reported sensitivity of 14.3% in seronegative group. Thus, anti-CCP antibody serves as a better diagnostic marker in the diagnosis of RA, especially in the seronegative group.

The sensitivity of anti-MCV in the sero-negative RA group was 39.2%, as 11 out of the 28 RF-ive patients were anti-MCV positive. This finding is supported by recent results of other authors, that the higher sensitivity of anti-MCV especially in the sero-negative patients makes it a more valuable marker in the diagnosis of RA.[16] Wagner, et al.,[17] reported sensitivity of anti-MCV in the sero-negative group of 43% and sensitivity of anti-CCP to be 30.8%. In seronegative RA patients, the sensitivity of anti-MCV was superior over anti-CCP. Soos, et al.,[18] reported sensitivity of anti-MCV in the sero-negative group to be 29.4%. Narvaez, et al.[19] documented sensitivity of 23% in their series of sero-negative RA patients.

Limitations of the Study

Small sample size was the limitation of this study. Further studies with greater number of RA patients are recommended.

Conclusion

Both anti-CCP and anti-MCV antibodies can be used for the early diagnosis of sero-negative patients of Rheumatoid Arthritis. Moreover anti-MCV antibody has a significantly higher sensitivity as compared to anti-ccp antibodies for the diagnosis of seronegative RA

Recommendations

Clinicians must be aware of the implications of delayed diagnosis in RA. Keeping in view the cost-effectiveness, this study emphasizes the utility of RF initially for the diagnosis of RA, reserving ACPA's for seronegative RA patients where strong clinical suspicion exists.

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REFERENCES

1. Lee DM, Weinblatt ME. Rheumatoid Arthritis. Lancet 2001; 358: 903-11.
2. Combe B. Progression in early rheumatoid arthritis. Best Pract Res ClinRheumatol 2009; 23: 59-69.
3. Saraux A, Berthelot JM, Chales G, Le Henaff C, Thorel JB, Hoang S, et al. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. Arthritis Rheum 2001; 44: 2485-91.
4. Marianna, MN. Rheumatoid factors: What do they tell us? J Rheumatol. 2002; 29: 2034-40.
5. Zendman AJ, van Venrooij MJ, Pruijn GJ. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology. 2006; 45: 20-5.
6. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis- specific autoantibodies. J. Clin Invest 1998; 101: 273-81.
7. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 2003; 25: 1106-18.
8. Bang H, Egerer K, Gauliard A, Berg W, Fredenhagen G, Feist E, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. Arthritis & Rheumatism. 2007; 56: 2503-11.
9. Coenen D, Verschueren P, Westhovens R, Bossuyt K. Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. Clin Chem. 2007; 53: 498-504.
10. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli, R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem. 2001; 47: 1089-93.
11. Alessandri C, Conti F, Conigliaro P, Mancini R, Massaro L,Valesini G. Seronegative autoimmune diseases. Ann NY Acad Sci. 2009; 1173: 52-9.
12. Alexiou I, Germenis A, Ziogas A, Theodoridou K, Sakkas LI. Diagnostic value of anti-cyclic citrullinatedpeptide antibodies in Greek patients with rheumatoid arthritis.BMC Musculoskeletal Disorders. 2007; 8: 37.
13. Mobini M, Kashi Z, Mahdavi, MR. The role of rheumatoid factor and anti-cyclic citrullinated peptide antibody in diagnosis of rheumatoid arthritis. IRCMJ. 2010; 12: 100-3.
14. Vanichapuntu M, Phuekfon P, Suwannalai P, Verasertniyom O, Nantiruj K, Janwityanujit S. Are anti-citrulline autoantibodies better serum markers for rheumatoid arthritis than rheumatoid factor in Thai population? Rheumatol Int. 2010; 30: 755-9.
15. Serdaroglu M, Cakirbay H, Deger O, Cengiz S, Kul S. The association of anti-CCP antibodies with disease activity in rheumatoid arthritis.Rheumatol Int. 2008; 28: 965-70.
16. Ladislav S, Walter G, Peter S. Laboratory biomarkers or imaging in the diagnostics of rheumatoid arthritis?BMC Medicine. 2014; 12: 49.
17. Wagner E, Skoumal M, Bayer PM, Klaushofer K. Antibody against mutated citrullinatedvimentin: a new sensitive

marker in the diagnosis of rheumatoid arthritis. RheumatolInt 2009; 29: 1315-21.
18. Soos L, Szekanecz Z, Szabo Z, Fekete A, Zeher M, Horvath IF, et al. Clinical evaluation of anti-mutated citrullinatedvimentin by ELISA in rheumatoid arthritis. J Rheumatol. 2007; 34: 1658-63.
19. Narvaez J, Sirvent E, Narvaez JA, Bas J, Gomez-Vaquero C, Reina D, et al. Usefulness of magnetic resonance imaging of the hand versus anticycliccitrullinated peptide antibody testing to confirm the diagnosis of clinically suspected early rheumatoid arthritis in the absence of rheumatoid factor and radiographic erosions.Semin Arthritis Rheum.2007; 38: 101-9.

ORIGINAL ARTICLE

Efficacy of Nasal Splints in Reducing the Incidence of Intranasal Adhesions Following Septoplasty

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ABSTRACT

Objective: To determine the efficacy of nasal splints in preventing adhesion formation in patients undergoing septoplasty for deviated nasal septum (DNS).

Study Design: Quasi experimental study.

Place and Duration of Study: The study was carried out at ENT department, Holy Family Hospital, Rawalpindi from January 2010 to January 2011.

Materials and Methods: Two hundred and sixty patients with DNS requiring septoplasty were included in this study. They were divided in two groups A and B with each group consisting of 130 patients. In group A splints were used postoperatively after septoplasty while in group B no splints were used. In group A nasal splints were removed on 14th postoperative day. All patients in group A and B were followed up regularly in OPD for six weeks for development of adhesions to determine efficacy of nasal splints in prevention of adhesions.

Results: In group A average age was 29.13 years+10.11SD while it was 27.6 years+ 9.5SD in group B. Male to female ratio was 1.15:1. There was no statistically significant difference in the incidence of adhesion formation between two groups with p-value of 0.734.

Conclusion: There is no significant advantage of routinely using intranasal splints in septoplasty patients for prevention of adhesion formation.

Key Words: *Nasal Splints, Septoplasty, DNS.*

Introduction

Deviated nasal septum is one of the most common ENT complaints and is treated by performing septoplasty.[1] Formation of intranasal adhesions is an important postoperative complication of septoplasty with an incidence of 10-36%.[1,2,3] Rhinologists all over the world routinely use intranasal splints after nasal septal surgery to prevent the adhesions and also tomaintain nasal stability and improve the results of septoplasty. This use of intranasal splints is not evidence based and splints are associated with increased pain and discomfort to patient.[3,4,5] Many studies have been done worldwide over the years to determine the efficacy of intranasal splints in prevention of intranasal adhesions to justify their use.[6,7,8]

Although many surgeons insert nasal splints routinely, no well-designed trials exist to support their use.[3] The nasal splint increases the post-operative pain and discomfort.[6,7]Although splints are in common practice but their use has shown limited benefit in terms of prevention of adhesions when compared withseptoplasty patients without splints.[6] So in this study we tried to find out efficacy of nasal splints in prevention of nasal adhesions to justify their routine use in patients undergoing septoplasty in our set up.

Materials and Methods

It is a quasi experimental study carried out in department of Ear, Nose and Throat of Holy Family Hospital, Rawalpindi from January 2010 to January 2011. Patient coming in outpatient department in age group of 16 to 50 years who were diagnosed with deviated nasal septum by anterior rhinoscopy were included in the study. Patients with turbinate hypertrophy, nasal polyps, previous surgeries or requiring other lateral wall nasal procedures or having comorbids like diabetes or hypertension were not included.

Two hundered and sixty patients with DNS requiring

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septoplasty were admitted in the ward. The purpose and benefits of the study was explained to patients and a written informed consent was obtained. All patients were worked up with detailed history and clinical examination followed by routine pre operative investigations. The patients were randomly allocated in two groups by convenient sampling technique. Patients in group A were subjected to use of splints after septoplasty and in patients in group B no splints were used after septoplasty.

All patients were operated by the same ENT surgeon having five years of experience in nasal surgery. After surgery nasal packing was removed after 24 hours and patients were discharged on 2nd postoperative day. Patients in both groups were given same oral antibiotic and analgesic for one week. Both groups were advised saline nasal douches three times a day for two weeks. In group A nasal splints were removed on 14 postoperative day. All patients in group A and B were followed up weekly in OPD till 6th week post operatively to determine intervention efficacy in terms of absence of adhesions.

All the above mentioned data was collected using performa. Data was analyzed by using SPSS version 20. Mean and standard deviation were calculated for age. Frequencies and percentages were calculated for gender and efficacy. Chi square test was used to compare the efficacy in both the groups. P value of <0.05 was considered significant.

Results

Average age was 27.6 years+ 9.5SD in group A while in group B it was 29.13 years +10.11SD. The age distribution among the groups was insignificant with p-value 0.860. Further demographic characteristics of both groups A and B are shown in table I and II.

In group A adhesions were seen in four patients (3.1%) and in group B five patients developed adhesion (3.8%). This difference between two groups was insignificant with p-vale of 0.734 (Table III).

Table I: Age Wise Distribution in Group 'A' and 'B'

	20 years or less	21-30years	31-40years	41 years or more
Group A	39(30%)	51(39.2%)	27(20.8%)	13(10%)
Group B	24(18.5%)	56(43.1%)	27(20.8%)	23(17.7%)

(P value= 0.860)

Table II: Gender Wise Comparison in Group 'A' and 'B'

Gender	Group A	Group B
Male	70	69
Female	60	61

(p value=0.901)

Table III: Adhesion Formation in Groups 'A' and 'B'

Adhesion formation	Group A	Group B
Yes	4(3.1%)	5(3.8%)
No	126(96.9%)	125(96.2%)

(p value = 0.734)

Discussion

In our study although there is higher incidence of adhesions in the non splinted group (Group B) but in comparison of the both groups statistically this difference was not significant and this may be due to intra operative surgical technique and postoperative nasal douching with normal saline to prevent crust formation and thus formation of adhesions.

Similar findings were seen in studies of some other authors. Tang and Kacker concluded that nasal splints cause significantly increased postoperative pain and there is not sufficient evidence that they are effective in decreasing the incidence of intranasal adhesions.[6] Von Schoenberg and Robinson found out on three months postoperative follow up that the splinted and non splinted groups had the same low rate of adhesion formation of 2% which in their opinion was because of early outpatient review with careful nasal toilet on weekly basis.[8]Cook et al showed a failure of intranasal splints in preventing intranasal adhesion(6.5% in splinted vs. 7.0% in non splinted group) and concluded that there is no clear advantage of using intranasal splints and they should therefore be used sparingly and recommended use of nasal toilet after septal surgery.[9] Pringle et al carried out a survey of 440 consultants and found that 33% of them never or rarely used intranasal splints and reported an adhesion rate of 5.2% in non splinted patients vs. 3.9% in the splinted patients, which is not statistically significant.[5]Like wise the results of the study done by Malki et al showed no statistically significant difference in the incidence of adhesions between the splinted and non-splinted patients.[10]Study of Almoflehi also concluded that the intranasal splints are not of significant value in preventing nasal adhesion(10% in splinted vs. 21% in non splinted group) and recommendation was that the use of intranasal splints in septal surgery has to be individualized. They stressed on Nasal irrigation using saline to prevent crusting and minimizing

occurrence of adhesion.[11]Almazrou and Zakzouk in their study found no significant difference in incidence of adhesion formation between splinted and non splinted groups (2% in splinted vs. 10% in non splinted group).[12]

On the other hand contrary to our results Deniz M et al. suggested that nasal splints were effective in reducing the incidence of nasal synechia formation.[13] Schoenberg et al. found a low incidence of adhesions in the first week post operatively when intranasal splints were used and found that the highest incidence of intranasal adhesions occurred in non splinted patients who had surgery to both walls of nasal cavity (3.6% in splinted vs. 31.6% in non splinted).[8] Campbell et al. inserted a nasals plint into one side of the nose of 106 patients undergoing a variety of intranasal procedures. All adhesions occurred on the non splinted side and were more common when bilateral nasal wall procedures had been performed (8% in splinted versus 26% in non splinted).[14] They concluded that splints were justified for bilateral wall procedures but their increased morbidity did not justify their use in single wall procedures.[14] Roberto et al. found nasal splints very effective inpreventing adhesion formation in patients under going septoplasty with turbinectomy (0% in splinted vs.10.6% in non splinted group).[15] Nabil-ur-Rahman concluded that complications were related to the type of procedure being performed and adhesions were common complication if intranasal splint were not provided.[16] The routine use of nasal splints in septoplasty patients is still a matter of debate. Splints addsignificantly to post operative pain and discomfort. It is recommended that their use should be individualized depending upon procedure being performed. They are more justified when surgical procedures are performed on both medial and lateral walls of nose simultaneously. Alternati velysaline nasal douching and regular outpatient follow up with suction toilet are very effective in preventing intranasal crusting and thus adhesion formation.

Conclusion

There is no significant advantage of using intranasal splints routinely in patients undergoing septoplasty for prevention of adhesions provided patients are closely followed up with saline nasal douches and regular nasal toilets.

REFERENCES

1. Shone GR, Clegg RT. Nasal adhesions. Cambridge Journal of Laryngology & Otology. 1987; 101: 555-7.
2. KhayatFJ, Yaseen A. the effect of intranasal splint on prevention of adhesions after septoplasty. Diyala J Med 2012; 2: 5-12.
3. Gaia R, Coelho J, Brandao FH, Carvalho MRMS, Aquino JEP, Paula SHP. Frequencia de Sinequia Nasal aposCirurgia de Septoplastia com Turbinectomia com e semUso de Splint Nasal. ArqIntOtorrinolaringol. 2008; 12: 24-7.
4. Sharif M, Ali S, Zaman T. Nasal septal surgery without splints, without packing. Ann AbbasiShaheedHosp Karachi Med Dent Coll. 2007; 12: 17-23.
5. Pringle MB. The use of intra-nasal splints: a consultant survey. UK.ClinOtolaryngol Allied Sci. 1992;17: 535-9.
6. Tang S, Kacker A. Should intranasal splints be used after nasal septal surgery? The Laryngoscope. 2012 Aug 1; 122: 1647-8.
7. GiJy, Won H, Young Gyu E, MyungGu K. Objective usefulness of thin silastic septal splints after septal surgery. Am J Rhinol Allergy. 2011; 25: 182-5.
8. Von Schoenberg M, Robinson P. The morbidity from nasal splints in 105 patients. Clin. Oto. Laryngol. 1992; 17: 528-30.
9. Cook AC, Murrant NJ, Evans KL, Lavelle RJ. Intra-nasal splints and their effects on intra- nasal adhesions and septal stability. ClinOtolaryngol. 1992; 17: 24-7.
10. Malki D, Quine SM, Pfleiderer AG. Nasal splints revisited. Cambridge J Laryngol Otol. 1999; 113: 725-7.
11. Almoflehi MS. The impact of using intranasal splints on morbidity and prevalence of adhesions. J Sc Tech. 2009; 10: 1-6.
12. Almazrou KA, Zakzouk SM. The impact of using intranasal splints on morbidity and prevalence of adhesions. Saudi Med J. 2001; 22: 616-8.
13. Deniz M, Ciftci Z, Isık A, Demirel OB, Gultekin E. The impact of different nasal packings on postoperative complications. American journal of otolaryngology. 2014; 35: 554-7.
14. Campbell JB, Watson MG, Shenoi PM. The role of intranasal splints in the prevention of post-operative nasal adhesions. Cambridge JLaryngolOtol. 1987; 101: 1140-3.
15. Roberto G, Fabiano H, Maria R. Frequency of nasal synechiae after septoplasty with turbinectomy with or without the use of nasal splint.2008. Arch otolaryngol. Sao Paulo. 2008; 12: 24-7.
16. Nabil ur Rahman MA. Complications of surgery for deviated septum.JColl Physicians Surg Pak. 2003; 13: 565-8.

ORIGINAL ARTICLE

Vitamin D Status in Diabetic and Non-Diabetic Patients of Coronary Artery Disease

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ABSTRACT

Objective: To assess and compare the level of hypovitaminosis D in diabetic and non-diabetic patients of coronary artery disease (CAD).

Study Design: Cross-sectional observational.

Place and Duration of Study: The study was conducted from May 2014 to November 2014 at Pakistan Railways Hospital, Islamic International Medical College, Rawalpindi.

Materials and Methods: A total of 149 patients of coronary artery disease were selected in this studyby convenient sampling technique including 77 male and 72 female patients. Out of 149 patients, 75 were diabetic and 74 non-diabetic. These participants were assessed for their individual serum vitamin D levels(ng/ml). Family history of coronary artery disease and treatment modalities was also obtained from all the patients. The data was analyzed through SPSS 21. Descriptive statistics and t test were used to analyze the data.

Results: The serum levels of Vit D among diabetic and non- diabetic patients suffering from CAD were 16.4(±9.7) ng/ml and 17.4(±7.9)ng/ml respectively. These values suggest a deficiency of Vit D in both diabetic and non-diabetic patients suffering from CAD. However the difference in Vit D levels of the two subgroups of CAD was statistically not significant (p = 0.4).However vitamin D levels were found to be significantly lower in patients with positive family history of CAD (p=0.04).

Conclusion: There is no significant difference in the level of Hypovitaminosis D in diabetic and non-diabetic CAD patients.

Key Words: *Vitamin D, Vitamin D Receptor, Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, Hypovitaminosis D, Islet β Cells, Insulin Secretion, Insulin Sensitivity.*

Introduction

Vitamin D is a secosteroid prohormone which is synthesized in the skin from 7-dehydrocholesterol by ultraviolet B-irradiation. Almost 80 percent of vitamin D is provided through cutaneous synthesis while 20 percent is obtained from various dietary resources.[1] Vitamin D has been known for its key role in bone metabolism and calcium homeostasis. However its extra-skeletal effects have attracted much attention because of recent discovery of Vit D receptor (VDR) expression in almost all tissues of the body including immune, endocrinal, vascular and myocardial cells.[2] Vit D deficiency is considered as a risk factor for diabetes type-2 diabetes and higher plasma levels of vitamin D are shown to decrease the risk for development of diabetes mellitus.[3,4]

Diabetes mellitus is a very common condition which affects over 300 million people worldwide and accounts for significant morbidity and mortality.[5] Ziegler et al found that vitamin D administration in type 1 diabetes can protect the function of pancreatic islet β cells. Deficiency of vitamin D has been investigated to affect the synthesis and secretion of insulin both in human and animal models.[6] Vitamin D supplementation not only restores the synthesis but also secretion of insulin and therefore decreases blood glucose concentration.[7] Palomer et al also reported that vitamin D deficiency can predispose to glucose intolerance and vitamin D supplementation improves insulin secretion in type 2 diabetes mellitus.[8]

Both genetic and environmental factors are known to be etiological in causing type 1 diabetes mellitus (T1DM), a chronic disorder that results from autoimmune response against the beta cells of the pancreatic islets of Langerhans at early stages of life. It is hypothesized that vitamin D has a role in the therapy of T1DM due to its immune-modulating effects. Chronic insulinitis is found in islet cells

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involving CD8+, CD4+ T cells, B lymphocytes and macrophages. It has been reported that vitamin D inhibits the production of IFN-γ and IL-2 cytokines which are responsible for the destruction of pancreatic islet cells.[9] Vitamin D also reduces the activity of APCs and regulates the production of CD4+ lymphocytes.[10] Cytokines cause apoptosis. 1,25(OH)2D3 protects beta cells by reducing exposure of MHC-1 molecules. As a result A20 protein which inhibits apoptosis is activated and a decrease in expression of a transmembrane cell surface receptor occurs.[9]

The major factors involved in the etiology of type 2 diabetes mellitus are insulin resistance, disorders of pancreatic beta cells and inflammation. Vitamin D facilitates pancreatic beta cell function through direct actions in which vitamin D is activated in pancreatic beta cells by intracellular 1-α-hydoxylaseenzyme. Vitamin D not only increases insulin secretion but also enhances beta cell survival through the effects of cytokines. Vitamin D also has indirect actions in which it regulates calbindin, a calcium-binding protein present in beta cells. Calcium then influences insulin secretion because this process is calcium dependent. In this way calcium indirectly affects insulin secretion via calcium binding protein calbindin. One more mechanism involved can be that low vitamin D levels cause secondary hyperparathy roidism. Parathyroid hormone then inhibits insulin secretion and also causes resistance in target cells because of its effects on regulation of calcium levels.[9]

An inverse relationship of insulin resistance was found at Vit D levels between 16 and 36ng/ml in arecent study.[10] Other cross-sectional, case control, prospective observational and longitudinal studies showed that higher Vit D concentrations were associated with a lower risk for diabetes mellitus type 2 and lower levels were negatively correlated with diabetes and glycosylated haemoglobin levels. However these studies did not reveal consistent results, neither vitamin D supplementation was found to improve glucose control in type 2 diabetes in all the subjects.[11]

The levels of blood glucose and vitamin D have been shown to be different in Third National Health and Nutritional Examination Survey and a negative correlation was found in healthy white post-menopausal women and in Mexican American men and women but not in Hispanic black population. This finding suggests that the effect of vitamin D may differ by ethnicity.[12]

The present study was undertaken to assess and compare the level of hypovitaminosis D in diabetic and non-diabetic patients of CAD because of paucity of research work on this therapeutically important subject.

Materials and Methods

This study was conducted at department of Biochemistry IIMC Rawalpindi. It was a cross-sectional observational study. The Study was conducted from May 2014 to November 2014.

Blood samples were drawn from 149 patients of coronary artery disease comprising of 77 males and 72 females from OPD, emergency department and ICU of Railways General Hospital Rawalpindi, a 480 bedded teaching hospital affiliated with IIMC. These patients were divided into two groups:

Group I consisted of diabetic patients of coronary artery disease while group II comprised of patients without diabetes. Patients of coronary artery disease between ages of 40 years to 55 years were included in the present study. Patients with renal, liver, parathyroid and thyroid disorders were excluded from the study. Patients taking vitamin D and calcium supplements and those on medication that affect vitamin D metabolism such as anticovulsants were also not recruited in this study.

Venous blood was drawn from the patients which was centrifuged at 300 rpm for separation of serum and then stored at -20 degree centigrade in laboratory freezer till further analysis. Serum levels of 25(OH)D was measured by enzyme linked immunosorbent assay. Statistical analysis was carried out for finding out significant p values ($p<0.05$) and t test was used to evaluate the data. SPSS version 21 was used for data processing.

A total of 149 patients with coronary artery disease were selected to participate in the study that fulfilled the inclusion criteria and gave informed consent. All the participants were assessed for their individual serum vitamin D levels (ng/ml). The diabetic patients were questioned about the mode of treatment, either oral hypoglycaemic agents or insulin therapy to control their diabetes.

Results

Out of a total of 149 patients selected to participate in the study, 77 were male and 72 were female patients. Of these, 75 male and female patients were diabetic whereas 74 were non-diabetic male and female participants. The mean age of the male participants was 49.1+4.1(in years) and of the female participants was 47.4+4.5(in years).

The mean serum vitamin D level in diabetics was 16.4 ng/ml and in non-diabetics was 17.4 ng/ml(Fig 1). However the difference was not statistically significant (p value= 0.4)(Table I). The serum Vitamin D level in the female diabetic participants was 16.8 ng/ml and in the male diabetic participants it was 16.0 ng/ml. The serum vitamin D level in non-diabetic female patients was 19.0 ng/ml and in male non-diabetic participants it was 15.9 ng/ml.

Vitamin D deficiency was considered when the serum vitamin D was < 20ng/ml, insufficiency if the serum vitamin D was between 21-29ng/ml and normal if serum vitamin D was > 30 ng/ml. Vitamin D deficiency was seen in 100(67.1%), insufficiency was seen in 39(26.2%) and normal Vitamin D was present in 10(6.7%) of the participants. The percentage of deficiency and insufficiency was 93% in the sample population.

Family history of coronary artery disease was present in 63(84.0%) of the diabetic patients and in 53(71.6%) of the non-diabetic patients. Vitamin D levels in patients with and without family history of CAD were 7.7(±9.2)ng/ml and 14.2(±7.1)ng/ml respectively. The difference was significant (p= 0.04)(Table II).

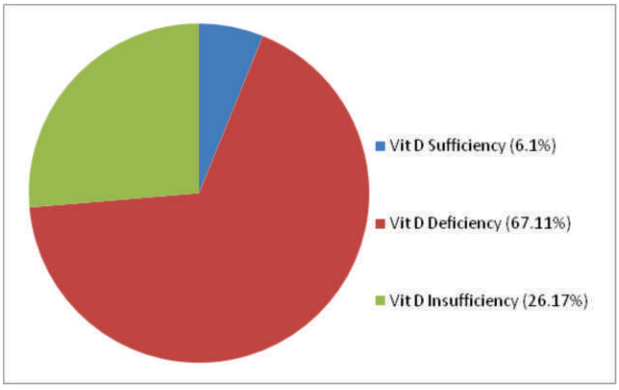

Fig 1: Percentage of hypovitaminosis in the study population

Table I: Comparison of Vitamin D levels between diabetic and non- diabetic patients

Disease	N=149	Vitamin D level (±SD)ng/ml	p- value
Diabetic population	75	16.4(±9.7)	0.4
Non-diabetic population	74	17.4 (±7.9)	

Table II: Comparison of vitamin D levels between patients with family history of coronary artery disease and patients with no family history of coronary artery disease

Family history of coronary artery disease	N=149	Vitamin D levels (±SD)ng/ml	P-value
Positive family history	116	7.7(±9.2)	0.04
No family history of CAD	33	14.2(±7.1)	

Discussion

This study was undertaken to assess the role of vitamin D in diabetes mellitus and to measure its levels in diabetic and non-diabetic patients of CAD. Our results did not reveal statistically significant difference in Vitamin D levels between diabetic and non-diabetic sample population (p= 0.4). This finding which is in contrast to many other observational studies may possibly be due to multiple confounding factors like obesity, sun exposure and/or methodological and measurement limitations. However cumulative percentage of vitamin D deficiency and insufficiency in the study population has been found to be about 93% which matches with one of the studies carried out by Bellen et al. in which hypovitaminosis D was reported in 95% of cases.[13]

A study performed by Daga et al. in North India showed that 91% of diabetic patients had hypovitaminosis D and the mean concentration was 7.88(±1.2)ng/ml. In non-diabetic patients the mean concentration was 16.46(± 7.83) ng/ml. This finding is in line with our results for non-diabetic patients.[14]

Hypovitaminosis D is reported worldwide mainly due to dietary insufficiency and use of sun protection clothing and sun screen. Studies on this subject in Saudi Arabia, Turkey, Australia, Arab Emirates and India have shown that 30-50% of children and adults had vitamin D levels of less than 20ng/ml. A Japanaese study has shown a prevalence of vitamin

D deficiency in 75% of the study population which is consistent with the findings of our study. The results of a study carried out in the United Kingdom also revealed the prevalence of vitamin D levels (below 20ng/ml) in more than 80% of the research participants.[15] Our study also revealed comparable results.

An interesting and therapeutically useful finding in this study is that patients taking oral hypoglycemic drugs had significantly lower vitamin D levels as compared to those on insulin therapy (p = 0.05). Another finding of the present research is a significant difference in Vit D levels while comparing results of patients with and without family history of coronary artery disease (p = 0.04).

The conclusive statement of a study carried out by Bellan et al. on this subject is "At variance with previous reports, we were unable to disclose negative effects of low 25(OH)D concentration on plasma glucose levels both in fasting conditions and after the oral glucose challenge after correction for multiple variables known to affect glucose metabolism".[16]

Another study postulates that vitamin D is needed to improve production and action of insulin in both diabetic and non-diabetic patients but the relationship between vitamin D, insulin resistance and β cell function is inconsistent. Additionally interventional studies with vitamin D in any form have also shown conflicting results. Therefore, interpretation of the results is difficult in view of lack of placebo-controlled studies.[17]

Conclusion

The results of the present study revealed that serum levels of Vit D in diabetic patients of coronary artery disease were not significantly lower than non-diabetic patients of coronary artery disease in our study. It is inferred from the current findings that diabetes mellitus is a multifactorial disease resulting from complex interaction of genetic and environmental factors. Vitamin D could only be one of the environmental variables and not an independent potential predictor of diabetes mellitus but rather could be a mediator of metabolic disturbances responsible for the long- term health outcomes.

Further large well-designed, randomized controlled studies are suggested to assess and clarify the correlation between vitamin D and glucose homeostasis in diabetes mellitus.

REFERENCES

1. Keinreich K, Tomaschits A, Verheyen N, Pieber T, Gaksch M, Glubler MR, et al. Vitamin D and cardiovascular disease. Nutrients. 2013; 5: 3005-21.
2. Goleniewska B, Kacprzak M, Zielinska M. Vitamin D level and extent of coronary stenotic lesions in patients with first acute myocardial infarction. Cardiol J.[Online] 2013. Available from: doi: 10.5603/CJ.a 2013.0048. [Accessed 16th February 2014].
3. Pittas A, L au J, Hu F, Dawson Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J ClinEndocrinolMetab. 2007; 92: 2017-29.
4. Pittas A, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan D, et al. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. Diabetes Care. 2012; 35: 565-73.
5. Ahmadieh H, Azar ST, Lakkis N, Arabi A. Hypovitaminosis D in Patients with type 2 Diabetes Mellitus: A Relation to Disease Control and Complications. ISRN Endocrinology. [Online] 2013. Available from: doi: 10.1155 /2013/641098. [Accessed 24th March 2015].
6. Gua J, Xiao Z, Xue X, Liu X, Lu Y, Yin X, et al. 25-hydroxyvitamin D is closely related with the function of the pancrejatic islet β cells. Pak J Med Sci.2013; 29: 809-13.
7. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretions of insulin. Science.1980; 209: 823-5.
8. Palomer X, Gonzalez Clemente JM, Blanco Vaca F. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes ObesMetab. 2008; 10; 185-97.
9. Harinayaran CV. Vitamin D and diabetes mellitus. Hormones. 2014; 13: 163-81.
10. Heaney R, French C, Nguyen S, Ferreira M, Baggerly I, Brunel L, et al. A novel approach localizes the association of vitamin D status with insulin resistance to one region of the 25-hydroxyvitamin D continuum. Adv Nutr. 2013; 4: 303-10.
11. Kostoglou Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P. Vitamin D and glycemic control in diabetes mellitus type 2. Ther Adv Endocrinol Metab. 2013; 4: 122-8.
12. Scragg R, Sowers M, Bell C. Serum 25 hydroxyvitamin D diabetes andethnicity in the Third National Health and Nutrition Examination Survey.Diabetes Care. 2004; 27: 2813-8.

13. Jehle S, Lardi A, Felix B, Hulter HN, Stellar C, Krapf R. Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: arandomised, placebo-controlled, prospective pilot study. Swiss Med Wkly. [Online] 2014.Available from: doi: 10.4414/smw.2014.13942. [Accessed 24th March 2015].
14. Riachy R, Vandewalle B, Belaich S. Beneficial effect of 1,25dihydroxyvitamin D3 on cytokine-treated human pancreatic islets. J Endocrinol. 2001; 169: 161-8.
15. Bellan M, Guzzaloni G, Rinaldi M, Merlotti E, Ferrari C, Tagliaferri A. Altered glucose metabolism rather than naïve type 2 diabetes mellitus (T2DM) is related to vitamin D status in severe obesity. Cardiovasc Diabetol. 2014; 13: 57.
16. Bayani MA, Banasaz B, Saeedi F. Status of vitamin D in diabetic patients and compared it with healthy individuals. Caspian J Intern Med. 2014; 5: 40-2.
17. Griz LHM, Bandeira F, Gabbay MAL, Dib SA, Freese de Carvalho E. Vitamin Dand diabetes mellitus-an update-2013.Arq Bras Endocrinol Metab.2014;58:1-8.

ORIGINAL ARTICLE

Frequency of Different Risk Factors of Autism Spectrum Disease: A Multicentre Comparative Study

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ABSTRACT

Objective: To determine the frequency of different prenatal risk factors associated with autism spectrum disease.

Study Design: A Case-control study.

Place and Duration of Study: The Study was conducted at Rawalpindi. Cases were recruited from Autism resource centre (ARC), Step to learn (STL) Rawalpindi, Hassan academy for special children, and Army public academy for special children Rawalpindi and controls from DHQ hospital Rawalpindi from February 2014 to January 2015.

Materials and Methods: Two groups of children were included in the study, Group one were diagnosed case of autism ASD (n=101) aged between 3 to 10 years and Group two (control n=233). The data was collected and entered in a self designed structured questionnaire. Data was analyzed by SPSS (Statistical Package for the Social Sciences) Vs 20.

Results: Over 30 risk factors have been identified. 67.3% cases were males and 32.6% were females whereas 34% controls were males and 66% controls were females. The factors associated with autism were male gender, advanced father's age at child birth, severe iron deficiency anemia during pregnancy, vitamin D deficiency, maternal hypothyroidism, asthma, hypertension, diabetes mellitus and obesity, high stress during pregnancy, C-section, valporoic acid use, antidepressants and antibiotics use in mothers, infection during pregnancy, no or minimum exposure of sunlight during pregnancy, premature birth, Meconium aspiration syndrome and late cry, blood group incompatibility with mothers and Rh incompatibility between mothers and fathers.

Conclusion: Prenatal risk factors like stress, vitamin D deficiency, iron deficiency anemia, hypothyroidism, obesity, diabetes, blood group incompatibility with mothers, children born in spring and C section are the strong causes to develop autism in children.

Key Words: *Autism, Neurodevelopmental Disorder, Risk Factors.*

Introduction

Autism is a wide-ranging term used to illustrate an assemblage of complex neurodevelopmental disorders also known as Pervasive Developmental Disorders (PDD) or Autism Spectrum Disorders (ASD). ASDs are a collection of developmental disabilities characterized by atypical development in socialization believe behavior and communication. Symptoms of ASDs are present before age 3 years and frequently are accompanied by difficulty in learning, cognitive functioning, sensory processing and attention.[1] The phrase "spectrum disorders" is used to point out that ASDs include a variety of behaviorally concerned conditions, which are diagnosed by clinical observation of development. These conditions encompass autistic disorder (i.e., autism), and pervasive developmental disorder and Asperger disorder.[2] ASDs, related to other neurodevelopment disabilities, are usually not curable and chronic maintenance, preservation and management is requisite. Regardless of the fact outcomes are diverse and specific behavioral characteristics vary over time, the majority of children with ASDs remains within the spectrum like adults and, disregarding of their intellectual level, undergo to experience problems with employment, independent and self-regulating living, communal relationships, mental health and societal problem.[3,4] The diagnostic criteria depend upon the symptoms that become evident before a child is three years old.[5] Causative factors for autism are inadequately

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understood. Both environmental and genetic risk factors are likely to commit etiology. Although autism appears to have its roots in very early brain development, mainly identifiable signs and symptoms are likely to appear between two and three years of age. Mostly parents are the first to note and identify that their child is presenting a typical behaviors such as deteriorating to make eye contact, not answering to his or her name or playing with toys in unusual, repetitive behavior.[6] The purpose of this study is to determine the frequency of different prenatal risk factors associated with ASD so that these new risk factors should be used to make recommendations for clinical practice and by the avoidance of these risk factors, prevalence of ASD decreases.

Materials and Methods

It was a multicentre case control study. There were total 334 children enlisted in this study in which 101 were diagnosed case of autism and 233 healthy controls were taken with no neurological, musculoskeletal or cardiopulmonary abnormalities. Non probability convenient sampling technique was used. The Case data was collected from ARC (Autism Resource Center, Rawalpindi), STL (Step to Learn), Hassan academy for special children, Rawalpindi and Islamabad branch and Army Public Academy for special children Rawalpindi. Control data was collected from DHQ Hospital, Fauji Foundation Hospital Rawalpindi, National Institute of Rehabilitation and Medicine Islamabad, Railway General Hospital Rawalpindi and Divisional Public School Rawalpindi from January 2014 to December 2014.Permission was taken from respective institutes.

Data Collection Tool and Procedure: A self designed structured questionnaire was used for data collection. The questionnaire had 33 items (Appendix A). The different risk factors causing autism included in our questionnaire were based on literature search different studies in different settings by different authors. It included Iron deficiency anemia, vitamin D deficiency in mother during pregnancy, older parents, C section, mother high stress level during pregnancy due to any reason, blood group incompatibility with mother and many more. Ethical permission was taken from Medical Superintendent of DHQ, FFH, NIRM and PRH. After obtaining informed consent from parents, data was collected using self designed structured questionnaires. Questions were first explain and then asked from parents of study groups. Data was filled personally by the authors.

Statistical Analysis: Data was analyzed by using SPSS (statistical package for social sciences) VS 20.

Results

30.2% ASD cases and 69.7% healthy controls were taken in this study. In ASD cases 6.9% mothers were suffering from obesity and 11.8% from diabetes and 21% from hypothyroidism (Fig 1). Table I show Stress mothers taken during pregnancy, infection during pregnancy, exposure of sunlight during pregnancy, premature births, C section, episiotomy, SVD, meconium aspiration syndrome, late cry of baby at birth, Different Rh and different blood group among parents and blood group incompatibility with mothers. Controls had lesser frequency of iron deficiency anemia during pregnancy whereas ASD cases had prevalence of moderate and severe iron deficiency anemia during pregnancy increases in mothers of cases (Fig 2). 70.27% mothers of children with ASD used folic acid and vitamin D during pregnancy whereas less usage in mothers of controls. Mothers of children with ASD had history of more usage of valproic acid, antidepressant and antibiotics i.e. 6.9%, 22.7% and 14.8% respectively whereas only 2.57% mothers of controls used antidepressant and 3.0% used antibiotics and none used valproic acid.

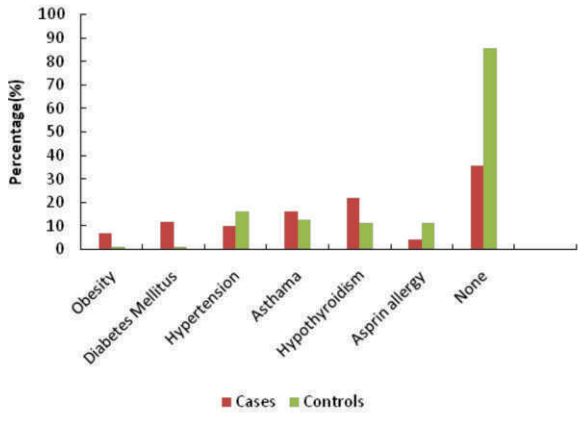

Fig 1: Comparison of Mother's medical history between cases and controls

Table I: Comparison of different conditions between cases and controls

	Cases (%)	Controls (%)	*P* value
Stress during pregnancy	42.5	14.5	0.032
Infection during pregnancy	13.8	1.71	0.045
Exposure of Sunlight during pregnancy	31.68	52.36	0.029
Premature birth	41.58	22.3	0.028
C-section procedure	81.18	18.82	0.030
Episiotomy	7.92	92.08	0.001
SVD	18.89	81.11	0.011
Meconium aspiration syndrome	26.7	73.3	0.029
Late cry at birth	13.8	86.2	0.015
Different Rh and different blood group among parents	55	31.7	0.034
Blood group incompatibility with mothers	73.26	13.30	0.025

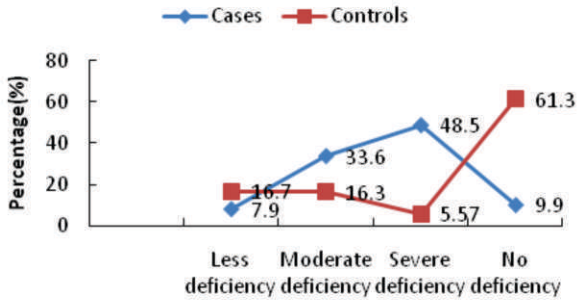

Fig 2: Comparison of Iron deficiency anemia between cases and controls

Discussion

In our study 67% cases of autism were found out to be males and only 29% were females hence supporting male gender as a risk factor of autism. Males are at higher risk for neurodevelopmental disorders, such as autism spectrum disorder (ASD), than females, but the fundamental reasons have been uncertain.[7]

Scientists have supposed a connection between iron deficiency and autism spectrum disorder. In a study maternal intake of supplemental iron and risk for autism spectrum disorders by Schmidt RJ, et al establishes a strong relationship between iron deficiency and autism.[8] In our study 48% severe deficiency of iron is found in cases and only 5% severe deficiency is found in controls. 9% of the cases had no iron deficiency while 61% of the controls also did not have any iron deficiency. This signifies the iron deficiency as one of the contributory risk factors for development of ASD. According to Kinney DK et al, in their study, "prenatal stress and risk for autism" stress of expectant mother is a noticeable reason for the symptoms of autism.[9] In another study "timing of prenatal stressors and autism" by Beversd of DQ et al, it was reported that prenatal stress has a role in causing autism.[10] In our study 42% cases had self-reported moderate to severe stress during their pregnancies and only 14.5% of controls also reported stress. So we can deduct the conclusion that stress is as one of the risk factor for ASD.

A prospective study of Exposure to valproic acid and risk for autism spectrum disorder indicates that valproic acid usage may be an important factor in causing autism.[11] In our study 6.9% cases taken valproic acid during pregnancy (anti- seizure medicine) whereas none of the control took valproic acid. This finding is in agreement with previous studies but further investigation is warranted.

Prenatal exposure to SSRIs, particularly during the first trimester, may moderately increase the risk of ASDs.[12] 22% cases in our study used antidepressant during pregnancy whereas only 2% controls were on antidepressant indicating either it's the antidepressant drugs or the depression which is hidden behind and need further investigation.

Studies showed that antibiotic use in pregnancy might cause autism.[13] In our study 14% cases took antibiotics during pregnancy whereas only 3% controls took antibiotics during pregnancy but the need is to investigate the group of antibiotic, dose, frequency and all the minor details, so further investigation is warranted. Drugs possibly will activate the disease, earlier researchers found that use of medications during pregnancy may have an effect on the developing fetus and may cause autism.[13] In our study 55% cases taken no drug during pregnancy emphasizing that it is not only the drugs that causes autism, there are some other factors that may in combination with the drugs causes autism.

Conclusion

Prenatal risk factors like stress, vitamin D deficiency, iron deficiency anemia, hypothyroidism, obesity, diabetes, blood group incompatibility with mothers, children born in spring and C section are the strong causes to develop autism in children. It is concluded that there is insufficient evidence to implicate any one prenatal factor in autism etiology, although there is some evidence to suggest that exposure to pregnancy complications may increase the risk.

REFERENCES

1. DeStefano F, Bhasin TK, Thompson WW, Yeargin-Allsopp M, Boyle C. Age at first measles-mumps rubella vaccination in children with autism and school matched control subjects: a population-based study in metropolitan Atlanta. Pediatrics. 2004; 113: 259-66.
2. Braun KVN, Pettygrove S, Daniels J, Miller L, Nicholas J, Baio J, et al. Evaluation of a methodology for a collaborative multiple source surveillance network for autism spectrum disorders -Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2002. MMWR Surveillance summaries: Morbidity and mortality weekly report Surveillance summaries/CDC. 2007; 56: 29-40.
3. Howlin P, Goode S, Hutton J, Rutter M. Adult outcome for children with autism. Journal of Child Psychology and Psychiatry. 2004; 45: 212-9.
4. Friedman ND, Warfield ME, Parish SL. Transition to adulthood for individuals with autism spectrum disorder: current issues and future perspectives. Neuropsychiatry. 2013; 3: 181-92.
5. Baharara J, Hojjati M, Rasti H. The Ratio of Second to Fourth Digit Length (2D: 4D) in Children with Autistic Disorder. International Journal of Pediatrics. 2014; 2: 5-11.
6. Lord C, Paul R. Language and communication in autism. Handbook of autism and pervasive developmental disorders. 1997; 2: 195-225.
7. Robinson EB, Koenen KC, McCormick MC, Munir K, Hallett V, Happe F, et al. Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%). Archives of general psychiatry. 2011; 68: 1113-21.
8. Schmidt RJ, Tancredi DJ, Krakowiak P, Hansen RL, Ozonoff S. Maternal Intake of Supplemental Iron and Risk of Autism Spectrum Disorder. American Journal of Epidemiology. 2014: kwu208.
9. Kinney DK, Munir KM, Crowley DJ, Miller AM. Prenatal stress and risk for autism. Neuroscience &Biobehavioral Reviews. 2008; 32: 1519-32.
10. Beversdorf DQ, Manning S, Hillier A, Anderson S, Nordgren R, Walters S, et al. Timing of prenatal stressors and autism. Journal of autism and developmental disorders. 2005; 35: 471-8.
11. Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. Jama. 2013; 309: 1696-703.
12. Croen LA, Grether JK, Yoshida CK, Odouli R, Hendrick V. Antidepressant use during pregnancy and childhood autism spectrum disorders. Archives of general psychiatry. 2011; 68: 1104-12.
13. Atladottir HO, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics. 2012; 130: 1447-54.

ORIGINAL ARTICLE

Demographics of Intestinal Parasitic Infections in Karachi: An Insight from Positive Stool Samples

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ABSTRACT

Objective: The objective of this study was to study the demographics of intestinal parasitic infections and find out the relative frequency of different intestinal parasites in clinical samples received for routine stool examination from symptomatic patients with diarrhea and/or dysentery.

Study Design: A Retrospective cross sectional study.

Place and Duration of Study: The study was conducted at Dow Diagnostics Research and Reference Laboratory, Karachi, Pakistan from January 2008 to December 2013 of stool analysis from the patients with symptoms of diarrhea and/or dysentery.

Materials and Methods: Microscopic stool examination was performed using direct samples by wet mount under low as well as high power bright field magnification. Tabulations were performed using Microsoft Excel while Chi-square test was applied using Open-epi software.

Results: Among the 1815 positive cases infected with single parasites, *Entamoebahistolytica* was the most commonly found parasite, followed by *Giardia labmlia*, *Blastocystishominis*, *Ascarislumbricoides* and *Hymenolepis nana*. Among these patients, 1081 (59.6%) were males and 734 (40.4%) were females. Chi-square test was applied using open-episoftware, which showed significant differences among males and females (p=0.03).

Conclusion: Protozoal intestinal parasites *Entamoebahistolytica* and *Giardia lambliaare* the commonest among all age groups followed by the helminths *Ascaris lmbricoides* and *Hymenolepis nana*. Other notable parasites include Entamoeba coli and Taenia species.

Key Words: *Intestinal Parasites, Parasitic Infections, Entamoeba Histolytica, Giardia Lamblia.*

Introduction

Parasitic infections are a major public health issue in developing world, which affects around 3.5 billion peopledirectly[1] and indirectly.[2] They are mostly endemic and specific parasites are prevalent in particular areas, therefore disease pattern is different in different geographical settings. These diseases are associated with climatic conditions, poor sanitation, unsafe drinking water and lack of personal hygiene in tropical and sub-tropical countries.[1,3] They are a major cause of morbidity and mortality and are responsible for chronic diseases such as iron deficiency anemia,[4] growth retardation in children,[5] physical and mental health problems and diarrheal diseases,[6] which are a major cause of death in Pakistan India, Bangladesh and other developing countries.[7]

Previous population based reports from Pakistan show Giardia lamblia to be the most common parasite in Karachi followed by *Ascaris lumbricoides*[8] and *Entamoeba histolytica*[9], though the reports from Bannu and Peshawar (cities situated in Northern Pakistan with a comparatively cold climate) showed Ascaristo be the commonest parasite infecting the school children followed by *Enterobiusvermicularis* and *Hymenolepis nana*.[10,11] Reports from Muzaffarabad, a city located further North showed Giardia to be the commonest followed by *E. histolytica*.[12] Karachi has a semi-arid warm and humid climate and it is home to a number of infectious parasites. Most common parasites reported up to date are *Entamoeba species*, *Ascaris, H. nana, Giardia*, *T. trichura* and *Taenia species*.[8,9,13] However, the present studies are either old[9] or do not cover the age groups other than children,[8,13] which is also affected by the parasitic infections and add a considerable bulk to the disease

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burden. Therefore, a retrospective study was performed to assess the relative disease burden of different parasites across different demographic groups, so that a snapshot can be generated regarding relative frequency of different parasites in symptomatic patients. To study the relative frequency of these parasites in samples from patients coming with diarrhea and/or dysentery results from laboratory records were analyzed for the period of 2008-2013 and were stratified according to age and gender.

Materials and Methods

The retrospective cross-sectional study based on the laboratory records was carried out in the Dow Diagnostic Research and Reference Laboratory of Health Sciences Karachi from January 2008 to December 2013.

All positive stool samples received for routine stool examinations were included in the study, while those which were negative for parasites, ova or cysts were excluded. A total of 1873 positive samples were included in data analysis.

Stool samples were collected in sterilized leak proof containers from patients after detailed informed consent. Departmental approval was taken for the study,diagnostic data were de-identified and tabulated without patients' particulars.

Each sample was initially examined physically for consistency, color, odor, blood and mucus. Slides were then prepared directly for wet mount by placing a drop of fresh physiological saline at one end of the clean side, while a drop of iodine was placed at the other end. Stool samples were mixed with wooden stick and a small amount was then taken for saline and iodine and covered with cover slip. These were microscopically examined under low (10× = 100 times magnification) and high (40× = 400 times magnification) power bright field for worms, ova, larvae, trophozoites, cyst, erythrocytes, leukocytes and any intestinal mucosal sloughing.

Diagnosis was made by observing the differentiating morphological features of protozoal cysts and trophozoites and parasitic ova and larvae. For *Entamoeba histolytica*, cyts were identified on the basis of presence of four or lesser number of nuclei, while trophozoites were identified as having ingested RBCs and differentiated from *Entamoeba coli* on the basis of more than five nuclei in cysts and trophozoites without RBCs. *Iodamoeba butschlii* was identified on the basis of cellular morphology and presence of a single nucleus in Iodine preparation.

Blastocystis hominis was identified on the basis of cyst-like forms having a large central body resembling like a large vacuole surrounded by small, multiple nuclei. Ova of *Hymenolepis nana* were identified on the basis of appearance as they are round to oval with two distinct walls and having 4-8 hair-like processes at each pole on the inner aspect along with three pairs of hookletswithin the inner wall. Ova of *Ascaris* were identified the basis of yellow brown color with thick shells having an albuminous outer coat and thick transparent wall under the shell.

Tabulations were performed using Microsoft Excel[14] while Chi-square test was applied using Open-epi software.[15]

Table I: Gender-wise proportion of infections in patients detected using stool examination

Parasite	Male (n=1081)	Female(n=734)	X^2	Degree of Freedom (Df)	p-value
Entamoeba histolytica	400	321			
Entamoeba coli	66	30			
Giardia lamblia	369	211			
Ascarislumbricoides	56	34			
Iodamoeba butchii	14	13	15.74	7	0.03
Hymenolepis nana	46	24			
Taenia species	9	5			
Blastocystis hominis	120	95			
Trichuristrichura	1	1			
Total	1081	734			

Table II: Gender-wise proportion of infections in patients using stool examination stratified by age

	<3		3-18		>18	
Parasite	Male	Female	Male	Female	Male	Female
Entamoebahistolytica	15	10	114	88	271	223
Entamoeba coli	2	0	13	4	51	26
Giardia lamblia	47	39	157	88	165	84
Ascarislumbricoides	3	5	20	11	33	18
Iodamoebabutchii	0	0	2	3	12	10
Hymenolepis nana	2	3	38	15	6	6
Trichuristrichura	0	0	0	0	1	1
Taenia species	2	1	0	1	7	3
Blastocystishominis	1	4	44	30	75	61
Total	72	62	388	240	621	432

Results

Among the 1815 positive cases infected with single parasites, 1081 (59.6%) were males and 734 (40.4%) were females. *E. histolytica* was the most commonly found parasite in these patients, followed by *G. labmlia, B.hominis, A. lumbricoidesand H. nana*. A chi-square test was appliedusing Open-episoftware, which showed significant differences among the two groups (p=0.03).

Data were stratified according to age groups and gender. It was observed that most commonly affected group was adult males, and altogether 1081 males were found to be mono-infected, most commonly with *E. histolytica*(n=400) followed by *G. lamblia*(n=369) and *B.hominis* (n=120). Similar pattern was observed in females and E. *histolytica*(n=321) *G. lamblia*(n=211) and *B. hominis* (n=95) were found to be the most common parasites infecting adult females.

However in patients, who were three years of age, *G. lamblia* was found to be the most common parasite, in both males (n=47) and females (n=39). In children, the most common parasites were again *Giardia* and *E. histolytica*, though trends were different in males and females as in males *Giardia*(n=157) infection rate was detected to be higher than *E. histolytica*(n=114), though in females both infections were found in equal frequency (n=88).

Some of the patients were found to be infected with two parasites (n=52), while very few were triply infected (n=6). Organisms found in these cases were mostly *G.iardialamblia, E. histolytica, H. nana, E. coli, I. butschlii* and *A. lumbricoides.*

Discussion

The study found a number of parasitic agents more common in the clinical samples received in Karachi. In our results protozoans like *E. histolytica* and *G. lamblia* are among the commonest finding in the local population, with a higher proportion in males as compared to females. Though these protozoal parasites are present in all ages, their high prevalence among children is particularly of concern as amoebiasis can lead to liver abscess, resulting in severe morbidity and mortality. These two parasites have also been reported to be among the commonest in other studies performed in Karachi, which shows that the pattern has not changed over the years.[8,13,16] Though the two studies performed earlier found *Giardia* to be more common than Entamoeba[8,9] the difference in our findings could simply be explained by the fact that these two studies were population based studies, while our study was mainly performed upon clinical samples. Since both these organisms are spread by contaminated food and water. It is unfortunate that the sanitation and water supply conditions have not improved over the years and is the major contributing factor towards parasitic spread. The lack of hygienic living, inadequate health literacy and poor health-related practices among the uneducated and low-socioeconomic classes could also be considered important contributing factors towards this situation. An improved water surveillance system and health advocacy activities among the affected population may help in reduction of the burden of these diseases. *Giardia* infections should be of particular concern in infants and toddlers because they may be related to malnutrition resulting in growth problems.[17]

These findings are somewhat slightly different from the Northern Parts of the country as reports published from Bannu and Peshawar showed *Ascaristo* be the commonest parasite infecting the school children followed by *E. vermicularis* and *H. nana*[10,11] which may be attributed to different climatic conditions or other unknown factors.

Presence of helminths such as *A. lumbricoides* and *H. nana* must be considered important as faecooral transmission of cysts and ova is common and untreated cases in the population may keep on passing the pathogens to those at risk.[3] Since, these parasites along with others have been related to malnutrition in children, their testing and eradication should be a priority for the community.[18,19]

Since our study is based on clinical samples, it has its limitations and it may not reflect the exact picture in the community, however it clearly shows the type of intestinal parasites present in the population and highlights the need to develop a strategy to deal with the issues associated with them. A detailed community based survey is necessary to get the exact prevalence measures of intestinal parasitic infection in the local population.

REFERENCES

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors. 2014; 7: 37-56.
2. Torgerson PR. One world health: Socioeconomic burden and parasitic disease control priorities. Veterinary parasitology. 2013; 195: 223-32.
3. Hotez PJ, Savioli L, Fenwick A. Neglected tropical diseases of the Middle East and North Africa: review of their prevalence, distribution, and opportunities for control. PLoS Negl Trop Dis. 2012; 6: e1475.
4. Ngui R, Lim YAL, Chong Kin L, Sek Chuen C, Jaffar S. Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of West Malaysia. PLoS Negl Trop Dis. 2012; 6: e1550.
5. DuPont HL. Giardia: both a harmless commensal and a devastating pathogen. The Journal of clinical investigation. 2013; 123: 2352-4.
6. Nazeer JT, Khalifa KES, Von Thien H, El-Sibaei MM, Abdel-Hamid MY, Tawfik RAS, et al. Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt. Parasitology research. 2013; 112: 595-601.
7. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. The Lancet. 2012; 379: 2151-61.
8. Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. PLoS One. 2008; 3: e3680.
9. Siddiqui MI, Bilqees FM, Iliyas M, Perveen S. Prevalence of parasitic infections in a rural area of Karachi, Pakistan. Prevalence. 2002; 52: 315-20.
10. Khan MS, Jehan S, Akram M, Rabnwaz, Zaib M, Lathif Z, et al. Prevalence of intestinal protozoan and worm infestation in primary school going children of 5-10 years of age in district Bannu. Annals of Pakistan Institute of Medical Sciences. 2012; 8: 243-8.
11. Ullah I, Sarwar G, Aziz S, Khan MH. Intestinal worm infestation in primary school children in rural Peshawar. Gomal Journal of Medical Sciences. 2009; 7: 132-6.
12. Chaudhry ZH, Afzal M, Malik MA. Epidemiological factors affecting prevalence of intestinal parasites in children of Muzaffarabad district. Pakistan Journal of Zoology. 2004; 36: 267-71.
13. Mumtaz S, Siddiqui H, Ashfaq T. Frequency and risk factors for intestinal parasitic infection in children under five years age at a tertiary care hospital in Karachi. J Pak Med Assoc.2009; 59: 216-9.
14. Microsoft. Microsoft Excel. Washington: Microsoft; 2010.
15. Dean A, Sullivan K, Soe M. OpenEpi: Open Source Epidemiologic Statistics for Public Health 2015 [updated 2015 5/4/2015; cited 2015]; Available from: www.OpenEpi.com
16. Ahsan ul Wadood AB, ur Rhman A, Qasim KF. Frequency of intestinal parasite infestation in children hospital Quetta. Pakistan J Med Res. 2005; 44.
17. Al-Mekhlafi MSH, Azlin M, Nor Aini U, Shaik A, Saiah A, Fatmah MS, et al. Giardiasis as a predictor of childhood malnutrition in Orang Asli children in Malaysia. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2005; 99: 686-91.
18. Mbuh JV, Nembu NE. Malnutrition and intestinal helminth infections in schoolchildren from Dibanda, Cameroon. Journal of helminthology. 2013; 87: 46-51.
19. Papier K, Williams GM, Luceres Catubig R, Ahmed F, Olveda RM, McManus DP, et al. Childhood malnutrition and parasitic helminth interactions. Clinical Infectious Diseases. 2014; 59: 234-43.

ORIGINAL ARTICLE

Comparison of Extracts of *Syzygium Aromaticum* on the Weight of STZ induced Diabetic Rats

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ABSTRACT

Objective: To observe the effect of different extracts of *Syzygium aromaticum* (50% ethanolic and 50% aqueous) on the weight of Streptozotocin (STZ) induced diabetic rats in comparison with insulin.

Study Design: Randomized control trial.

Place and Duration of Study: The study was conducted at National Institute of Health Islamabad from July 2011 to December 2011.

Materials and Methods: Forty adult rats of Sprague dawaley specie with weight ranging between 200-250 g were selected and were equally divided into 5 groups (I-V) with eight rats in each group. Group- I was control. Diabetes was induced to group II- V by a single intraperitoneal injection of STZ and rats with fasting blood glucose above 200mg/dl were selected. After receiving the injection of STZ, the animals were weighed on Day zero i.e 48 hours (post STZ). Group-II was diabetic control , group III received 50% aqueous extract while group IV received 50% ethanolic extract of *Syzygium aromaticum* at a dose of 750 mg/kg body weight respectively for sixty days. Group V received 70/30 humulin insulin, 0.6 units/kg body weight subcutaneously, bid for sixty days. After two months of study on Day 60 the animals were weighed again, changes in the weight which occurred with both extracts were compared with the changes in the weight which occurred with the standard drug insulin.

Results: Group-IV receiving 750 mg/kg body of 50% ethanolic extract showed marked improvement in the weight as compared to group III receiving 50% of the same dose of aqueous extract. Group V receiving insulin showed improvement in the weight which is almost closer to the increase in weight which occurred in group III diabetic rats.

Conclusion: 750 mg/kg body weight of 50% ethanolic extract of *Syzygium aromaticum* caused more improvement in the weight of diabetic rats than the increase in the weight with the 50% aqueous extract of the same dose and the drug insulin.

Key Words: *Diabetes Mellitus, Weight Improvement , Syzygium Aromaticum Extract.*

Introduction

Diabetes mellitus (DM) is a very common disease throughout the world. In Pakistan the incidence of diabetes mellitus is 22.04%.[1] In this disease there is destruction of the insulin producing beta cells of pancreas (Type1DM) and resistance to insulin action in liver and peripheral tissue leading to hyperglycemia (Type2DM).[2] It is a complex disorders that disturbs the metabolism of protein, fat and carbohydrates leading to weight loss. Several oral hypoglycemic agents lowers blood glucose and improve weight but have many side effects. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, with less side effects and can counter the high cost and poor availability of antidiabetic drugs for rural populations.[3] Medicinal plant like *Syzygium aromaticum* has antidiabetic potential, its effect on STZ induced diabetic rats have been studied and its extract lowered the blood glucose level.[4] Streptozotocin (STZ) a nitrosourea compound is used to induce insulin-dependent diabetes mellitus in experimental animals.[5] It causes irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin.[5] The cytotoxic action of STZ is mediated by reactive oxygen species, which causes a rapid destruction of B cells leading to

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hyperglycemia.[6] A single intraperitoneal injection of STZ with the dosage range between 40-60 mg/kg body weight is very effective.[6] This high blood glucose causes the formation of advanced glycosylation end-products which injures the pancreatic beta cell through oxidative stress.[7] Increased serum glucose causes increase catabolism in skeletal muscles leading to increase breakdown of muscle protein and loss of adipose tissues causing a decrease in body weight.[8] A herbal plant *Syzygium aromaticum* (clove), with the scientific name (L)Merrill and Perry syn Eugenia caryophyllata, also known as "laung" belongs to the species *aromaticum*, family *myrtacea* and genus *Syzygium*.[9] It is widely cultivated in Indonesia, Sri Lanka. *Syzygium aromaticum* is commonly used as local anesthetic in dentistry and as flavoring agent in food. It treats gastrointestinal symptoms and act as ant-inflammatory, insecticidal, and antioxidant agent.[10] *Syzygium aromaticum* extract acts like insulin in hepatocytes by reducing phosphoenolpyruvate carboxykinase and glucose 6-phosphatase gene expression.[11] The active ingredient in clove is eugenol.[12] It improves gut motility by increasing the secretion of gastro-intestinal enzyme and relieves indigestion.[13] Many studies have shown that ethanolic and aqueous extract of *Syzygium aromaticum* improves and increases the weight of diabetic rats. The aim of the present study isto compare the improvement in the weight of the diabetic rats which occurs with both extracts of *Syzygium aromaticum* in comparison with the changes in the weight which occurs with the standard drug insulin.

Materials and Methods

Present study was carried out in National Institute of Health Sciences Islamabad in the department of plant and science from July to December 2011. It was a randomized comparative trial in which forty adult healthy male sprague dawley rats were selected, rats belonging to both genders with weight more than 250 gm and less than 200 gm were not included in the study. For acclimatization the rats were kept at animal house of NIH for one week at room temperature of 260C and humidity of 70% with 12 hours dark and light cycle was maintained. Rodent pellet containing proteins, fats, minerals, vitamins, fibre and water were available throughout the study.[14] Dried *Syzygium aromaticum* buds about 250gms were verified from the National University of Science and Technology Islamabad, department of Plant Sciences (NUST/NCVI/MQH/ZRC/001). About 125g of *Syzygium aromaticum* buds were soaked into 50% aqueous solution and the same amount was soaked in 50% ethanolic solution. Each was stirred with magnetic stirrer for twenty four hours in the flask at room temperature. After 24 hours the filtrates were separated and kept in a separate flask. This process was repeated thrice , the filtrates were then concentrated under reduced pressure of 40°C in a rotary evaporator. The prepared extracts was stored at a temp of -20°C to be used for further experiment.[15]

After one week of acclimatization, all the experimental groups with fasting blood glucose level between 70-135 mg/dl were induced diabetes with a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/ kg body weight) in 0.1ml citrate buffer (pH 4.5).[16] Forty eight hours after receiving the injection of STZ blood samples were taken from the tail vein of experimental animals. Quantitative estimation of blood glucose was done by using glucometer and glucose oxidase based test strips.[17](Abbott AxSYM system, USA). Rats with fasting blood glucose above 200mg/dl were selected. The animals were divided into five groups with eight rats in each group. All the rats received rodent pallets and water ad libitum for sixty days in addition 10 ml/kg of 0.9% saline solution was given to first group . Second group was (diabetic control. Third and fourth group received 750mg/kg body wt of 50% aquoeus and 50% ethanolic extract respectively by gavage. Insulin (humulin 70/30) with the dose of 0.6 units/kg body wt , subcutaneously twice daily was adminstered to fifth group.[18] The weight of the diabetic rats was estimated on Day zero i.e forty eight hours after receiving Streptozotocin and after 2 months of the study at Day60.[19] Data was analyzed using SPSS version 20. Weight of diabetic rats were expressed in Mean ± SD and the results were compared by using One Way ANOVA followed by Post-hoc Tukey test p-value of < 0.05 was considered significant.

Result

In the beginning of the study on Day zero there was significant difference in the body weight between all

the groups (p<0.001). Group I had higher body weight as compared to other groups (p < 0.001). There was insignificant difference in the body weight between all the four diabetic groups (p > 0.05). At the end of the study on Day 60 body weight was significantly higher in group-I as compared to all the four diabetic groups (p < 0.001). The weight of group II diabetic rats was significantly lower than other groups (p < 0.001). Group-III and group V showed significantly lower body weight than group-I and group-IV but higher than group II . However there was insignificant difference between group-III and group-V(p=0.941) The rats belonging to group-IV showed significantly higher body weight than other diabetic groups (p < 0.001) but significantly lower than group I.

From the above result significant increase in the weight of group IV rats, receiving 750 mg/kg body weight of 50% ethanol extract of *Syzygium aromaticum* was seen. Simultaneous administration of Insulin (humulin) also resulted in significant (p<0.001) improvement in the weight of group V as compared to the group II (diabetic control) but this increase in weight is closer to the increase in weight which occurred in group III diabetic rats .

Table I Shows the mean and standard deviation of weight taken on Day zero and Day 60.

Table I: Body weight of all study groups on Day zero and Day 60

Groups	Body Weight (grams) / Mean ± SD	
	Day zero	Day 60
Group-I (n = 8)	220.62 ± 4.56 ¶¥€ π	226.50 ± 4.75¶¥€ π
Group-II (n = 8)	174.12 ± 6.73*	170.88 ± 7.08*¥€π
Group-III (n = 8)	175.12 ± 5.28*	190.25 ± 5.18*¶€
Group-IV (n = 8)	175.75 ± 6.14*	200.38 ± 6.02*¶¥π
Group-V (n = 8)	177.38 ± 6.04*	189.25 ± 5.70*¶€
p-value	< 0.001**	< 0.001**

All values have been expressed as mean±SD

** = Highly Significant * = Significant from group-I

¶ = Significant from group-II ¥ = Significant from group-III

€ = Significant from group-IV π = Significant from group-V

Discussion

In the present study the results have proved that *Syzygium aromaticum* ethanolic extract improves the weight of diabetic rats. In this study the effect of differents extracts of *Syzygium aromaticum* on the weight of streptozotocin induced diabetic rats is seen and the results are compared with the standard drug insulin at a dose of 0.6 units/kg body weight. We used 50% aqueous and 50% ethanol extract with the dose of 750mg/kg body weight respectively. There is significant reduction in the weight of group II diabetic rats as compared to control group I. On comparing the improvement in the weight of the group III, group IV and group V with the diabetic group II it is observed that *Syzygium aromaticum* ethanol extract causes 8% improvement and the *Syzygium aromaticum* aqueous extract causes 5.6 % increase in the weight which is close to the increase in the weight which occurred with insulin receiving group 5.5% . Tajuddin et al., (2003) used 50% ethanol extract of clove in rats.[20] in our study Similar concentration of ethanolic extract showed more impovement in the weight as compared to aquoeous extract and insulin. Namasivayam et al., (2008) conducted a study on the genus *Syzygium* and concluded that the aqueous extract of the *Syzygium* also possess the potential of improving the weight of diabetic rats.[21] Singh et al., (2007) studied the effect of ethanolic extract of *Syzygium* on diabetic rats and concluded that ethanolic extract lowered the blood glucose and improved the weight of diabetic rats.[22] In diabetes mellitus free radicles and reactive oxygen specie are produced which damages nucleic acid, carbohydrates , proteins and lipids, this may lead to weight loss.[23] Musabayane et. al.,(2010) on doing the analytical chemistry of constituents of *syzygium aromaticum* mainly eugenol and olaenic acid suggested that both act as antioxidant and are the major scavenger of free radicals, thus preventing the weight loss.[24] Many studies have reported that aqueous and ethanolic extracts of plants causes glucose lowering effect by activation of pancreatic beta cells , improving its granulation, increasing insulin production and have insulin mimetic effect.[25] Khan et al.,(2006) in one of his study on the *Syzygium aromaticum* suggested that this herbal plant has the potential to stimulate the functioning cells of islet of langerhans and causes regeneration of pancreatic beta cells thus increasing insulin release.[26] Laizuman et al., (2010) suggested that improvement in the weight of diabetic rats by *Syzygium* can be by either due to release of insulin from pancreatic beta cell or from the bound form and insulin also inhibit gluconeogenesis from proteins thus preventing loss of proteins and maintan body weight.[27] Insulin causes increase food intake and retains energy leading to

the improvement in the weight of diabetic rats. Willing et al.,(1990) suggested that insulin stimulates daily food intake and body weight gain in diabetic rats.[28] So we can say that improvement in the weight of diabetic rats by *Syzygium aromaticum* can be due to the stimulation of functioning pancreatic beta cells, to increase the release of insulin or this may be due to regeneration of beta cells. Admin, (2013) suggested that *Syzygium aromaticum* activates digestive enzymes , aid in digestion , smoothens the lining of digestive tract and has myorelaxant action. He further reported that high amount of carbohydrates, protein , dietary fibers, multivitamins and minerals are present in clove which maintain the weight of body.[29] Lester et al., (2004) suggested that eugenol the main constituent of *Syzygium* causes stimulation of gastric secretion and improves digestion.[30] In our study the ethanolic extract caused more improvement in the weight than the aqueous extract we can say that this effect is probably because the constituents of the *Syzygium aromaticum* are more soluble in the ethanol solution than the aqueous solution . San et al., (1995) suggested that *Syzygium aromaticum* compounds have more solubility in the ethanolic solution .[31] We can say in our study that the possible mechanism by which ethanol extract improves the weight of diabetic rats is that the free radical scavenging property of ethanolic extract is more than aqueous or it may be due the presence of antioxidant active principles in the ethanol extract in excess amount than aqueous extract. This improvement in weight can also be due to the induction of insulin secreting pancreatic β cells of islets of langerhan or it may be due to enhanced transport of blood glucose to the peripheral tissue and proper glucose utilization by diabetic rats.[32] The antispasmodic and myorelaxant property of the compounds of *Syzygium aromaticum* can also lead to the improvement in the body weight.[33]

Conclusion

Our results from this study indicate that 50% ethanolic extract of *Syzygium aromaticum* at a dose of 750 mg/kg body weight has more potential to improve the weight of diabetic rats than the 50% aqueous extract of the same dose and the drug insulin because of its insulin mimetic action , free radical scavenging property and the increased solubility and presence of excess amount of antioxidant active principles in it.

REFERENCES

1. Shera AS, Jawad F, Maqsood A. Prevalence of diabetes in Pakistan. Diabetes Res.Clin.Pract.2007; 76: 219-22.
2. Marlon E. Beta Cell Dysfunction and Insulin Resistance. Front Endocrinol (Lausanne). 2013; 4: 37.
3. Prasad SK, Kulshreshtha A, Qureshi TN. Antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats. Pak J Nutr. 2009; 8: 551-7.
4. Chaudhry ZR, Naseer A, Chaudhry ER, Chaudhry SR. Comparison of Aqueous and Ethanolic Extract of Syzygium Aromaticum on Blood Glucose in Diabetic Rats. JIIMC. 2015; 10: 214-18.
5. Zafar M, Naeem ul hassan naqvi S. Effects of STZ-Induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int. J. Morphol. 2010; 28: 135-42.
6. Szkudelski T. The mechanism of alloxan and Streptozotocin action in B cells of the rat pancreas. Physiol Res.2001; 50: 536-46.
7. Lin N, Zhang H, Su Q . Advanced glycation end-products induce injury to pancreatic beta cells through oxidative stress. Diabetes and Metabolism. 2012; 38: 250-7.
8. Niels M, Sreekumaran N. Diabetes and Protein Metabolism. American Diabetes Association . Diabetes . 2008; 57: 3-4.
9. Cortes Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pacific Journal of Tropical Biomedicine. 2014; 4: 90-6.
10. Alqareer A, Alyahya A, Andersson L. The effect of clove and benzocaine versus placebo as topical anesthetics. J Dent.2006; 34: 747-50.
11. Prasad RC, Herzog B, Boone B, et al. An extract of Syzygium aromaticum represses genes encoding hepatic gluconeogenic enzymes.Journal of Ethnopharmacol. 2005; 96: 295-301.
12. Kuroda M, Mimaki Y, Ohtomo T, et al . Hypoglycemic effects of clove (Syzygium aromaticum flower buds) on genetically diabetic KK-Ay mice and identification of the active ingredients. J Nat Med .2012; 66: 394-9.
13. Umesh R. Medicinal properties and health benefits of cloves. www.nutrition and you.com 2009.
14. Nwanjo HU. Studies on the Effect Of Aqueous Extract of Phyllanthus Niruri leaf on plasma glucose level and some hepatospecific marker in diabetic wister rats .The Internet Journal of laboratory Medicine. 2006; 2: 2.
15. Ankit G, Madhu N, Vijay K. Modern extraction

methods for preparation of bioactive Plant extract. International Journal of Applied and Natural Sciences 2012; 8: 8-26.
16. Tesh G, Aleen T. Rocket model of Streptozotocin induced diabetic nephropathy . Nephrology. 2007; 12: 261-2.
17. National Committee for Clinical Laboratory Standards. Statistical Quality Control for Quantitative Measurement; Principle and Definition.1999.
18. Horsfall A, Alyegbusi A, Noronha C, et al. Morinda citrifolia fruit juice augments insulinaction in Sprague Dawley rats with experimentally induced diabetes. Nigeria Nig Q Journal Hosp Med. 2008; 18: 162-5.
19. Chinwe E, Uchechukwu D, Joel O, et al. Estimation of glucose level and body weight in Alloxan induced diabetic rat treated with aqueous extract of Garcinia Kola Seed. Ulutas Med J. 2015; 1: 26-30.
20. Tajuddin A, Ahmad S, Latif A et al. Aphrodisiac activity of 50% ethanolic extracts of Myristica fragrans Houtt.(nutmeg) and Syzygium aromaticum (L) Merr. & Perry. (clove)in male mice: BMC. Complement Altern Med. 2003; 3: 10-20.
21. Namasivayam R, Ramachandran B, Munuswamy D. Effect of Aqueous extract of Syzygium cumini Pulp on antioxidant defense system in STZ induced diabetic rats. Iranian Journal of Pharmacology and Therapeutics IJPT. 2008; 7: 137-45.
22. Singh N, Gupta M. Effect of ethanolic extract of Syzygium cumini (linn) seed powder on the pancreatic islet of alloxan diabetic rats. Indian Journal of Exp Biol. 2007; 45: 861-7.
23. Chaillou, Nazareno. New method to determine antioxidant activity of polyphenols. J. Agric. Food Chem. 2006; 54: 8397-8402.
24. Musabayane C, Tufts M, Mapanga R. Synergistic antihyperglycemic effects between plant-derived oleanolic acid and insulin in streptozotocin-induced diabetic rats. Renal Failure. 2010; 32: 832-9.
25. Lukmanul H, Girija S, Senthil k. Effect of aqueous and ethanol extracts of Cassia auriculataL flowers on diabetes using alloxan induced diabetic rats. Int J Diabetes & Metabolism. 2007; 15: 100-6.
26. Khan A, Qadir SS, Nawaz K. Cloves improve glucose, cholesterol and triglycerides of people with type 2 diabetes mellitus.The FASEB journal.2006; 20: 990.
27. Laizuman N, Farhana AP, Abu H. Comparative study of antidiabetic effect of Abroma augusta and Syzygium Cumini on alloxan induced diabetic rats. ABJNA. 2010; 1: 1268-72.
28. Willing AE, Walls EK, Koopmans HS. Insulin infusion stimulates daily food intake and body weight gain in diabetic rats. Physiol Behav. 1990; 48: 893-8.
29. Admin A. Health Benefits of Cloves. Food to fitness. 2013.
30. Lester P, Sissi WG, Choon NO. Herbal and Traditional Medicine,Biomolecular and Clinical Aspects. 2004; 2: 80-4.
31. San M, Wan R, Abubakar M. Separation and identification of eugenol in ethanol extract of cloves by reversed-phase high-performance liquid chromatography. Journal of the American Oil Chemist Society. 1995; 72: 1231-3.
32. Kanter M, Meral I, Yener Z, et al. Partial regeneration/proliferation of the beta-cells in the islets of langerhans by Nigella sativa in streptozotocin-induced diabetic rats. Tohoku J Exp Med. 2003; 201: 213-19.
33. Wang R, Yang B. Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. Innovative Food Science and Emerging Technologies. 2009; 10: 289-92.

ORIGINAL ARTICLE

Outcome and Complications of Trans-Sternal Thymectomy for Myasthenia Gravis

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ABSTRACT

Objective: To determine outcome and postoperative complications of trans-sternal thymectomy for myasthenia gravis.

Study Design: Experimental, prospective.

Place and Duration of Study: The study was conducted at Department of Surgery, Pakistan Institute of Medical Sciences (PIMS), Islamabad from June 2009 to June 2012.

Materials and Methods: We included 30 consecutive patients from all age groups either coming to Surgical outpatient clinic or referred from Neurolgy unit having generalized myasthenia gravis between 12-55 years of age, thymic mass on radiology or poor medical control of disease with no contra indications to surgery. Patients unfit for anaesthesia due to any reason or inoperable thymic tumour were excluded. We studied outcome and post-operative complications in all patients after total thymectomy through trans-sternal approach for 24-40 months. All patients were assessed for haemorrhage, transfusion requirement, shock, myasthenia crisis, respiratory infection, wound infection, delayed healing of wound and dehiscence and pulmonary embolism. Remission of disease was assessed according to De Filippi classification. The data was collected by post graduate trainees on a pre-designed proforma and analysed by SPSS 10. The descriptive statistics were applied and results were shown in percentage.

Results: In total 30 patients, mean age was 37±8.6 years. All patients needed at least two units of blood transfusion. We observed pleural damage in 20 patients (66.67%), respiratory infection in 20 (66.67%), myasthenia crisis in 8 (26.7%), minor wound infection in 6 (20%), mortality in 4 patients with carcinoma(13.33%). Artificial ventilation discontinued within 24 hours in 22, at 7th day in 4 and at 14th days in 4 patients. Wound dehiscence occurred in 1(3.33%), cardiac arrest in 1 (3.33%). Mean hospital stay was 13.6 (7-45) days. Six patients (20%) were symptom free without drugs and 9 patients (30%) were symptom free with drugs at 2 years.

Conclusion: Trans-sternal total thymectomy provides good results for symptom control in benign thymic conditions. The major complication is haemorrhage needing transfusion. Other complications can be managed successfully by conservative treatment.

Key Words: *Trans Sternal Thymectomy, Myasthenia Gravis, Thymic Tumour.*

Introduction

Myasthenia Gravis (MG) occure due to formation of antibodies against acetylcholine receptors at myoneuronal junctions and presents with muscle weakness and fatigability.[1] Most of patients develop generalized weakness involving bulbar, limb musculature, extensors of neck and diaphragm.[2] Antibody to muscle-specific kinase (MuSK) may be positive in sero-negative MG patients and they develop involvement of respiratory muscles, shoulder and neck without ocular weakness.[3,4]

Treatment comprises of four methods: anticholinesterase drugs, immune-suppressive agents, removal of thymus, and immunotherapies, like exchange of plasma and intravenous immuno globulin (IVIG).[5,6]Blalock et al found the successful outcomes of thymectomy in MG in 1941.[7]Since then surgery is widely being used as a for such patients.[8,9] In fact, thymectomy is considered, by neurologists, as a first-line therapy in most patients with thymoma or generalized myasthenia to achievesustained improvement or remission.[10,11]

About three fourth of such people have abnormality in thymus; of them, hyperplasia in 85% andthymoma in 15%.[2,12]Thymoma is the most common mediastinaltumour (25%).[13]Median sternotomy is considered safe and effective approach for

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thymectomy, especially for ectopic locations.[14,15] Data of the efficacy and complications of thymectomy in patients with MG via median sternotomy is limited in Pakistan. The authors, in the current study, made an effort to determine the safety of median sternotomy in patients with MG and then outcome of thymectomy.

Materials and Methods

This experimental, prospective study was conducted between June 2009 and June 2012 in department of Surgery, Pakistan Institute of Medical Sciences (PIMS). We included 30 consecutive patients from all age groups either coming to Surgical Out Patient Department or referred from Neurolgy unit of PIMS with generalized myasthenia gravis having the disease for less than 5 years, thymic mass on radiology (mediastinal widening) or poor medical control of disease with no contra indications to surgery usually grade II B & III. The patients unfit for anaesthesia due to any reason or inoperable thymictumour were excluded. The data was collected by post graduate trainees on a pre-designed proforma. After detailed history, thorough examination and routine investigations, specific investigations including Tensilon test, Electromyography (EMG), X-ray chest and CT Scan of chest to assess anatomical location, thymoma and vascular relationships were carried out.

All patients remained in care of a multidisciplinary team including surgeon, neurologist, pulmonologist, respiratory therapist, anaesthetist and intensivist. The patients were stabilized on drugs preoperatively but anticholinesterase drugs were stopped 6-8 hours before surgery. Patients taking oral steroids were tapered gradually. Plasmapheresis was done 3 days before surgery. Blood group, cross match, and ventilator in intensive care unit (ICU) were arranged and informed consent taken. The antibiotic prophylaxis consisted of three intravenous doses of 1g of Ceftriaxone, at induction, at 12 and 24 hrs post operative period respectively.

General anaesthesia without muscle relaxants and median sternotomy approach was used in all patients. The vascular pedicles tied and gland separated by blunt dissection from surrounding structures. Total thymectomy was done removing all tissue of thymus and mediastinal fat from the diaphragm and pericardium inferiorly to the thymic extension in neck superiorly upto thyroid and from one phrenic nerve to the other. Pleural damage, when occurred, repaired and chest drain placed in right chest wall, across the mediastinum and upto contra-lateral apex connected to under water seal. All surgeries were performed by one experienced surgeon with a dedicated team.

In post operative phase, all patients were monitored in ICU until their vital signs got stable and anaesthetist declared safe to shift to ward. Neurologists reviewed them and anticholinesterase drugs started if any signs of disease were found. We avoided, in all patients, sedatives, narcotics and muscle relaxants. We studied the post operative complications by assessment of haemorrhage, transfusion requirement, shock, myasthenia crisis, respiratory infection, wound infection, delayed healing of wound and dehiscence and pulmonary embolism.

On the basis of remission of disease according to De Filippipost operative classification, we assessed the outcome.[16]

Class I: remission complete, without medication
Class 2: symptom free, less medication
Class 3: improved, decreased symptoms or medication
Class 4: no improvement
Class 5: worsening symptoms

Remission was defined as no symptom of myasthenia gravis or cessation of medical treatment without reappearance of any symptoms.

All patients were followed for 24-40 months (average 27) in surgical OPD and advised to consult on appearance of any of the signs and symptoms of myasthenia again. The data was analysed by SPSS 10. The descriptive statistics were applied and results were shown in percentage.

Results

Mean age of patients was 37±8.6 years. Neuromuscular blocking drugs, atropine and morphine were avoided during anaesthesia and no significant complication of anaesthesia was observed. Pleura was damaged in 66% of patients which was managed by chest intubation. Cardiac arrest occurred in one patient which was managed by internal cardiac massage and patient revived. Myasthenia crisis was observed in 8 patients, which was successfully managed in 4 patients. Three

patients died within 2 weeks and one survived for next 6 weeks. All 4 had carcinoma of thymus (Osserman's group III). Respiratory infection was noted by symptoms of cough with sputum and irritation in throat in 6 (20%). All were successfully managed by medical treatment and physiotherapy. In post operative period, nasal endotracheal tube was kept for ventilation in ICU for 24 hours. In 22 patients, vital capacity was found to be 1.5-2 litres within 24 hours and artificial ventilation discontinued and patient ambulated. Endotracheal tube kept in place for further 3-4 days as a precaution. Four patients were extubated at 7th day while 4 patients needed re-intubation and ventilator support for next 14 days. Chest radiograph taken to exclude hemo/pneumothorax. Skin stitches removed on 7th-10th post operative day and patient discharged home.

Minor wound infection was observed in 6 cases (20%) and was managed successfully by local antiseptics. One patient on steroids for long period developed wound dehiscence, which was managed by pectoral myoplasty by a plastic surgeon. Mean hospital stay was 13.6 (7-45) days. Further results are in table I.

Table I: Post operative complications (n= 30)

Complication	Number of Patients	Percentage
Haemorrhage needing transfusion 2 units	30	100
Pleural damage	20	66.6
Respiratory infection	20	66.6
Myasthenia crisis	8	26.7
Wound infection	6	20
Mortality	4	13.3
Wound dehiscence	1	3.4
Cardiac arrest	1	3.4

Table II: Symptoms control of myasthenia after thymectomy (n= 30)

Status of Symptoms	Number of Patients	Percentage
Symptom free without drugs	6	20
Symptom free with drugs	9	30
Mild symptoms with drugs	9	30
Severe symptoms with drugs	1	3.3

Discussion

Thymectomy is one of the most effective treatment modalities to stop progression of symptoms in MG. The observations in different parts of the world, of the disease and the outcome of procedures are not the same.

In the study of Remes-Troche JM, mean age was 32.10 +/- 14.42 years.[10] NiazHussain, found mean age at presentation to be 35.2 ± 14.5 years. Out of 22 patients, having persistent generalized or ocular myasthenia gravis, 16 (72.7%) were females and 6 (27.2%) males. Ali Soleimani, in a study of 110 patients in Iran, noticed mean age at thymectomy to be 30.3 ± 12.8 years.[2] In our study, the mean age of patients presented for thymectomy was 37±8.6 years. The younger patients (<55 years) having MG for less than 5 years are most benefited by surgery.

Trans sternal approach is considered standard and safe by many surgeons.[17] In the current study, only median sternotomy was used to gain access to thymus and remained a safe technique. Kas et al found similar results. Pneumothorax was the most common minor complication. Very few patients developed any major or intra-operaitve complication.[18] Zielinski et al reported no difference in morbidity after less invasive procedures like manubriotomy as compared to median sternotomy.[19]

Xiang-yang C reviewed 243 patients with MG who underwent thymectomy. Forty-four patients (18%) experienced postoperative myasthenic crisis within thirty days post thymectomy. Myasthenic crisis,in postoperative patients, was significantly higher in those having history of myastheniccrisis (P=0.016), thymoma (P <0.0001), and major postoperative complications (P <0.0001) than in patients without these three conditions.[20] Ali Soleimani, in a study of 110 patients in Iran (76 cases done via trans-sternal approach and 34 done via trans-cervical route),

showed that 32% developed myesthenia crisis. Lack of drug compliance and pneumonia causes were main triggering factors for myasthenic crises.[2] Remes-Troche JM found myasthenic (9.3%) and cholinergic (2.7%) crises, the most serious complications.[10] In our study, histopathology reports showed 16 cases of thymoma. Half of them (8 cases) had experienced crisis, which makes 26.7 % of total patients and 40% of those with previous history of

crisis. Major postoperative complications related to thymectomy in the literature include pneumonia, pneumothorax, hydrothorax and ARDS. We found that the frequency of postoperative myasthenic crisis increases when the patients had severe postoperative complications. Multivariate logistic regression analysis in China revealed that major postoperative complications related to thymectomy are a predictor of postoperative crisis.[20]

In the work of Remes-Troche J Met al in Mexico, no surgery-related deaths occurred in 75 patients[10]. Marulli et al demonstrated the safety and efficacy of this procedure, after a review of 100 consecutive patients who underwent left-sided robotic thymectomy for MG. No deaths or intra-operative complications occurred.[21] In our study, 3 patients died within 2 weeks and one survived for next 6 weeks. All 4 had carcinoma of thymus (Osserman's group III).

Spath G et al operated upon 75 patients. Impaired wound healing was noticed in 6.7% of the patients, with complete sternal instability in one patient, while 5.3% developed pneumonia.[22] Ali Soleimani showed that 20 of 110 (18%) developed attacks of respiratory failure.[2] Niaz Hussain, out of 22 patients studied in Karachi, mentioned the most common major wound complication was sternal bleeding, encountered in 2 (9%) patients. This was followed by 1 case of disruption of the wound. Pneumothorax was the most common minor complication observed in 7 (31.8%), followed by wound infection in 4 (18%) patients, and 2(9%) each of haemothorax, and seroma.[23] We found wound infection in 6 (20%), and wound dehiscence in 1 patient (3.4%). Respiratory infection in 20(66%). The wound complications are common in patients on long term steroids and pneumo / hemothorax is a common complication of surgery on thymus secondary to pleural damage.

Spath G et al documented nerve paresis. Phrenic nerve in 2.7% and recurrent laryngeal nerve in 1.3%, noticed only in thymoma patients.[22] NiazHussain reported that a total of 2 (9%) patients out of 22 had intra-operative complications including phrenic nerve and innominate vein injury. No long term morbidity occurred.[23] In the current study, no nerve damage was noticed. Probably nerve damage is rare and associated with tumours.

Patients often experience some transient worsening of symptoms early in the postoperative period. Improvement usually is delayed for months or years. We followed the patients for next 24-40 months (average 27 months). Scott and Dtterbeck reported that 78% patients became better by one or more modified Osserman class and 69% had complete remission in Osserman class I, II and III.[24] Takanami I found thymectomy to induce remission, more frequently in young patients with a short duration of disease, hyperplastic thymus, more severe symptoms, and a high antibody titer.[25] We found remission of symptoms within 3 months. 20% became symptom free without drugs and 30% became symptom free with medication. Symptoms of further 30% became mild and only 1 patient (3.3%) remained unresponsive. All patients having complete remission were 45 yrs old or less. In a study by Nieto et al, the rate of remission in the presence of thymic hyperplasia was 42% compared to 18% in patients with thymoma.[26] Shrager et al. reported an actual 50% complete remission with Kaplan-Meier estimate of 5 years of total remission of 57% in patients with ocular MG. After thymectomy there was no disease progression.[27]

Masaoka reported a 46% remission rate at 5 years, 67% at 15 years and 90% overall palliation for the non thymoma group. They noted 32% remission rate and 82 % palliation rate for thymomas. The drug free remission was achieved primarily in early Osserman classes.[28] According to Nason and Maddaus, thymectomy for thymomas results in improvement or resolution of symptoms only in 25% of MG patients. But patients with no thymoma, MG symptoms improve in 90% and 50 % have complete resmission.[29] Marulli et al demonstrated, after 5-year clinical follow-up of 100 consecutive patients who underwent left-sided robotic thymectomy, that 28.5% of patients had complete stable remission, and 87.5% showed overall improvement. Remission was significantly more likely in patients with preoperative class I to II MG according to standards of Myasthenia Gravis Foundation of America.[21]

Conclusion

Trans-sternal total thymectomy provides good results for symptom control in benign thymic conditions. The major complication is haemorrhage needing transfusion. Other complications can be managed successfully by conservative treatment.

REFERENCES

1. Felix G. Fernandez FG, Battafarano RJ. General Thoracic surgery. In: Klingensmith ME, Chen LE, Glasgow SC, editors.Washington Manual of Surgery. 5th Ed.St. Louis, Missouri :Lippincott Williams & Wilkins; 2008.
2. Soleimani A, Moayyeri A, Akhondzad eh S, Sadatsafavi M, Shalmani HT, Soltanzadeh A. Frequency of myasthenic crisis in relation to thymectomy in generalized myasthenia gravis: A 17-year experience BMC Neurology 2004, 4: 12.
3. Pasnoor M, Wolfe GI, Nations S. Clinical findings in MuSK-antibody positive myasthenia gravis: a U.S. experience. Muscle Nerve. 2010; 41: 370-4.
4. Martignago S, Fanin M, Albertini E, Pegoraro E, Angelini C. Muscle histopathology in myasthenia gravis with antibodies against MuSK and AChR. NeuropatholApplNeurobiol. 2009; 35: 103-10.
5. http://emedicine.medscape.com/ [homepage on the internet]. Yale University School of Medicine ; 2012 [updated May 2014; cited 30 Nov 2014]. Available from; http://emedicine.medscape.com/article/1171206-treatment.
6. Vanderpluym J, Vajsar J, Jacob FD, Mah JK, Grenier D, Kolski H. Clinical Characteristics of Pediatric Myasthenia: A Surveillance Study. Pediatrics. 9, 2013.
7. Blalock A, Harvey AM, Ford RF, Lilienthal JL Jr. The treatment of myasthenia gravis by removal of the thymus gland. J Am Med Assoc 1941; 117: 1529-33.
8. Waitande SS, Thankachen RJ, Philip MA, Shukla V, Korula RJ. Surgicaloutcome of thymectomy for myasthenia gravis. Indian J ThoracCardiovasc Surg 2007; 23: 171-5.
9. Baraka A, Anesthesia and critical care of thymectomy for myasthenia gravis. Chest Surg Clin N Am 2001; 11: 337-61.
10. RemesTroche JM, Tellez Zenteno JF, Estanol B,GardunoEspinoza J, Garcia Ramos G. Thymectomy in Myasthenia Gravis: Response, Complications, and Associated Conditions. Arch Med Res. 2002; 33: 545-51.
11. www.bmj.com [homepage on the internet]. British Medical Journal; 2010 [updated July 2012; cited 6 December 2014]. Available from; thorax.bmj.com/content/15/3/262.full.pdf .
12. Castleman B: The pathology of the thymus gland in myasthenia gravis. Ann N Y AcadSci 1966; 135:496-505.
13. Hunt I, Tan C. Thorax. In: Williams NS. Bulstrode CJK, O'Connell PR, editors. Bailey and Love's short practice of surgery. 26th ed. London: CRC press; 2013.
14. Lee CY, Lee JG, Yang WI, Haam SJ, Chung KY, Park IK. Transsternal maximal thymectomy is effective for extirpation of cervical ectopic thymic tissue in the treatment of myasthenia gravis. Yonsei Med J 2008; 49: 987-92.
15. Khan A, Bilal A, Baseer A. Thymectomy for Myasthenia Gravis: Peshawar experience of 27 cases in five years. J Post grad Med Inst 2007; 21: 238-41.
16. De Camp MM, De Hoyos A, Transternal, Transcervical and thoracoscopicthymectomy. In: Fischer JE, Jones DB, Pamposelli FB, editors. Fischer's Mastery of surgery. 6th edition. Philadelphia:Lippincott Williams &Wilkins ; 2012.
17. Kondov G, Trajkovska T, Ljapcev R, Gogova L, Kondova I. Thymectomy in Myasthenia Gravis: Response and Complications. Turkish Respirat J 2005; 6: 144-8.
18. Kas J, Kiss D, Simon V, Svastics E, Major L, Szobor A. Decade-long experience with surgical therapy of myasthenia gravis: early complications of 324 transsternalthymectomies. Ann Thorac Surg 2001; 72: 1691-7.
19. Zielinski M, Kuzdza J, Szlubowski A, Soja J. Comparison of late results of basic trans-sternal and extended trans-sternal thymectomies in the treatment of myasthenia gravis. Ann Thorac Surg 2004; 78: 253-8.
20. Xiang-yang C, Zhi-qiang X, Ru-wen W, Qun-you T. Predictors of postoperative myasthenic crisis in patients with myasthenia gravis after thymectomy. Chin Med J 2011; 124: 1246-50.
21. Marulli G, Schiavon M, Perissinotto E. Surgical and neurologic outcomes after robotic thymectomy in 100 consecutive patients with myasthenia gravis. J ThoracCardiovasc Surg. 2013; 145: 730-5.
22. Spath G, Brinkmann A, Huth C, Wietholter H. Complications and efficacy of transsternalthymectomy in myasthenia gravis. Thorac Cardiovasc Surg. 1987; 35: 283-9.
23. Hussain N, Ahmed SW, Ahmed T, Hafeez AB, Baloch R, Ali S. Experience of thymectomy by median sternotomy in patients with myasthenia gravis. J Pak Med Assoc.2010; 60: 368-70.
24. Goldstein SD, Yang SC. Assessment of robotic thymectomy using the Myasthenia Gravis Foundation of America Guidelines. Ann Thorac Surg. 2010; 89: 1080-5.
25. Takanami I, Abiko T, Koizumi S. Therapeutic outcomes in thymectomied patients with myasthenia gravis. Ann ThoracCardiovasc Surg. 2009; 15: 373-7.
26. Nieto IP, Robledo JP, Pajuelo MC, Montes JA, Giron JG, Alonso JG, et al: Prognostic factors for myasthenia gravis treated by thymectomy: review of 61 cases. Ann Thorac Surg. 1999; 67: 1568- 71.
27. Shrager JB, Deeb ME, Mick R, Brinster CJ, Childers HE, Marshall MB, et al. Transcervicalthymectomy for myasthenia gravis achieves results comparable to

thymectomy by sternotomy. Ann Thorac Surg 2002; 74: 320-6.
28. Masaoka A, Yamakawa Y, Niwa H, Fukai I, Kondo S, Kobayashi M, et. al:Extended thymectomy for myasthenia gravis patients: a 20-year review. Ann Thorac Surg 1996; 62: 853-9.
29. Nason KS, Maddaus MA, Luketich JD. Chest wall, lung, mediastinum and pleura. In: Brunicardi FC, Andersen DK, Billiar TR, editors. Schwartz's Principles of surgery. 9thed. New York: McGraw- Hill ; 2010.

ORIGINAL ARTICLE

Students' Perceptions towards Formative and Summative Assessment: A Single Institution Study

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ABSTRACT

Purpose of the Study: The purpose of this study was to get an understanding of the MBBS students' perception and experience about their formative and summative assessment. Since the implementation of integrated curriculum students need to adopt deep learning approach contrary to traditional learning. We were interested to explore what approach our students take towards the preparation of their exam. We were also interested to get their views about formative and summative assessment currently being undertaken in our medical college.

Objectives of the Study: To collect data using a Pre-validated questionnaire and then analyze it, regarding:

a) Students study efforts towards passing the exam.

b) Students' perception about the importance of formative assessment held in our college.

c) Students experience and their views about conduction of summative assessment.

Study Design: A quantitative cross-sectional analytical study.

Place and Duration of Study: The Study was conducted from Jan 2015 to June 2015 at Islamic International Medical College Rawalpindi.

Materials and Methods: This was a quantitative cross sectional study using a structured, pre-validated questionnaire. The questionnaire was administered to all present students of 2nd to 5th year students. Data was entered in MS Excel sheet and analyzed using one sample binomial test.

Results: The response rate was 75% (5th year) to 84% (3rd year). Only 27% students performed same amount of study regularly. Whereas 73% put more effort near the assessment. Almost 50% students still performed selective studies for the assessment. 60-80% students opined that formative assessment experiences stimulated learning and were useful in the preparation for summative assessment.

Except for final year, more than 50% students were satisfied with the general atmosphere, structure and conduct of summative assessment. More than 70% admitted that they learn more when preparing for summative assessment near the examination. Almost 90% resented the inclusion of SEQs in methods of summative assessment and were contended with MCQs and OSCE/OSPE.

Conclusion: Students like formative assessment mainly because of feedback as it helps them in learning as well as preparing for summative assessment. All aspects of summative assessment were more satisfactory for the students.

Key Words: *Learning Styles, Student Perceptions, Assessment.*

Introduction

The importance of training future doctors through relevant and effective curriculum cannot be overemphasized[1] Effectiveness and relevance of the curriculum not only depends upon the curriculum design, its implementation methods and assessment but also on how it is perceived by the students. It may be below optimum if the students have poor perceptions[2] The students' perception largely depends upon how the curriculum is implemented[3] Positive perceptions are known to enhance the motivation level of students therefore it is important to evaluate students' perception regularly at frequent but predefined intervals.[4]

The importance of regular evaluation is further emphasized because of the change involved in learning style in transition from Higher Secondary/A Level schooling to University/Professional Education level education. The learning style at school level is Strategic Learning Style characterized by motivation to be successful, to compete with others and to achieve high grades. The learning processresults in

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patchy and variable understanding. In contrast University/Professional education requires deep learning style in which student is motivated by interest in the subject, vocational relevance and personal understanding. It involves processes of identifying general principles, relating ideas to evidence and integrating material across courses.[5]

In Pakistan admission to medical school still remains the dominant desire among Secondary School and A Level students therefore the learning style in school is strategic learning. Majority of the medical schools are still following traditional subject based curriculum and old assessment methods and hence no change is required in learning style of the students to be successful. At Islamic International Medical College reformed integrated curriculum and new methods of assessment were introduced in 2009 and one 5 years course has been completed. This curriculum demand deep learning style from the students who have to struggle hard to meet this demand. Hence it was important to evaluate the changes in students' perception of new assessment methods and the time which they took to acquire these changes in order to improve the curriculum design, implementation methodology and learning environment to make their education more relevant and effective. With the above mentioned objectives this quantitative cross-sectional analytical study was designed and conducted among four years (2nd year to 5th year) of MBBS students of Islamic International Medical College (IIMC).

Materials and Methods

This was a quantitative cross sectional study using a structured, pre-validated questionnaire. The questionnaire had three parts; one with 5 questions recorded the study habits/effort by the students, second of 8 questions recorded students' perceptions towards formative assessment and the third with 15 questions dealt with perceptions towards summative assessment, total of 28 questions (Annex A). The questionnaire was administered to all present students of 2nd to 5th year (100 students each) and collected back after completion by them. Data was entered in MS Excel sheet. For meaningful comparison 2nd and 3rd year (first half of the program) students have been grouped together (Group A) and 4th year and 5th (second half of the program) have been grouped together (Group B). Those responding as strongly agree and agree were pooled together and those who responded as disagree and strongly disagree were collected together. Those who were uncertain were excluded. Results were analyzed using SPSS 17.0. One sample binomial test was applied and significance was calculated to three places of decimal.

Results

Study Habits/Effort (Table I): Responses to question 1 and 3 (complimentary questions) suggest that majority of the students still are in the habit of giving more time to studies near the assessment and are not uniformly committed to regular study hours as demanded by deep learning style. Similarly results did not reveal any significant change in perception towards selective study to pass summative assessment.

Table I: Question Regarding Study Effort

Q	2ND YEAR		3RD YEAR		C	
	A	D	A	D	A	D
1	32.53	51.81	32.14	50.00	32.34	50.90
2	43.37	44.58	40.48	44.05	41.92	44.31
3	87.95	9.64	86.90	9.52	87.43	9.58
4	53.01	32.53	52.38	30.95	52.70	31.74
5	37.35	51.81	35.71	47.62	36.53	49.71

Q	4TH YEAR		FINAL YEAR		C	
	A	D	A	D	A	D
1	36.78	49.43	22.67	57.33	29.72	53.38
2	43.68	36.78	24.00	46.67	33.84	41.72
3	79.31	5.75	85.33	10.67	82.32	8.21
4	54.02	22.99	48.00	42.67	51.01	32.83
5	41.38	29.89	42.67	41.33	42.02	35.61

Response to question 5 indicates some change in desire to work for a position in the class, while progressing in the program, but the change is not significant statistically. It implies that Strategic Learning Habits acquired at Higher Secondary School and A Level education still prevail and students have not adapted to deep learning style required for effective implementation of the new curriculum.

Formative Assessment (Table II): The level of interest in formative assessment remained high from the beginning and was maintained throughout the program (responses to Q1). Majority of the students

is satisfied with diversity of methods of formative assessment (Q2) as well throughout the program. Satisfaction with feedback (Q3) diminished significantly as students progressed in the program (p=0.001 for agreement and 0.002 for disagreement).

Both groups overwhelmingly agree that they take feedback seriously to remedy their weaknesses (Q4). This also supported by response to Q5 by group A. However significantly higher number of group B students tend to take marks seriously as well, (p=0.029 and 0.035) for agreement and disagreement. Both groups strongly agree that formative assessment is very helpful in stimulating deep learning (Q6). Same perception is portrayed in response to Q7. The interest of students in formative assessment is further substantiated in response to Q8 where majority of students of group B do not agree with the statement that formative assessment is more frequent.

Table II: Questions Regarding Formative Assessment

Q	2ND YEAR		3RD YEAR		C	
	A	D	A	D	A	D
1	89.16	8.43	85.71	3.57	87.44	6.00
2	78.31	10.84	80.95	10.18	79.63	10.51
3	67.47	22.89	71.43	22.62	69.45	22.76
4	77.11	14.46	75.00	14.29	76.05	14.37
5	42.17	39.76	39.29	39.29	40.73	39.52
6	89.16	6.02	88.10	4.76	88.63	5.39
7	93.98	2.41	96.43	2.38	95.20	2.40
8	45.78	38.55	45.24	38.10	45.51	38.32

Q	4TH YEAR		FINAL YEAR		C	
	A	D	A	D	A	D
1	74.71	8.05	81.33	10.67	78.02	9.36
2	55.17	24.14	68.00	13.33	61.59	18.74
3	34.48	48.28	49.33	38.67	41.91	43.47
4	60.92	18.39	80.00	17.33	70.46	17.86
5	40.23	40.23	82.67	10.67	61.45	25.45
6	68.97	14.94	82.67	10.67	75.82	12.80
7	74.71	10.34	93.33	1.33	84.02	5.84
8	26.44	60.92	22.67	56.00	24.55	58.46

To summarize responses to this segment indicate that the students not only like formative assessment but also agree that it is more effective in deep learning and preparing for summative assessment. At the same time responses suggest to introduce more methods of formative assessment and to improve feedback mechanism.

Summative Assessment (Table III): This segment comprises questions mainly to evaluate assessment policy of the program. Although majority in both groups do not agree that the number of summative assessments are too many but students in group B significantly (p=0.019) surpass those in group A in this disagreement (Q1). This is probably because introduction of clinical clerkship in 4th and 5th year increases the number of summative assessments in this period. Students in both groups equally support

Table III: Questions Regarding Summative Assessment

Q	2ND YEAR		3RD YEAR		C	
	A	D	A	D	A	D
1	38.55	48.19	38.10	42.86	38.32	45.52
2	69.88	20.48	78.57	17.38	74.23	18.93
3	80.72	18.07	66.67	19.05	73.69	18.56
4	54.22	36.14	51.19	34.52	52.70	35.33
5	53.01	28.92	51.19	30.95	52.10	29.93
6	62.65	22.89	64.29	21.43	63.47	22.16
7	49.40	38.55	47.62	34.52	48.51	36.54
8	12.05	85.54	10.71	85.71	11.38	85.63
9	40.96	44.58	41.67	40.48	41.32	42.53
10	49.40	39.76	44.05	47.62	46.72	43.69
11	36.14	54.22	34.52	55.95	35.33	55.08
12	60.24	19.28	57.14	20.24	58.69	19.76
13	49.40	36.14	48.81	36.90	49.10	36.52
14	95.18	2.41	78.57	2.38	86.88	2.40
15	50.60	39.76	47.62	38.10	49.11	38.93

Q	4TH YEAR		FINAL YEAR		C	
	A	D	A	D	A	D
1	17.24	70.11	18.67	64.00	17.95	67.06
2	59.77	27.59	82.67	12.00	71.22	19.79
3	60.92	25.29	46.67	34.67	53.79	29.98
4	27.59	45.98	46.67	46.67	37.13	46.32
5	19.54	56.32	44.00	29.33	31.77	42.83
6	26.44	50.57	32.00	60.00	29.22	55.29
7	41.38	25.29	60.00	24.00	50.69	24.64
8	16.09	58.62	24.00	66.67	20.05	62.64
9	49.43	33.33	68.00	21.33	58.71	27.33
10	16.09	62.07	45.33	34.67	30.71	48.37
11	17.24	57.47	56.00	32.00	36.62	44.74
12	42.53	32.18	49.33	26.67	45.93	29.43
13	50.57	28.74	48.00	29.33	49.29	29.03
14	73.56	6.90	86.67	9.33	80.11	8.11
15	37.93	19.54	53.33	21.33	45.63	20.44

the weightage given to combined assessment (semester examination) in annual examination (Q2). Students in group B as compared to group A agree less to next three questions Q3, 4, 5 pertaining to conduct of summative assessment. This difference is highly significant (p=0.019, 0.021, <0.001 respectively). Students in group B also do not agree that there are adequate number of MCQs for each module and subject (p=<0.001). This necessitates reviewing the process of summative assessment for clinical subjects.

A significantly higher number in group A is against inclusion of other types of MCQs and SEQs (p=0.047 and 0.002 for Q7 and 8 respectively). More students in group B (p=0.02) disagree to the statement in Q9 regarding correlation of contents of OSCE with learning objectives. Significantly less number in group B (p=0.01) thinks that the time for response is adequate (Q10). Both groups are not satisfied with quality of OSCE material (Q11).

Both groups agree that the examination is fare (Q12, 13). They also agree that they learn new things while preparing for examination but are of the opinion that they tend to forget these afterwards (Q14, 15).

To summarize students are contended in general with present system of summative assessment, while at the same time they have pointed out areas requiring improvement particularly in clinical subjects.

Discussion

The purpose of assessment is understood to be to test what students have learnt. However it is now widely accepted that the students orient their learning in relation to what they are assessed. This led to formulation of well-known rule that "assessment drives learning".[6] Students attune their learning habits according to the demands of assessment which, in turn is an integral part of any educational system. In addition to learning, assessment also has an impact on how and what the teachers teach which, in turn directly affect student learning thus completing the cycle.[7] After introducing integrated modular curriculum it was imperative to evaluate students' perceptions about various aspects. This study dealt with students' perceptions towards assessment.

The efficacy of integrated modular system of medical education is greatly enhanced if we can promote deep learning style in our students. It contrasts markedly with Strategic Learning Style, hallmark of learning styles in pre-medical school education. The results of this study indicate that learning style of the students has hardly changed in the medical school as they are still studying more near the examination and doing selective studies. Study habits a learning styles acquired over a long period of schooling take their toll in the University/Professional education which demand a change in learning style. This has also been highlighted by other investigators. Endstrom et. al., have reported that students achieving high grades support the concept of studying more and selectively near the examinations.[8] Doing selective studies is also highlighted by Vos as well. He found that students give fewer study hours to those subjects which are less frequently assessed.[9]

Students should be assessed throughout the academic year at regular intervals when there is still time to intervene to improve students' learning.[10,11] Formative assessment was therefore introduced early in the program. Results of this study indicate that Formative assessment was highly favored approach towards learning. This is also concluded by other researchers.[12]

Another clear message from this study is that students take feedback seriously for improving their learning. Feedback is recognized as a single most powerful influencing factor since years as described in a review by Hattie in 1087.[13] About 89% students in this study confirm that formative assessment is very useful for preparing for summative assessment. This fact has been substantiated by other investigators as well.[14]

Regarding frequency of formative assessment, a significantly higher number of senior students are in agreement with existing frequency. Junior students (who are not tuned to frequent assessments) do not agree to that extent. This finding is supported by Ghiatau et al and Kadri et al and.[11, 15] Most of the senior students were of the opinion that OSCE correlated well with the learning objectives. This is in conformity with results of another study in which 81% students thought OSCE to be more interesting and educative.[16]

The time allowed for different stations needs to be optimal or conversely stations should be piloted

before assessments to see whether they can be undertaken in the stipulated time. Most students are of the opinion that time allocation in the OSCE was insufficient. Similar observations have been reported in other studies.[17] Students in this study have not favoured inclusion of Short Essay Questions in assessment, whereas Rafique et al have reported contrary results.[17]While in a study by Ibrahim et al 56.8% of respondents preferred MCQs for written assessment.[18]

Students' satisfaction with the overall conduct of the assessment, in comparison was greater in our study.

Conclusion

Like others this study confirms that study habits at the time of admission to medical school are difficult to change but can be modified. It also confirms that students like formative assessment mainly because of feedback as it helps them in learning as well preparing for summative assessment. It has also indicated some weaknesses in design, conduct and provision of feedback which necessitate further faculty training. Summative assessment, on the other hand, was more satisfactory for the students except that they opposed inclusion of more methods of assessment.

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REFERENCES

1. Chastonay P, Brenner E, Peel S, Guilbert FF. The need for more efficacy and relevance in medical education. Med Educ. 1996; 30: 235-8.
2. Morgan Jones RL, Wade R, Richardson JB. The motivation to learn: efficacy and relevance of the Oswestry postgraduate orthopaedic training programme. Ann R Coll Surg Engl. 1998; 80: 271-3.
3. Tyler IV, Hau M, Buxton JA, Elliott LJ, Harvey BJ, Hockin JC, et.al. Canadian medical students' perceptions of public health education in the undergraduate medical curriculum. Acad Med. 2009; 84: 1307-12.
4. Brooks JG, Brooks MG. In Search of Understanding: The Case for Constructivist Classrooms.Alexandria, Virginia USA: ASCD; 1999.
5. McManus IC, Richards P, Winder BC, Sproston KA. Clinical experience, performance in final examinations, and learning style in medical students: prospective study. BMJ 1998; 316: 345-50.
6. Biggs J. Assessment and classroom learning. A role for summative assessment? Assessment in Education 1998; 5: 103-110.
7. Havnes A. Examination and learning: An activity-theoretical analysis of the relationship between assessment and learning. Paper presented at the Learning Communities and Assessment Cultures Conference organised by the EARLI Special Interest Group on Assessment and Evaluation, University of Northumbria, 28-30 August 2002. Available at http://www.leeds.ac.uk/educol/documents/00002238.htm.
8. Endstrom DW, Nilas WN, Josephson A, Wahlgren C. A phenomenographic study of students' conception of learning for a written examination. Int J Med Educ 2015; 6: 40-6.
9. Vos P. Curriculum control of learning processes in Higher Education. 13th International Forum on Higher Education of the European Association for Institutional Research. Edinburgh 1987.
10. Swearingen R. A primer: Diagnostic, formative and summative assessment. Heritage University, 2002; 4: 1-14.
11. Ghiatau R, Diac G, Curelaru V. Interaction between summative and formative in higher education assessment: students' perception. Procedia Social and Behavioural Sciences. 2011; 11: 220-4. Labarca J.
12. Figueroa C, Huidobro B, Wright AC, Riquelme A, Moreno R . Perception of medical students about formative assessments during clinical courses. Rev Med Chil. 2014; 142: 1193-9.
13. Hattie JA. Identifying the salient facets of a model of student learning: a synthesis of meta-analyses. IJERN, 1987; 11: 187-212.
14. Jain V, Agrawal V. Use of Formative assessment as an educational tool. J Ayub Med Coll 2012; 24: 3-4.
15. Kadri HM, Al-Moamery MS, Magzoub ME, Roberts C, Vleuten CPM. Students' perceptions of the impact of assessment on approaches to learning: a comparison between two medical schools with similar curricula. International J Medical Education. 2011; 2: 44-52.
16. Sadia S, Sultana S, Fareesa Waqar F. OSCE as an Assessment Tool: Perceptions of Undergraduate Medical Students. Anaesth Pain & Intensive Care 2009; 13: 65-7.
17. Rafique H. Students' feedback on teaching and assessment at Nishtar Medical College, Multan. JPMA 2013; 63:1205-9.
18. Ibrahim NK, Al Sharabi BM, Al Asiri RA, Al Otaibi NA, Abdullah N, et al. Perceptions of Clinical Years' medical students and interns towards assessment methods used in King Abdulaziz University, Jeddah. Pak J Med Sci 2015; 31: 757-62.

Annex A: ASSESSMENT EXPERIENCE QUESTIONNAIRE

Please answer every item quickly by giving your immediate response. Circle the appropriate code to show your response to assessment. SA=Strongly Agree, A=Agree, U=Uncertain, D=Disagree, SD=Strongly Disagree

REGARDING STUDY EFFORT						
1.	I do the same amount of study each week regardless an assessment is due or not	SA	A	U	D	SD
2.	I can do well in the assessment by doing selective study	SA	A	U	D	SD
3.	In weeks when the assessment is due, I put in more hours in study	SA	A	U	D	SD
4.	I omit to prepare subjects which are likely to be minimally represented in the assessment	SA	A	U	D	SD
5.	I am not very keen on obtaining position in the class	SA	A	U	D	SD
REGARDING FORMATIVE ASSESSMENT						
1.	I take formative assessments regularly and seriously	SA	A	U	D	SD
2.	Methods used in formative assessment are multiple, diverse and stimulate learning	SA	A	U	D	SD
3.	Feedback is regularly provided by the faculty on formative assessment	SA	A	U	D	SD
4.	I take the feedback seriously to remedy my weaknesses.	SA	A	U	D	SD
5.	I tend to read the marks only	SA	A	U	D	SD
6.	Formative assessment is very help full in stimulating deep learning	SA	A	U	D	SD
7.	Formative assessment is very helpful in preparing for summative assessment	SA	A	U	D	SD
8.	There are too many formative assessments in a year	SA	A	U	D	SD
REGARDING SUMMATIVE ASSESSMEN						
1.	There are too many summative assessments	SA	A	U	D	SD
2.	Weightage of CBA is appropriate in professional result	SA	A	U	D	SD
3.	General atmosphere and conduct of examination is satisfactory	SA	A	U	D	SD
4.	The contents of the examination correlate well with the learning objectives	SA	A	U	D	SD
5.	The questions are easy to understand	SA	A	U	D	SD
6.	There are adequate number of MCQs for each module and subject in a paper	SA	A	U	D	SD
7.	There should be more types of MCQs in addition to one best/correct type.	SA	A	U	D	SD
8.	There should be SEQs in the assessment as these let us apply deep learning	SA	A	U	D	SD
9.	The contents of OSCE correlate well with the learning objective being tested.	SA	A	U	D	SD
10.	The time allotted to each station is adequate for the response.	SA	A	U	D	SD
11.	The slides and images placed on OSCE are of good quality.	SA	A	U	D	SD
12.	The observers and invigilators are impartial	SA	A	U	D	SD
13.	There is hardly any room for use of unfair means in the assessment	SA	A	U	D	SD
14.	I learn new things while preparing for assessment	SA	A	U	D	SD
15.	I forget most of what I learnt after the assessment	SA	A	U	D	SD

CASE REPORT

Hypokalemic Periodic Paralysis

Abidullah Khan[1], Muhammad Farooq[2]

ABSTRACT

Hypokalemic Periodic Paralysis is a group of rare inherited disorders that can cause temporary and often recurrent episodes of acute flaccid paralysis. Several conditions e.g. thyrotoxicosis, increased carbohydrate and salt dietare known to cause or precipitate it. A case of a 26 years age male is presented here who reported with sudden onset of muscle weakness with concomitant hypokalemia after a high carbohydrate diet. The patient's paralysis resolved upon replacement of potassium. Periodic Paralysis must be differentiated from other causes of sudden onset paralysis, so that the proper treatment can be initiated in time to prevent complications due to this rare disease.

Key Words: *Hypokalemia, Hypokalemic Periodic Paralysis, Flaccid Paralysis.*

Case Report

Male soldier 26 years of age presented to the emergency room with sudden onset weakness of all four limbs. The patient had gone to sleep after taking a heavy dinner andwoke upnext morning with inability to move all his four limbs. The weakness involved both proximal and distal muscles. There was no respiratory or swallowing difficulty. He denied any muscle pain and there were no sensory symptoms. Prior to this episode, the patient had been healthy and denied any recent diarrhea, sore throat, vaccination, shortness of breath, palpitation, weight loss or heat intolerance. There was no history of psychomotor illness. Two years ago he had experienced a similar episode of muscle weakness for which he was admitted in hospital and recovered completely after potassium replacement. Duration of attack both times was about 4-6 hours and both times it occurred after a carbohydrate-rich meal. He did not take any medications and denied use of alcohol. There was no family history of neuromuscular disease.

On examination, BPwas 130/80 mmHg, pulse74/ minute, RR14/minute, temperature 99F. Neurological examination revealed flaccid paralysis of all limbs with power 2/5. Deep tendon reflexes were overall diminished and plantars were normal. There was no sensory loss, cranial nerve involvement, bladder/bowel or higher function disturbance. There were no signs of hyperthyroidism and rest of the systemic examination was un remarkable.

Baseline investigations such as blood complete picture, urinalysis, random blood sugar, serum urea, serum creatinine and liver enzymes were all normal. Serum electrolytes revealed hypokalemia with a potassium level of 3.2 mmol/l; however serum sodium, calcium, magnesiumand phosphate levels were normal. Creatinine phosphokinase(CK) levels, thyroid profile, serum Aldosterone / Renin and 24 hour urinary sodium and potassium levels were also sent and were subsequently found to be normal. The patient was meanwhile managed in ICU with potassium replacement and cardiac monitoring. Within few hours his symptoms resolved completely and muscle power was 5/5. A repeat electrocardiogram revealed a normal sinus rhythm and rate with normal T waves.

Based on the typical presentation and the dramatic response to potassium replacement, a clinical diagnosis of hypokalemic periodic paralysis (HypoKPP) was made. Considering the nature of his job, he was counseled in detail regarding trigger identification, such as avoiding carbohydrate-rich meals, long distance travel and strong physical exercise, in order to prevent future attacks.

Discussion

Hypokalemic paralysis is characterized by episodes of acute muscle weakness associated with hypokalemia.[1] Possible etiological factors include

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decreased potassium intake, urinary losses (e.g. primary/secondary aldosteronism, cushing's syndrome, diuretics, hypomagnesemia, renal tubular acidosis etc), GI losses (e.g. diarrhea, vomiting etc.), and potassium shift into the cells (e.g. by insulin, alkalosis or periodic paralysis). Hereditary defects causing hypokalemia are Bartter's syndrome and Gitelman syndrome. In this case, secondary causes of hypokalemia and thyrotoxicosis were sequentially ruled out on the basis of clinical evaluation and battery of laboratory investigations. Although desirable, NCS/EMG studies were not available at our facility. Hencea clinical diagnosis of HypoKPP was made on the basis of typical presentation and recurrent episodes triggered by high carbohydrate diet.

HypoKPP is a genetic disorder/channelopathy in which affected individuals (majority of them young males) may experience episodes of acute flaccid paralysis with concomitant hypokalemia. The paralytic attacks are reversible, usually leading to paraparesis or quadriparesis. They can last several hours and sometimes days. Some individuals have only one episode in a lifetime; more commonly, crises occur repeatedly. The major triggering factors are carbohydrate-rich meals and rest after strenuous exercise; hence symptoms frequently occur early morning on awakening. Other triggers include stress, viral illness, fatigue, and medications such as beta-agonists, insulin or steroids. HypoKPP is caused by mutations in the genes that control the development and function of certain ion channels in the muscle membrane.[2] Although the serum potassium level is often low, other electrolytes usually remain normal. Infact, total body potassium is actually normal with the change in the serum level merely reflecting an acute shift of potassium into cells.[3] Many variants are recognized including thyrotoxic periodic paralysis (TPP) which is mainly observed in Asians and familial periodic paralysis.[1-5] They are of sudden onset and sometimes even with life-threatening respiratory failure. As this is primarily a problem with muscle contraction rather than nerve conduction, tendon reflexes are diminished but sensation is intact. Electrocardiographic changes are common, but changes do not correlate well with the measured serum level. Diagnosis in between the paralytic episodes is difficult as the patient will have normal strength and potassium levels. Electromyography may reveal abnormalities in some patients but is often normal especially in between the episodes.[2-5]

Management of HypKPP includes potassium replacement and identifying and avoiding triggers of attack. Potassium may be replaced orally or intravenously depending on potassium levels and clinical condition. When given intravenously the rate of potassium administration should not exceed 20 mmol/hour. To calculate the amount of potassium supplementation one should have an estimate of potassium deficit. On average a reduction of serum potassium by 0.3 mmol/L suggests a total body deficit of 100mmol/L.[2,4-6] Biochemical parameters including electrolytes such as potassium, sodium, magnesium, calcium and phosphate, ECG and cardiac rhythm monitoring are essential components of management of HypoKPP. Although completely reversible on potassium replacement in the majority of the affected individuals, some of the patients with HypoKPP may develop myopathy leading to exercise intolerance in later life; others may be at increased risk of post anaesthetic weakness.

Conclusion

A high index of suspicion is required for the timely diagnosis of HypoKPP in any patient presenting with acute flaccid paralysis. Identifying and avoiding triggers of attack are integral component of the management of HypoKPP.

REFERENCES

1. Sharma CM, Nath K, Parekh J. Reversible electrophysiological abnormalities inhypokalemicparalysis: Case reportof two cases.Ann Indian Acad Neurol. 2014; 17: 100-2.
2. Mukhtar H, Ahmad S, Javaid A, Qayyum K. Hypokalemic periodic paralysis. Ann King Edward Med Uni 2006; 12: 598-9.
3. Suetterlin K , Mannikko R, Hanna MG. Musclechannelopathies: recent advances in genetics, pathophysiology and therapy. CurrOpin Neurol 2014; 27: 583-90.
4. Boissier E, Georgin-Lavialle S, Cochereau D. Thyrotoxicperiodicparalysis: a case series of four patients and literaturereview. Rev Med Interne 2013; 34: 565-72.
5. Alkaabi JM, Mushtaq A, Al-Maskari FN. Hypokalemic periodic paralysis: a case series, review of the literature and update of management. Eur J Emerg Med 2010; 17: 45-7.
6. Soule BR, Simone NL. Hypokalemic Periodic Paralysis: a case report andreviewof the literature. Cases J. 2008; 1: 256.

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