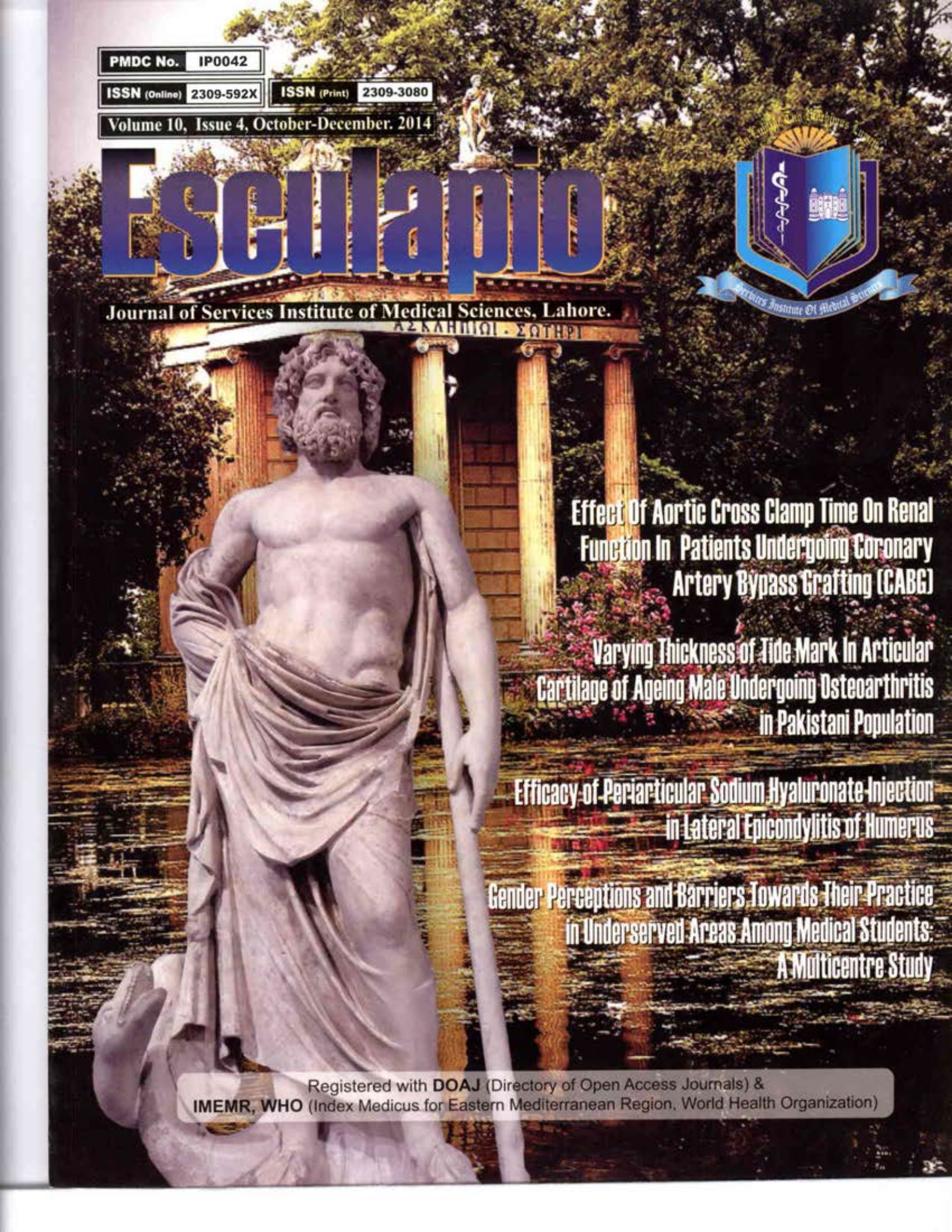
PMDC No. IP0042

ISSN (Online) 2309-592X ISSN (Print) 2309-3080

Volume 10, Issue 4, October-December. 2014

Esculapio

Journal of Services Institute of Medical Sciences, Lahore.



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ESCULAPIO

JOURNAL OF SERVICES INSTITUTE OF MEDICAL SCIENCES, LAHORE.

VOLUME. 10 OCTOBER - DECEMBER 2014 ISSUE. 04

PUBLISHED BY

SERVICES INSTITUTE OF MEDICAL SCIENCES, LAHORE.

ONLINE EDITION

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Original Article

EFFICACY OF PONSETI METHOD IN MANAGEMENT OF CLUB FOOT

Rana Dilawaiz Nadeem, Mohammad Arif, Shafqat Wasim, M. Tasneem Javed and Ali Raza Hashmi

Objective: To determine the efficacy of ponseti method in the treatment of club foot.

Material & Methods: This descriptive case series was conducted at Department of Orthopaedic Surgery, Services Institute of Medical Sciences (SIMS) / Services Hospital Lahore. Sample size of 100 was calculated with 95% confidence using non probability purposive sampling technique. Children of age 6 month to 1 year with club foot of either sex were included, while those with any associated congenital anomaly were excluded. Children with idiopathic club foot having skin disease and pressure sore due to POP cast were also excluded. All children had six casts at weekly interval by Ponseti's technique. Prior to the fifth cast percutaneous Achilles tenotomy was done under local anesthesia if dorsiflexion was not possible beyond neutral. Following the removal of the last cast all the feet were placed in a Foot Abduction Orthosis (AFO). Sixth months after the completion of plaster treatment all feet were assessed by Pirani score.

Results: A total of 100 children were included in the study. The mean age was 5.78 ± 3.21 months. Majority of the patients were between 0-9 months of age i.e. 63% (n=63), and between 10-12 months 37% (n =37). Male to female ratio was 1.127:1. Percutaneous Achillies tenotomy was done in 82% of cases. Pirani score six months after cast treatment was calculated which showed 88% (n=88) children had good results and 12 % (n=12) didn't have effective results. Efficacy of Ponseti method in the treatment of club foot reveals Pirani score <1 in 88% (n =88) while >1 in 12% (n=12). Stratification for efficacy of Ponseti method with regards to age before treatment reveals that out of 88 cases 68.18% (n=60) were between 0-9 months of age and 31.82% (n=28) were between 10-12 months of age. Stratification for efficacy of Ponseti method with regards to initial Pirani score before treatment reveals that out of 88 cases 88.64% (n=78) had < 4 and 11.36% (n =10) had >4 Pirani score.

Conclusion: We concluded that Ponseti method is highly effective for the management of club foot in children up to 12 months of age and need for extensive corrective surgery is greatly reduced. We recommend the Ponseti method as standard therapy in clubfoot management.

Key words: Clubfoot, Congenital talipes equinovarus, pirani scoring, ponseti method.

Introduction

Clubfoot or Congenital talipes equinovarus (CTEV) is a multifaceted deformity of the foot, in which one or both feet are excessively plantar flexed, with the forefoot swung medially and the sole facing inward.[1] The prevalence of clubfoot in developing countries is estimated to be approximately one in 1000 births.[1] This condition occurs during development in the womb so it is one of the commonest congenital conditions requiring treatment.[2] The male-to-female ratio is 2:1. Bilateral involvement is found in 30-50% of cases. There is a 10% chance of a subsequent child being affected if the parents already have a child with a clubfoot.[3]

The incidence varies considerably between races. Clubfoot is particularly rare, for example, among Chinese and Japanese (approx. 0.5/1000), but common in black people (3.5/1000 in South Africa), Australian Aborigines (3.5/1000) and Polynesians (6.8/1000).[4]

Treatment for clubfoot should begin almost immediately after birth to have the best chance for a successful outcome without the need for surgery.[5] Recently there has been an enthusiastic embracing of the Ponseti technique. Recent research has improved and refined the Ponseti technique that must now be appreciated and incorporated by clinicians. This technique is used across the world in both developed and developing countries and is universally regarded as the best management method for clubfoot deformities.[6,7] The purpose of this study was to access the early outcome of the management of club foot with Ponseti technique using Pirani scoring system.

Material & Methods

This descriptive case series was conducted at department of Orthopaedic Surgery, Services Institute of Medical Sciences (SIMS)/ Services Hospital, Lahore. Sample size of 100 was calculated with 95%

confidence, 7% margin of error and assumed percentage of efficacy 85% (i.e., Pirani score 1 or <1) of Ponseti method in the management of club foot. The data was taken using non probability purposive sampling. In this study we included 100 children of either sex with age of 6 months to 1 year with clubfoot. Patients having any other congenital anomalies that lead to club foot like myelomeningocele and babies with idiopathic club foot having skin disease and those having pressure sores due to POP cast were excluded. Clubfoot was diagnosed clinically by the consultant orthopaedic surgeon in the outpatient department. Their parents were informed about the treatment and its possible outcome. Informed consent was obtained from the parent of the infants. All manipulations, casts and tenotomies were done by the surgeon himself. Pirani score was applied before manipulation and then first cast was applied in theater without anesthesia. Weekly cast was applied after the manipulation and stretching by Ponseti technique and Pirani score was applied before each cast. Prior to the fifth cast percutaneous Achilles tenotomy was done if dorsiflexion was not possible beyond neutral. If tenotomy was required then this cast was applied for three weeks that allowed the tendon to regenerate. Following the removal of the last cast the foot was placed in Foot Abduction Orthosis (FAO). The orthosis was used on full time basis for first three months and at night for last three months. Six months after the removal of the last cast all feet were assessed by final Pirani Score. The score of 1 or <1 was considered as good outcome and >1 was considered as poor outcome. All collected data was entered into SPSS (Statistical Package for Social Sciences) version 18 and analyzed descriptively. Frequency and percentages were calculated for qualitative variables like gender and efficacy (i.e., Pirani score 1 or <1), while mean and standard deviation was calculated for quantitative variables like patient age, Pirani score. Data was stratified for Pirani score uphill 4 at initial presentation.

Results

A total of 100 cases fulfilling the inclusion/ exclusion criteria were enrolled to determine the efficacy of Ponseti method in the treatment of clubfoot. Majority of the patients i.e. 63% (n=63) were between 0-9 months of age, 37% (n =37) were between 10-12 months. The mean age was 5.78 ± 3.21 months. Males were 53% (n = 53) while females were 47% (n =47). Pirani score after treatment was calculated, which showed 88% (n =88) children had ≤ 1 score and 12% (n=12) had >1 score, mean ± SD was calculated as 0.64 ± 0.36. So, Ponseti method was found effective in the treatment of clubfoot in 88% (n=88) while 12% (n=12) did not show effective result. Stratification for efficacy of Ponseti method with regards to age reveals that out of 88 cases 68.18% (n=60) were between 6-9 months of age and 31.82% (n=28)

Table-1: Demographical data and efficacy of treatment.

		Categories	Percentage
Age distribution (months) [n=100]		0-9	63 (63%)
		10-12	37 (37%)
Gender [n=100]		Male	53 (53%)
		Female	47 (47%)
Pirani score after treatment [n=100]		≤ 1	88 (88%)
		> 1	12 (12%)
Efficacy of procedure [n=100]		Yes	88 (88%)
		No	12 (12%)
Stratification for efficacy of procedure [n=88]	**Age in months at start of treatment**	6 - 9	60 (68.18%)
		10 - 12	28 (31.82%)
	Pirani score at start of treatment	≤ 4	78 (88.63%)
		> 4	10 (11.36%)

were between 10-12 months of age. Stratification for efficacy of Ponseti method with regards to Pirani score before treatment reveals that out of 88 cases 88.64% (n=78) had <4 while 11.36% (n=10) had >4 Pirani score.

Discussion

Clubfoot is a common congenital anomaly in the neonate.[9] This deformity is difficult to treat, having a marked tendency to recur & causes a real disability.[10] Neglected clubfoot is common, disabling and contributes to poverty in developing nations. The management of clubfoot is conservative at early age by serial casts.[3] There are still reports of early recurrence of the deformity, and it is likely that a small number of clubfeet will require surgery even after expertly applied non-operative treatment.[11-16] The main objective of the treatment for clubfoot is to obtain pain free, plantigrade foot with good mobility and without callosities.

The Ponseti clubfoot treatment has high efficacy in correcting the clubfoot deformity but is demanding on parents in developing nations and healthcare system. Its effectiveness as the best method of care remains unknown. The Ponseti method requires fewer casts and shorter duration of casting to achieve correction. Tenotomy of the Achilles tendon enables better ankle dorsiflexion. The incidence of residual deformity and recurrence is also reported but it is lower using the Ponseti method.

Our results are in agreement with a local study conducted by Din et al who found that 81.24% had excellent results with Ponseti's method.[17] Another study conducted by Mukhdoom et al shows that efficacy (Pirani score 0-0.5) was achieved in 97.18% cases at one year followup.[8] Our results regarding, efficacy according to age group are in contrast as we recorded significantly lower efficacy rate in 9-12 months of age. Makhdoom et al included the cases of all ages i-e. <1 month to 36 months but did not stratify the results to see the effects of age (early and late presentation). Moreover as far as efficacy is concerned, we assume it to be 85% in older children.

It was observed that if the initial Pirani's score was > 4 it is likely that this foot will require percutaneous Achilles tenotomy and it was required in 82% of cases.[23] In a series by Laaveg and Ponseti[19] 78% and Dobbs & Gurnett [2] performed tenotomy in 91% cases.

Ponseti technique has been reported having 92- 98% successful results for the treatment of the clubfoot.[13] Studies have shown that after adopting this technique the need for surgery has dropped from 94% to 3%.[22] Since 2002, several studies have demonstrated the successful use of Ponseti method in club foot correction, so much that this method is now becoming an ideal treatment of idiopathic clubfoot all over the world[18]. Laaveg and Ponseti reported that 90% of their patients were satisfied with the function and appearance of the feet.[19] It has been written rather convincingly by Cooper & Dietz that Ponseti method is more effective in treating congenital clubfoot non-operatively.[20]

This method has reported effective not only in clinical correction, but has also shown to correct the individual tarsal anlagen and their relationship seen on magnetic resonance imaging.[21] In our study it became evident that the success rate with Ponseti's method was significantly higher in our settings also. It corrected severely affected feet in a significantly shorter time period, thereby reducing the agony and distress to children as well as their parents. Superior results may be attributed to correcting all deformities simultaneously; the correction of cavus in the supinated position is called the magic move of Ponseti.[22] In future analysis of long term results (function and appearance) of the patient corrected by Ponseti method may be conducted for further evidence of higher success rate in Ponseti method.

Conclusion

We concluded that Ponseti method is highly effective for the management of clubfoot in children up to 12 months and need for extensive corrective surgery is greatly reduced. We recommend Ponseti method as standard therapy in clubfoot management.

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Original Article

ROLE OF CECAL GURGLING IN DIAGNOSIS OF ACUTE APPENDICITIS

Ghulam Mustafa, Aasim Malik and Ghazia Qasmi

Objective: To assess the role of Cecal Gurgling (CG) as a clinical sign to diagnose acute appendicitis.

Material & Methods: This study was conducted at the Allied teaching hospitals of FMH College of Medicine and Dentistry Shadman Lahore, between June 2011 and May 2013. Two hundred patients were included in the study between 13 and 50 years of age, presenting with pain right iliac fossa, irrespective of gender. All the patients were grouped according to the Alvarado scoring system into two groups; group I with score more than 7 and group II score less than 7, which was further subdivided onto CG positive and CG negative.

Results: Mean age of the patients was 18.5 years (13-50). 104 (52%) patients were male and 96 (48%) female. In Group I, 78 (93.97%) were confirmed to have acute appendicitis on histopathology (HP) and 5 (6.02%) were normal. In group II CG positive, 67 (95.7%) patients were confirmed to have acute appendicitis on HP.

Conclusion: We concluded from this study that CG is a good diagnostic sign in patients with borderline diagnosis and can be used in conjunction with Alvarado score to increase its yield. However, large randomized trial is required to strengthen this important clinical sign.

Keywords: Cecal gurgling, Acute appendicitis, Alvarado score.

Introduction

Acute appendicitis is a very common disease. It can cause great difficulty for patient and surgeon especially when the diagnosis is not clear.[1] It remains one of the most common diseases treated by the general surgeons. Appendectomy is the most commonly performed emergency surgery in industrialized countries. The incidence of acute appendicitis is much less in areas of Africa, Asia, and South America, probably due to difference in diet and lifestyle.[2] The pathophysiology of acute appendicitis has long been thought to be the result of luminal obstruction by a fecolith, hyperplastic lymphoid tissue, parasitic infestation, or tumor, with subsequent localized venous ischemia resulting in mucosal disruption followed by invasive bacterial infection. Infection limited to the appendix itself results in localized inflammation and simple, or suppurative, appendicitis.[2] Progression to full thickness necrosis and gangrene of the appendiceal wall may result in complications of appendicitis e.g., free perforation, abscess formation if the process is contained by adjacent structures, or even fistula formation if the inflammatory process continues unabated.[3]

The diagnosis of appendicitis has long been thought to be clinical. The definitive description of the clinical findings of acute appendicitis was made by Fitz in 1886[4] and McBurney's report to the New York Surgical Society in 1899.[5] A classic clinical presentation of acute appendicitis starts with the onset of poorly localized abdominal pain that eventually localizes to the right lower quadrant; typically, the pain becomes increasingly severe and constant. It is usually associated with nausea, anorexia and occasional vomiting. The presence of hunger and fever usually rules out appendicitis. Recently the scoring system like Alvarado[6,7] got popularity for the diagnosis of acute appendicitis. With the advent of newer radiographic modalities, the diagnosis of acute appendicitis has become increasingly controversial, and continues to evolve. CT scan[8] seems to be diagnostic modality, however this is not always available in our setup and high cost of scanning keeps us away from its routine use. Keeping in view these difficulties, we studied the role of Cecal Gurgling (CG) as a diagnostic tool in the emergency settings where the diagnosis was in doubt.

Material & Methods

This descriptive study was conducted at the allied teaching hospitals of FMH College of Medicine and Dentistry Shadman Lahore between June 2011 to May 2013. Over a period of 2 years 200 patients were included in the study. All the patients between 13 and 50 years of age, presenting with pain right iliac fossa, were included in the study irrespective of gender. Patients having co-morbid conditions or obvious

cause of pain abdomen were excluded from the study. CBC, urine complete and ultrasound abdomen were done for all patients, whereas ECG, chest x-ray, LFTs, RFTs, and serum electrolytes for patients more than 40 years of age for anesthesia fitness.

All the patients were analysed clinically and grouped according to the Alvarado scoring system into two groups. Group I with score more than 7 and group II score less than 7. For all the patients with score more than 7 appendicectomy was done and specimen sent for histopathology as gold standard post operative diagnostic tool.

The group II patients were further subdivided into two categories, (a) with positive CG and (b) with negative CG. The patients with negative CG were discharged with follow up instructions and positive CG were operated and appendix were sent for histological confirmation. The data was recorded on preformed proforma and results anlaysed using SPSS 17.

Results

Mean age of the patients was 18.5 years (13-50) and gender distribution of patients is shown in **Table 1.** There were 83 patients in group I and 117 in group II. Among 117 of group II, 70 were CG positive hence operated. In group I, 78 (93.97%) were confirmed to have acute appendicitis on histopathology (HP) and 5(6.02%) appendix were normal. In group II with positive CG, 67 (95.7%) were confirmed to have acute appendicitis on HP, **Table 2.**

Other diagnoses (other than appendicitis) during surgery were Meckel's diverticulum, mesenteric lymphangitis and acute salpingitis.

Table-1: Gender distribution of patients.

Gender	Male	Female	Total
Group - I	45 (54.21%)	38 (45.78%)	83
Group - II with CG	33 (47.14%)	37 (52.86%)	70
Group II without CG	26 (55.32%)	21 (44.68%)	47

Table-2: Histopathological distribution of patients.

Groups	HP Positive	HP Negative	Total
Group - I	78 (93.97%)	5 (6.02%)	83
Group - II with CG	67 (95.7%)	3 (4.3%)	70

Table-3: Alvarado score for acute appendicitis

Features	Score
Migration of pain	1
Anorexia	1
Nausea	1
Tenderness in right lower quadrant	2
Rebound pain	1
Elevated temperature 37.3°C	1
Leucocytosis >10,000	2
Shift of white blood cell count to the left	1

Discussion

Acute appendicitis is a common surgical condition encountered in surgical emergencies and is often the first procedure to be done by surgical interns and residents. The diagnosis is purely clinical and can be diagnosed up to 97% when proper history and examination is done.[2] Unfortunately, atypical clinical presentations are not uncommon. Abdominal tenderness may be absent or minimal in early appendicitis. Very young and very elderly patients are notorious for atypical or delayed presentation. Diarrhea or urinary symptoms related to inflammation of adjacent structures may mimic other disease processes. Negative or equivocal studies do not rule out appendicitis. Because of the significant increases in morbidity and mortality associated with perforation, a certain rate of negative appendectomies is acceptable,[9] especially in those with atypical presentations.

Rates of negative appendectomies have been reported in the literature from as low as 5% to as high as 40%. We see in our study very low negative appendicectomies probably due to (a) late presentation by patients (b) self medication & (c) multiple surgical consultations.

Due to excellent (over 95%) accuracy rates associated with abdominopelvic computed tomography (CT), some have advocated routine use of CT for all patients with possible acute appendicitis, even questioning the utility of any clinical assessment.[10] A prospective randomized comparison of a scoring system using traditional clinical markers versus CT failed to show a statistically significant difference in accuracy rates.[10] We noted in our study that if combined with Alvarado score the diagnostic accuracy of CG was comparable to CT alone, although the role of CT cannot be challenged, especially in atypical

cases. The high cost of CT precludes it as routine investigation in our setup. So on the basis of our observations we can confidently say that CG combined with Alvarado is the best available modality to diagnose acute appendicitis.

Conclusion

We concluded from this study that CG is a good diagnostic sign in patients with borderline diagnosis and can be used in conjunction with Alvarado score to increase its yield. However, large randomized trial is required to strengthen this important clinical sign.

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Original Article

Effect of Aortic Cross Clamp Time on Renal Function In Patients Undergoing Coronary Artery Bypass Grafting (CABG)

Usman Javed Iqbal, Majid Kaleem, Tahira Kanwal and Hamid Hassan

Objective: To see the effect of aortic cross clamp time on renal function in patients undergoing CABG post operatively.

Material & Methods: In a prospective study 90 patients were selected for CABG first time with normal renal function. A questionnaire was made to see the effect of aortic cross clamp time on post operative renal function. Demographic factors, pre-operative, intra-operative and post operative variables were evaluated. The patient were divided into two groups according to aortic cross clamp time, patient with aortic cross clamp time 50 minutes and patients with aortic cross clamp time > 50 minutes; ultimately they were evaluated to see the occurrence of acute kidney injury (AKI).

Results: AKI was observed in 6 patients with aortic cross clamp time ≤ 50 minutes and 36 patients with aortic cross clamp time > 50 minutes. The aortic cross clamp time was highly associated with blood urea post operatively at day 1 (p = 0.002), day 2 (p=0.000) and day 3 (p=0.000). It had significant effects on serum creatinine postoperatively day 1 (p=0.000), day 2 (p=0.005) and day 3 (p=0.001). It also had significant effect on reduction of GFR post operatively day 1 (p= 0.011), day 2 (p=0.003) and day 3 (p=0.001).

Conclusion: The greatest likelihood of developing acute kidney injury (AKI) after CABG was observed with prolonged aortic cross clamp time. The levels of blood urea and serum creatinine were increased in patients with aortic cross clamp time > 50 minutes. Glomerular filtration rate (GFR) was reduced with aortic cross clamp time of more than 50 minutes.

Key words: Cardiopulmonary bypass, acute kidney injury, aortic cross clamp, ischemic period

Introduction

Coronary artery disease (CAD) is narrowing of coronary arteries which supply oxygen and nutrients to the heart. Three fourths of global deaths due to CAD occur in the low and middle income countries.[1] There are three methods of intervention of CAD: Medical treatment, percutaneous coronary intervention PCI and CABG.[2] Randomized trials proved that CABG is superior to both of these therapeutical regimen.[3] The aorta at abdominal or thoracic level is clamped to provide dry operative field with good visibility during surgical intervention; however it is complicated by ischemia of lower extremities and vital organs such as kidneys. The high complication rate results in part from pathophysiologic disturbances that occur during cross clamping and unclamping of the aorta. The duration of aortic cross clamp not only affects some vital organs but also the overall results of surgical intervention.[4]

Acute kidney injury (AKI) after cardiac surgery is a major health issue. Lacking effective therapies, risk factor modification may offer a means of preventing this complication.[5] Acute renal failure (ARF) is a recognized complication following cardiac surgery. ARF was defined as doubling of serum creatinine concentration to >0.13mmol/L if serum creatinine was <0.13 mmol/L pre-operatively, or else a rise in serum creatinine of 2:0.10 mmol/L after cardiac surgery.[6] Some data indicate that 10 to 20% of patients who undergo CABG have a serum creatinine of more than 1.5 mg/dl.[7] Renal dysfunction is an important predictor of outcome in terms of in hospital mortality, morbidity, and midterm survival in patients undergoing CABG.[8] ARF occurs in up to 30% of patients who undergo cardiac surgery, with dialysis being required in approximately 1% of all patients. The development of ARF is associated with substantial morbidity and mortality independent of all other factors. The pathogenesis of ARF involves multiple pathways. Hemodynamic, inflammatory and nephrotoxic factors are involved and overlap each other in leading to kidney injury. And one of them is prolonged aortic cross clamp time.[9] The purpose of this study was to facilitate the understanding the pathophysiologic derangements in kidneys during clamping and unclamping of aorta and to provide a basis for rational therapy to reduce the complications and improve the outcome.

Material & Methods

It was a prospective longitudinal study of 90 patients undergoing CABG in Punjab Institute of Cardiology with three days follow-up after surgery. Study was completed in six months. We used non probability purposive sampling. All patients from age 30 years onwards regardless of gender who underwent CABG were included in the study. Reoperation and all those patients that were having previous renal function impairment were excluded from the study. Patients were followed up for three days after surgery. In addition to collecting basic demographic details, postoperative creatinine, urea levels, GFR and other related perfusion parameters were noted down on a short structured questionnaire. Patients were asked about the presence of hypertension and diabetes mellitus. Information was also obtained regarding smoking and history of heart disease in first degree relatives. Patients were divided into two groups i.e; those with aortic cross clamp time ≤50 min and those with aortic cross clamp time >50 min. Both descriptive and inferential statistical analyses were done in Statistical Package for Social Sciences SPSS version 16.0. Categorical data were presented as percentages and in form of graphs while descriptive and frequency distribution was used for quantitative analyses. Independent sample t-test was used to compare the means of two groups in pre & post-op conditions. p-value ≤ 0.05 was considered as significant.

Results

The mean age of patients who underwent CABG, was 54.36±9.8 years with a female predominance 40% males and 60% females. Out of 90 patients, 36 (40%) were diabetics, 56 (62.2%) were hypertensive, 34 (37.8%) were smokers and 20 (22.2%) had a strong family history of heart disease. Mean cardiopulmonary bypass (CPB) time was recorded as 97.5±45.1min. All patients were perfused with the mean pressures of 60.5±10.2 mmHg. Out of 90 patients 38 were having aortic cross clamp time ≤50 minutes and 52 were having aortic cross clamp time >50 minutes. Of 38 patients with cross clamp time ≤ 50 minutes; AKI was observed in only 6 patients while in patients with aortic cross clamp time >50 minutes AKI was observed in a significant number of patients i.e. 34 out of 52; as shown in **Fig-1.**

With respect to urea levels there was a significant effect of aortic cross clamp time in successive postoperative days 1, 2 and 3. Patients with aortic cross clamp time ≤ 50 minutes had mean blood urea

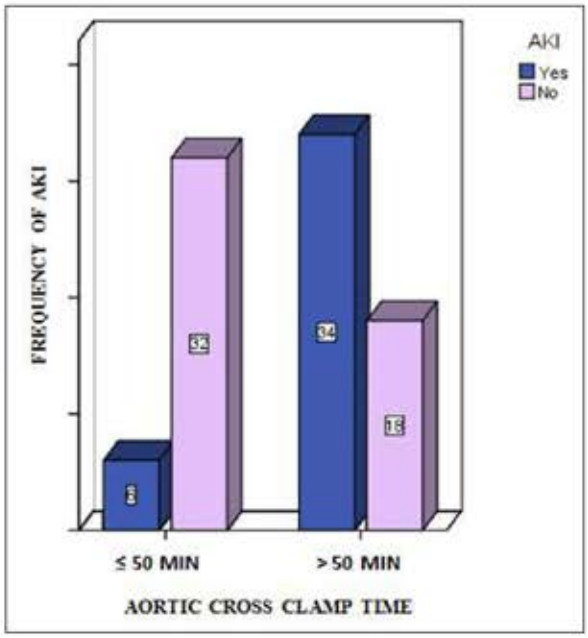

Fig-1: Frequency of acute kidney injury with respect to cross clamp time.

levels of 38.10±19.27, 47±22.58 and 47.05±24.60 mg/dl for day 1, 2 and 3 respectively. For patients with aortic cross clamp time >50 minutes mean blood urea levels were 53.16±25.91, 78.5±50.64 and 77.34±35.183 mg/dl for postoperative day 1, 2 and 3 respectively. The p-values 0.002, 0.000 and 0.000 for these respective days are significant.

Similarly for serum creatinine levels there was a significant effect of aortic cross clamp time in successive first three postoperative days. Patients with aortic cross clamp time ≤ 50 minutes had mean serum creatinine levels of 1.18±0.44, 1.33±0.87 and 1.29±0.78 mg/dl for postoperative day 1, 2 and 3 respectively. For patients with aortic cross clamp time > 50 minutes mean serum creatinine levels were 1.58±0.55, 1.80±0.56 and 1.81±0.49 mg/dl for post-operative day 1, 2 and 3 respectively. The p-values 0.002, 0.000 and 0.000 for these respective days signify an association between aortic cross clamp time and serum creatinine levels.

With respect to GFR there was also a significant effect of aortic cross clamp time in first three successive postoperative days. Patients with aortic cross clamp time ≤50 minutes had mean GFR 76.24±35.25, 76.74±43.53 and 73.69±34.39 mL/min/1.73m^2 for postoperative day 1, 2 and 3 respectively. For patients with aortic cross clamp time >50 minutes mean GFR was 59.13±21.76, 52.24±23.17 and 50.86±23.11 mL/min/1.73m^2 for postoperative day 1, 2 and 3 respectively. The p values

Table-1: Comparision of two groups in different postoperative days.

		X-Clamp time< 50 min Mean± S.D	X-Clamp time > 50 min Mean± S.D	p value
Day-1	Urea	38.1±19.2	53.1±25.9	0.002
	Creatinine	1.18±19.2	1.5±0.55	0.000
	GFR	76.2±35.2	59.1±21.7	0.011
Day-2	Urea	47±22.5	78.5±50.6	0.000
	Creatinine	1.33±0.87	1.80±0.56	0.005
	GFR	76.7±43.5	52.2±23.1	0.003
Day-3	Urea	47±24.6	77.3±35.1	0.000
	Creatinine	1.29±0.78	1.81±0.49	0.001
	GFR	73.6±34.3	50.8±23.1	0.001

p-value ≤ 0.05 significant

0.002, 0.000 and 0.000 for these respective days were significant.

Discussion

Acute kidney injury is a major complication after cardiac surgery.[10] According to another research risk factors associated with postoperative ARF were advanced age, diabetes mellitus, hypertension, high preoperative serum creatinine levels, impaired left ventricular function, urgent operation or reoperation, concomitant procedures, low cardiac output state, re-exploration for bleeding or pericardial tamponade and prolonged cardiopulmonary bypass CPB and aortic cross clamp periods.[11] The causes of renal dysfunction are multifactorial with cardiopulmonary bypass[6] producing harmful effect on renal function.[12] The non physiological state of cardiopulmonary bypass triggers inflammatory cascade and coagulation disorders that change renal function. Renal dysfunction preoperatively affects long term survival.[8] Recently it has been discussed that not using the CPB during surgery may protect renal function.[13] Some authors conclude that preoperative renal insufficiency and postoperative hypotension, CPB time greater than 140 minutes, prolonged aortic cross clamp time and old age, history of diabetes mellitus, and preoperative congestive heart failure are independent risk factors for development of renal dysfunction.[14]

In this prospective study, 90 patients were selected for CABG. Patients were divided into two groups according to the aortic cross clamp time, less than or equal to 50 minutes, and more than 50 minutes and they were evaluated for occurrence of acute kidney injury after cardiac surgery. According to Walhberg et al there is a 10 fold increased risk of post operative renal dysfunction when suprarenal aortic clamping time is greater than 50 minutes as compared with 30 minutes or less. If suprarenal clamp duration (renal ischemia time) is brief, patients with normal preoperative creatinine levels exhibit no increase or a marginal increase in BUN or creatinine levels after surgery. Accordingly, suprarenal aortic clamping less than 50 minutes in this patient group appears safe and well tolerated.[15] In patients with preoperative creatinine >4.0 mg/dl, the risk for acute renal failure rises to 25 to 28%.[16] The development of acute renal failure is associated with 40-50% reduction in renal blood flow. Following cross clamp of aorta there is high risk of tubular necrosis. Svensson et al reported an overall hospital incidence of dialysis following ARF of 5.5% and hospital mortality of 63%.[17]

According to Zakeri et al a raised serum creatinine or a reduced estimated GFR were both independent and strong predictors for adverse outcomes. It is known that there is a group of patients in whom, despite a near normal creatinine, the GFR is reduced and thus, GFR may be a more accurate parameter than serum creatinine to predict long term outcome.[8] According to our study there is increase in serum creatinine and reduced GFR in patients with prolonged aortic cross clamp time. The patients with aortic cross clamp ≤ 50 min did not showed significant rise in serum creatinine, blood urea levels and no effect on GFR. Patients with aortic cross clamp time more than 50 min showed significant rise in serum creatinine, urea and reduced GFR postoperatively at day 1, 2 and day 3. So the results of our study are same as that of previous literature.[8,11,15] There were several limitations in this study; it is an observational study done for short duration in a single center. During this study no patients were on dialysis, because patients were

assessed for 3 days after operation and dialysis may have been started after fifth post operative day.

Conclusion

The study concludes that aortic cross clamp time has significant effect on post operative renal dysfunction. Aortic cross clamping time less than 50 minutes in the patients appears safe for renal protection. As aortic cross clamping time increases the safety margin decreases and there is more risk of developing renal dysfunction post operatively.

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Original Article

Comparison of Serum Anti-mutated Citrullinated Vimentin Antibody With Anti-cyclic Citrullinated Peptide Antibody As A Diagnostic Marker In Local Pakistani Rheumatoid Arthritis Patients

Bushra Gohar Shah, Hamid Javaid Qureshi and Izaz ur Rehman

Objective: To compare the diagnostic value of antibodies against mutated citrullinated vimentin (anti-MCV) and antibodies to cyclic citrullinated peptides (anti-CCP) in patients with rheumatoid arthritis.

Material & Methods: A total of 88 subjects were included in the study, comprising of 58 known patients of rheumatoid arthritis (fulfilling the American College of Rheumatology Criteria).Thirty age and sex matched normal healthy volunteers were included as controls in the study. Sera of all study subjects were tested by Elisa for presence of anti-MCV and anti-CCP antibodies.

Results: The sensitivity and specificity of serum anti CCP antibodies for RA was calculated to be 58.6% and 86.7% respectively. The sensitivity and specificity of serum anti MCV antibodies for RA was calculated to be 34.5% and 70.6% respectively at the manufacturer's cutoff value of 25U/L.

Conclusion: Anti-cyclic citrullinated peptide antibodies have higher sensitivity and specificity for the diagnosis of RA as compared to anti-mutated citrullinated vimentin antibodies.

Key words: Rheumatoid Arthritis (RA), anti-mutated citrullinated vimentin antibody (anti-MCV), anti-cyclic citrullinated peptide antibody (anti-CCP)

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of multifactorial etiology characterized by chronic joint inflammation that often leads to joint destruction.[1] Early diagnosis of rheumatoid arthritis is a challenge for physicians especially for early implementation of treatment with disease modifying drugs.[2] Diagnosis of RA relies chiefly on clinical manifestations of the disease and the presence of several diagnostic markers. The American College of Rheumatology (ACR)[3] criteria of RA are rarely met in early arthritis cases, and rheumatoid factor positivity is present in fewer than 50% of all patients with early RA in the first few months after disease onset. Therefore, additional diagnostic markers with higher sensitivity and specificity for the diagnosis of RA are required. Currently available data suggests 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).[4] All these antibodies recognize the antigenic epitope containing citrulline,[5] which is generated by post-translational modification of naturally occurring amino acid arginine by the activity of enzyme peptidyl arginine deiminase (PAD).[6] Citrullinated peptides have been synthesized as antigens for diagnostic immunoassays.[5] 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 citrullinated vimentin (MCV-assay).[7] The Anti-MCV assay (ELISA) for the detection of antibodies against citrullinated vimentin uses an antigen with a genetically modified sequence, which is most abundant in patients with rheumatoid arthritis.[7] Anti-CCP antibodies seemed to fulfill the requirements of an ideal marker for diagnosis of early RA, but in recent years, the interest has focused on anti-MCV antibodies because of a higher diagnostic value in comparison with anti-CCP antibodies and rheumatoid factor.[7] The aim of this study was to investigate and compare the diagnostic value of antibodies against mutated citrullinated vimentin (anti-MCV) with antibodies to cyclic citrullinated peptides (anti-CCP) in patients with rheumatoid arthritis.

Material & Methods

Study Design: Cross-sectional analytical study.

Duration and Settings: This study was conducted over a period of one year from January, 2010 to

December, 2010. Subjects were recruited from Fatima Memorial Hospital, Rheumatology Out-patient Department, Lahore. The research work was conducted at the Department of Physiology and Cell Biology of University of Health Sciences, Lahore.

Subjects: A total of 88 subjects were included in the study, comprising of 58 known patients of rheumatoid arthritis (fulfilling the ACR Criteria) diagnosed by the rheumatologist. Thirty age and sex matched normal healthy volunteers were included in the study.

Written informed consent was taken from each study subject. 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. The data obtained was analyzed by using SPSS version 16.0

Serum anti-MCV antibody levels were determined by ELISA[8] 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[8,9] 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.

Results

The study population (n=88), comprised of 58 rheumatoid arthritis patients and 30 normal healthy (age and sex matched) controls. Mean ±SEM age of the RA group was 44±1.2 years and that of the control group was 44.1±1.58 years. In the control group (n=30), 23 were females and 7 were males. In the RA group (n=58), 38 were females and 20 were males.

In RA group, 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). All the patients were using methotrexate, while 35 were using steroids. **(Table-1)**

In the RA group (n=58), 34 (58%) were aCCP+ ive and 24 (41.4%) were aCCP -ive. In the control group (n=30), 26 (86.7%) were aCCP-ive and only 4 (13.3%) were aCCP +ive. In the RA group (n=58), 20 (34.5%) patients were aMCV+ive and 38 (65.5%) were aMCV-ive, at cutoff value of 25U/L. In the control group (n=30), 9 (30%)were aMCV+ive and 21(70%) were aMCV-ive **(Table 2).**

The sensitivity and specificity of serum aCCP antibodies for RA was calculated to be 58.6% and 86.7% respectively. The Positive Predictive Value (PPV) of serum aCCP antibodies for RA was found to be 0.895 (89.5%) with 95% CI of 0.76-0.96. The Negative Predictive Value (NPV) of serum aCCP antibodies for RA was 0.52 (52%) with 95% CI of 0.39-0.65. Positive likelihood ratio was 4.39 (43.9%) and negative likelihood ratio was 0.48 (48%) **(Table-3).**

The sensitivity and specificity of serum anti-MCV antibodies for RA was calculated to be 34.5% and

Table-1: Characteristics of patients with RA

Characteristics	Mean±SEM/ Median(IQR)
Drug treatment	
Methotrexate (MTX)	58
Steroids	35
Disease duration (years)	5 (4-8)
Serum aCCP titer (IU/ml)	10.8 (0.00-340.5)
Serum aMCV titer (IU/ml)	19.7 (14.2-30.06)

Table-2: Serum aCCP and aMCV status in the RA and control groups.

Parameters	RA group (n=58)	Controls (n=30)
Serum aCCP +ive	34 (58.5%)	4 (13.3%)
Serum aCCP -ive	24 (41.4%)	26 (86.7%)
Serum aCCP +ive	20 (34.5%)	9 (30%)
Serum aCCP -ive	38 (65.5%)	21 (70%)

Table-3 Diagnostic characteristics of anti-CCP and anti-MCV.

	aCCP	aMCV
Sensitivity	58.6%	34.5%
Specificity	86.7%	70.6%
PPV (95% CI)	89.5% (0.76-0.96)	68.9% (0.51-0.83)
NPV (95% CI)	52% (0.39-0.65)	35.6% (0.25-0.48)
Diagnostic accuracy	68.2%	46.6%
Positive LHR	4.39	1.15
Negative LHR	0.48	0.94

70.6% respectively at the manufacturer's cutoff value of 25U/L. The PPV of serum anti-MCV antibodies for RA was found to be 68.9% with 95% CI of 0.51-0.83. The NPV of serum anti-MCV antibodies for RA was 35.6% with 95% CI of 0.25-0.48. Positive likelihood ratio (LHR) was 1.15 and negative likelihood ratio was 0.94. The diagnostic accuracy was calculated as 46.6% (**Table-3**). At a cutoff value of 20U/L, the sensitivity improved to 51.7% but the specificity decreased to 56.6%.

For direct comparison of the diagnostic values of anti-MCV and the anti-CCP assays, we performed Receiver Operating Characteristic (ROC) analysis and its accuracy was measured by area under the curve (AUC). The calculated area under the curve (AUC) for anti-MCV was 0.513. The area under the curve (AUC) for anti-CCP was 0.77 and was considered to be good. **(Figure 1 & 2)**

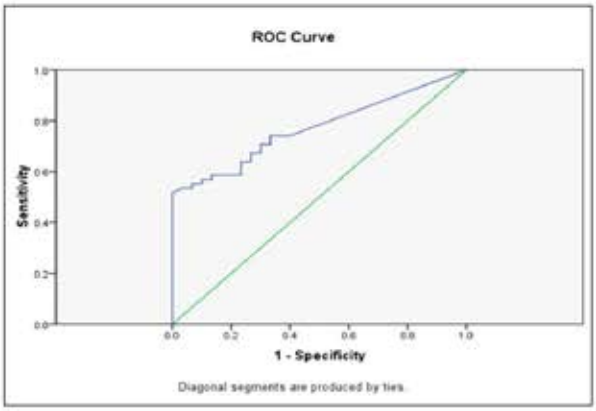

Fig-1: Receiver Operating Characteristic Curve for anti-CCP in the diagnosis of RA.

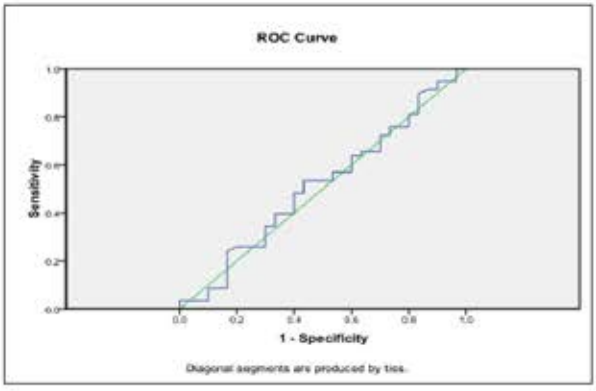

Fig-2: Receiver Operating Characteristic Curve for anti-MCV.

Discussion

The modern trend of RA treatment has been changed to start it as early as possible. Early control of inflammation in RA results in reduced joint damage. It is therefore important to differentiate between RA and other forms of arthritis earlier 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 outweigh the risk. Anti-CCP antibodies were the first citrullinated antibodies that were established as diagnostic tools in clinical use. Anti-MCV is also described as an antibody with high specificity for RA. The clinical performance of this marker has not been evaluated thoroughly. We therefore designed this study to evaluate the clinical value of anti-MCV determination as compared to anti-CCP in the diagnosis of RA in local Pakistani subjects.

In the present study RA group (n=58), 20 were positive for anti-MCV with diagnostic sensitivity for RA to be 35%, at a manufacturer cutoff of 25U/L. When value of 20U/L was considered as the cutoff, the sensitivity improved to 52%. Out of 58 RA patients 34 were positive for anti-CCP antibodies. So diagnostic sensitivity of anti-CCP reactivity was 59%. The results of this study show that anti-CCP has a higher sensitivity for the diagnosis of RA as compared to anti-MCV (59% *vs* 35%). The results of our study were not in concordance with the studies of most of other authors who reported higher sensitivity of anti-MCV antibody as compared to anti-CCP antibody. Result of study by Bang et al[7](sensitivity 82% vs 72% of anti-CCP), Coenen et al[8] (several assay tested: 74.5% vs 70-77% of anti-CCP), Soos et al[10] (75.6% vs 66.4% of anti-CCP). Similar results have been found in study by Dejaco[11] et al who reported a slightly higher sensitivity of 70.1% of anti-CCP vs 69.5% for anti-MCV.

Lower sensitivity of anti-MCV for RA in the present study could be attributed to the difference in the anti-MCV assay kits used. Anti-MCV assay kits utilized in all the reported studies was manufactured by the ORGENTEC Diagnostica GmbH, Mainz, Germany, whereas the anti-MCV assay kits utilized in the present study were manufactured by IMMCO Diagnostics, USA. Moreover, the cutoff value recommended by the manufacturer of our kit was higher (25U/L) as compared to 20U/L which was recommended cut-off value by ORGENTEC Diagnostica in all other studies. Further studies with a larger sample size would help in better evaluation of this antibody in our population.

Nine out of the 30 normal healthy controls were

positive for anti-MCV and four were positive for anti-CCP. So the specificity of anti-MCV was 70.6% while that of anti-CCP was 87%. Specificity of anti-CCP for RA was higher as compared with anti-MCV (87% vs 70.6%) in our cohort. Our results are comparable with the results of other authors: (Dejaco et al[11] anti-CCP 98.7%, anti-MCV 90.8%; Coenen et al[8] anti-CCP 93-96.4%, anti-MCV 91.5%; Soos et al[10] anti-CCP 98.3%, anti-MCV 91.5%; Wagner et al[12] anti-CCP 97.6%, anti-MCV 81.3%). Only Bang et al[7] found higher specificity of anti-MCV as compared to anti-CCP. Specificity of anti-MCV is lower in our cohort due to more frequent positivity in healthy controls. The analysis of ROC curve confirms the finding of higher specificity of anti-CCP. These results are in agreement with that reported by Dejaco et al.[11]

Limitation of our study was the small sample size; larger sample would have been better representative of the diagnostic spectrum of these antibodies. One more limitation of the present study was the cross-sectional study design, with lack of follow up data of the controls with positive auto-antibodies; controls with positive antibodies might end up having the disease, as it has been shown that ACPA's might be present years before the onset of the disease.[13] Prospective study design would help to evaluate the prognostic and diagnostic value of this test.

Conclusion

Anti-cyclic citrullinated peptide antibodies have higher sensitivity and specificity for the diagnosis of RA as compared to anti-mutated citrullinated vimentin antibody. So, it is stated that anti-CCP antibody is a more sensitive and specific marker for the diagnosis of RA as compared to anti-MCV antibody.

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Original Article

VARYING THICKNESS OF TIDE MARK IN ARTICULAR CARTILAGE OF AGEING MALE UNDERGOING OSTEOARTHRITIS IN PAKISTANI POPULATION

Shaista Ali, Ayesha Intisar and Muhammad Amin

Objective: To study increasing thickness of tide mark in articular cartilage of male with advancing age.

Material & Methods: Forty samples of articular cartilage of knee joint from male dead bodies of estimated age between 21- 60 years, brought to forensic department within six hours of death and undergoing autopsy within 12 hours of death were collected. The samples were grouped as A, B, C and D depending upon the estimated age. The thickness of tide mark was recorded in different age groups.

Results: With advancing age, the thickness of tide mark was increased and highly significant statistically (p-value = 0.000).

Conclusion: As the age advances the thickness of the tide mark increases in articular cartilage of knee joint of male undergoing osteoarthritic changes & such changes are set in about a decade earlier in Pakistani population.

Keywords: Tide mark (TM), articular cartilage (AC), osteoarthritis (OA).

Introduction

Articular cartilage is a specialized connective tissue that covers the ends of the long bones in synovial joints. Its structural, biochemical and metabolic properties are unique in providing the joints with extraordinary resilience, smooth movements and ability to resist enormous physical pressure.[1-3] Articular cartilage follows the path of the bone morphogenesis. Roughly at the beginning of endochondral bone formation in an embryo, articular cartilage also becomes an independent entity. It is separated from the growth plate by the layer of proliferating chondrocytes that develops into mineralized calcified cartilage. This mineralized cartilage present at the bottom of the adult articular cartilage is separated from the upper layers by the tide mark, a microanatomical acellular structure with as yet undefined composition.[4]

The Chondro-osseous junctional region of articular cartilage is very complex and is considered to consist of the deepest layer of non-calcified cartilage that is the tide mark, the layer of calcified cartilage, a thin cement line between the calcified cartilage and subchondral bone.[5]

The tide mark is a clearly defined boundary separating uncalcified from calcified cartilage. It is not a straight line across articular cartilage, but a complex 3D structure and has a distinct micro anatomical trilaminate appearance suggesting that this region is more complex and less understood.[6] It is rich in collagen and contains hyaluronan but appears to lack glycosaminoglycans of conventional proteoglycan. It expresses a very distinctive and limited lectin staining glycoprofile, which is due to specific glycoprotein. Biochemical data has shown a high concentration of calcium phospholipid phosphate complexes in the tide mark.[7] Three distinct variations of the collagen framework of the tidemark were identified under scanning electron microscope by Redler in 1975: (i) a band of randomly arranged compacted fibrils that appeared to be continuous with fibres of the non-calcified and calcified zones. (ii) A band of flattened fibrils running parallel to the undulating surface of the calcified cartilage. (iii) A band of perpendicularly oriented fibrils showing a continuous transition between the non-calcified and calcified zones.[8] The thickness of the single line of tide mark is up to 10 μm and its replication and thickness is taken to be an important feature of osteoarthritis. It is a calcification front advancing in the direction of non-calcified cartilage that reduces the thickness of hyaline cartilage and increases the thickness of calcified cartilage zone.[5]

Degeneration of articular cartilage leads to many diseases such as osteoarthritis, a most common age related, non inflammatory degenerative joint disease and a primary cause of pain and disability.[5,9,10] Knee joint is most commonly involved. The incidence of osteoarthritis is very high and it is only second to ischemic heart disease.[11] It is anticipated that by the

year 2020 about 18% of the US population will be affected.[12] Nigel Arden (2006) reported that 60% of men living up to seventh decade suffer from knee osteoarthritis.[11] Although the epidemiology of osteoarthritis in the developing world is much less known, the prevalence of osteoarthritis knee in 2008 was 5.8% in India, 9.6% in China, 11.3% in Thailand, 5.1% in Indonesia and 7.5% in Bangladesh.[13] The prevalence of osteoarthritis increases in aged people and it appears that aged cartilage incurs changes which directly predispose it to the development and progression of osteoarthritis.[14]

This study was aimed to observe the thickness of tide mark in ageing articular cartilage indicating severity of osteoarthritis.

Material & Methods

A descriptive study in which changes in tide mark of aging articular cartilage were observed & compared with each other. It was conducted in Anatomy Department of King Edward Medical University, Lahore.

Forty samples of articular cartilage of knee joint were collected from the autopsies of unknown male dead bodies brought to the Forensic Department of KEMU Lahore, of estimated ages between 21 & 60 years within six hours of death. Dead bodies showing signs of trauma, surgery and gross abnormality and deformity of the knee were not included in the study.

The collected samples of articular cartilage were placed in four groups A, B, C and D depending upon the estimated age of the cadaver.

Group A: Estimated age ranging from 21-30 years
Group B: Estimated age ranging from 31-40 years.
Group C: Estimated age ranging from 41-50 years.
Group D: Estimated age ranging from 51-60 years.

After doing dissection, knee joint was flexed and 1 cm x 1 cm full thickness chip of articular cartilage was removed from the tibial surface of femoral condyle, 1cm medial to the medial margin of intercondylar fossa.

The specimens were immediately placed in 10% neutral buffered formalin for 48 hours. Three random tissue samples of articular cartilage from each specimen were taken and processed for paraffin embedding. Five micrometer (5um) thick sections were made on rotary microtome and mounted on clear albumin coated slides. Sections were stained with Hematoxylin and Eosin for routine histological study and Masson's Trichrome stain was used for the evaluations of the histological features such as thickness of tide mark and zones of the articular cartilage.

Results

The mean thickness of tide mark was 4.87±1.90 µm in group A , 8 ± 1.46 µm in group B, 14±1.74 µm in group C and 15 ± 3.16 µm in group D. The mean thickness of the tide mark increased from group A to group D and was statistically significant in all study group (p-value = 0.000). Moreover the mean thickness in group A was less as compared to group B, C and D, significant statistically (p-value < 0.05). The mean thickness in group B was also less than group C and group D and was statistically significant (p-value < 0.05). The mean thickness in group C is less in comparison to group D (p-value = 0.04)

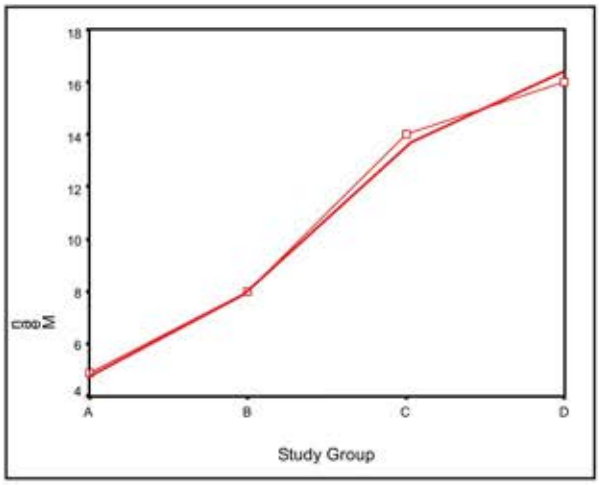

Fig-1: Mean of thickness of TM in UM.

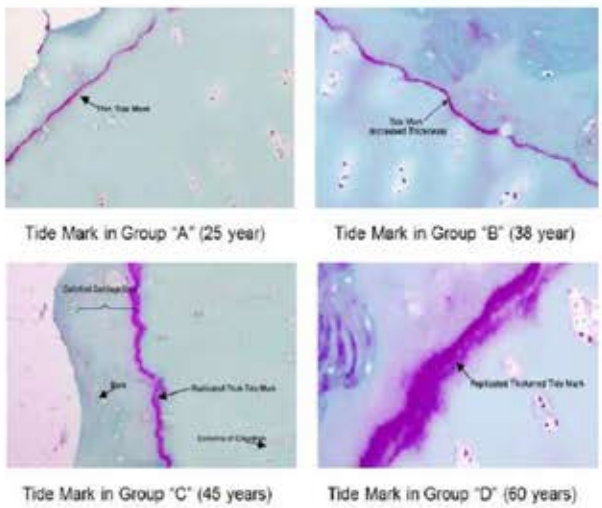

Fig-2: Histomicrograph showing comparison of thickness of tide mark in articular cartilage in different study group (Masson's Trichrome).

Table-1: Descriptive analysis of thickness of tide mark and multiple comparison test of thickness in different study groups.

		N	Mean (um)	Std. Deviation	Minimum	Maximum
Study Groups	**A**	10	4.8750	1.90485	2.50	7.50
	B	10	8.0000	1.46723	6.25	10.00
	C	10	14.000	1.74801	12.00	16.10
	D	10	15.0000	3.16228	10.00	20.00
Total		40	10.7188	4.99108	2.50	20.00
			p value			
Over all			**0.000**			
Pair Wise	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
	0.003	0.000	0.000	0.000	0.000	0.047

Discussion

This study describes the morphology of ageing male articular cartilage of knee joint and the degenerative changes that are related to the advancing age. In the present study, in calcified cartilage zone, the chondrons in all study groups were either isolated or arranged in groups. These findings were similar to the study of Bhosale and Richardson (2008) and Wang et al (2009) who noted that calcified cartilage zone with its two interfaces, the tide mark and the cement line was a zone with low metabolic activity having a small volume of dispersed cells embedded in calcified matrix.[15,16] It was noted that the thickness of calcified cartilage zone increased with the advancing age. Findings of present study were same as Hayami et al in 2006[17] who observed that thinning of articular cartilage with ageing was due to the surface degradation as well as increase in the thickness of calcified cartilage zone which resulted in reduction of the thickness of hyaline cartilage (upper three zones).[18,19]

In this study the tide mark was clearly visible in Masson's Trichrome stain. When the thickness of tide mark was compared in different study groups it was seen that the thickness increased with age. The mean thickness of tide mark was 4.8750 µm in group A, 8.0000 µm in group B, 14.0000 µm in group C and 15.0000 µm in group D. In addition to that, clear replications of the tide mark were also observed. So it was noted that increase in the thickness of calcified cartilage zone is at the expense of decrease in the thickness of hyaline cartilage and this is due to the advancement of tide mark in the direction of hyaline cartilage. The duplications of tide mark and its advancement towards non calcified cartilage was also noted by Burr BD in 2011[19] while Oda et al in 2007[18] described that the thickness of upper three zones as well as cellularity decreased with age, the findings that are very similar to this study in which it was observed that the thickness of the calcified cartilage zone and tide mark increased with age.

Conclusion

Increased thickness of calcified cartilage zone, tide mark and its advancement towards hyaline cartilage are age related degenerative changes which are set in a decade earlier in articular cartilage of Pakistani population.

Factors affecting these changes need to be explored. These findings may act as guideline for modulation of preventive measures and treatment trends.

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Original Article

MORPHOLOGICAL EFFECTS OF RIBAVIRIN ON ADULT OVARY OF ALBINO RAT

Hafiz Moeen-ud-Din, Muhammad Suhail and G.P.William

Objective: To evaluate the morphological effects of ribavirin on adult ovary of albino rats
Material & Methods: This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of Punjab. Doses of 20,100, 200mg/kg of ribavirin were given to albino rats orally for five consecutive days at intervals of 24 hrs . The ovaries were processed for histopathological analysis on day 14 after the last exposure. The gross parameters studied were body weight, paired ovarian weight, relative tissue weight index (RTWI). Data was analysed by ANOVA and Tukey's test for statistically significant difference.
Results: There was decrease in body weight of experimental groups compared with control at higher dose-levels i.e 100 and 200mg/kg. When paired ovarian weights were compared among groups the overall difference was significant. Post hoc analysis revealed that low dose group had significantly low ovarian weight as compared to control. When RTWI was compared among groups the overall difference was significant. Post hoc analysis revealed that low dose group i.e 20mg/kg had significantly low RTWI as compared to control.
Conclusion: Ribavirin administration in adult female rat showed significant harmful effects on ovary, and these gonadotoxic effects of ribavirin may cause infertility in females during reproductive period.
Key words: Ribavirin, gonadotoxicity, antiviral drugs, guanosine analogue nucleoside.

Introduction

Ribavirin is a guanosine analogue nucleoside with broad spectrum antiviral activity.[1] It acts by inhibiting inosine monophosphate dehydrogenase (IMPDH), which is the key step in de novo guanine synthesis responsible for viral replication.[2,3] Acute control of HCV RNA levels occurs through a brisk intra hepatic T-helper & T-suppressor cell response, a shift towards a T-helper (Th_1) cytokine profile and up-regulated natural killer cell activity.[2,3] Ribavirin is rapidly absorbed after oral administration (time to maximum concentration = 1.5 hours) followed by rapid distribution and prolonged elimination phases. Uptake from the proximal small bowel is active via concentrative N1 sodium-dependent nucleoside transporters.[4] Ribavirin has been used against flavi viruses such as yellow fever which is a life threatening mosquito-borne flaviviral hemorrhagic fever characterized by severe hepatitis, renal failure, hemorrhage, and rapid terminal events with shock and multi-organ failure.[5] The recommended ribavirin dose is based on body weight, being generally 1000 mg/day if less than 75 kg and 1200 mg/day if more than 75 kg. The oral LD50 of ribavirin is 2 and 5.3g/kg in mice and rats respectively and intra peritoneal LD50 has been reported 0.9-1.3 and 2g/kg respectively.[6]

Anaemia, neutropenia, thrombocytopenia, skin rashes, anorexia, pulmonary edema & depression are the major adverse effects.[6,7] Pre-natal exposure of ribavirin in pregnant women suggests birth defects with torticollis, hypospadiasis, polydactyly, natal teeth, glucose-6-phosphate dehydrogenase deficiency (G6PD), ventricular septal defect and cyst of 4th ventricle of brain.[8]

Material & Methods

This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Post Graduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of Punjab.

The sample size was estimated by using 5% level of significance and 80% power of test with expected mean body weight increase of 40± 5 gm, 35± 5 gm, 30± 5 gm and 29± 4 gm in rats of control group, groups with 20 mg/kg, 100 mg/kg and 200 mg/kg respectively at day 14.[9] Based on this a total of 40 female Albino rats Wistar Strain weighing between 175 and 230 grams were used after randomization by balloting method. All these animals were kept in cages for 14 days in the animal house of Zoology Department, University of Punjab Lahore for the purpose of acclimatization. A twelve hour light and dark cycle was maintained at room temperature between 22-25°C. The food and water was provided

to these animals ad libitum. The food given was in the form of chick feed.

After 14 days the animals were randomly divided into four groups. Group A was labelled as control, the other three groups were experimental, groups B, C & D. Each group comprised of 10 animals. All the rats were weighed and properly recorded in proforma. These rats were then marked with a permanent marker for identification and placed in their respective cages labelled with allocated tags. Ribavirin (Xolox, Ferozsons Ltd. Pakistan) 20mg/kg, 100mg/kg and 200mg/kg were used respectively in this study which was given to the rats through the nasogastric tube (NG tube). Control group A was having ten female rats and were not given any medication except for equivalent proportion of distilled water according to body weight by nasogastric tube for 5 consecutive days. Experimental groups B, C & D were given 20,100 & 200mg/kg of ribavirin dissolved in 1ml of distilled water by Nasogastric tube respectively for 5 consecutive days.

Ribavirin tablets were removed from the blister pack and were placed in a small mortis. These tablets were then crushed to powder form using a small wooden crusher. The powder form of medicine was transferred to electronic weighing machine. The dose was calculated according to body weight of animal, transferred to a beaker and 1ml of distilled water was added. Using a glass mixer the contents were mixed together. This was then given to each of the experimental rat with the help of insulin syringe by an NG tube.

At 14th day of experiment, after the cessation of ribavirin, the animals of all the groups were weighed and recorded in proforma. These animals were then euthanized by injecting sodium pentobarbital as anaesthetic intraperitoneally in doses of 45mg/kg [9,10] and morphine as analgesic in doses of 0.3-0.5mg/kg intraperitoneally.[11] This is the most acceptable agent for anaesthesia, less expensive, acts quickly and humanely kills all types of rodents.

By making an incision at the base of abdomen, it was opened. The anterior abdominal viscera were reflected and the ovaries were removed after identifying the uterine tubes for detailed morphological study. Ovaries were weighed and fixed in 10% formalin.

The following gross parameters were observed in this study.

A. Quantitative parameters.

1. Weight of the rat, normal or any abnormality.
2. Paired ovarian weight, normal or any abnormality.
3. Relative tissue weight index, normal or any abnormality.

It was calculated as follows:-

$$RTWI = \frac{\text{Mean weight of ovary}}{\text{Mean body weight of animal}} \times 100$$

The obtained results were tabulated and compared using computer software Statistical Package for Social Sciences (SPSS) version 17.0. The arithmetic mean of observations and standard deviation values were calculated. The significance between three means for body weight, paired ovarian weight, RTWI was calculated by analysis of variance (ANOVA) and Tukey. p-value <0.05 was considered statistically significant.

Discussion

This study was conducted to evaluate the harmful effects of ribavirin, an antiviral drug, on the morphology of the ovaries. The drug literature of ribavirin did not mention any side effects related to the ovarian cycle and there is lack of research work on its effects on the morphology of female gonads. As younger population of Pakistan, especially females of reproductive age group have been affected by viral infections especially hepatitis C, so this research study was carried out to investigate the effects of Ribavirin on the morphology of the adult ovaries.

Ribavirin has known effects on adults such as anemia, neutropenia, thrombocytopenia, weight loss, pulmonary embolism, pulmonary edema, myocardial infarction, cerebral haemorrhage, hypothyroidism etc.[6,12] It also has known effects on rat testis regarding abnormal morphology of sperms and decrease in the weights of seminal vesicle and prostate.[9]

Table-1: Mean body weight (g) for female albino rats in various groups at start of experiment.

Groups	Mean	SD	Minimum	Maximum
Group A	203.0	±13.37	180	220
Group B	197.0	±13.37	180	220
Group C	197.0	±13.91	175	221
Group D	210.0	±15.15	1183	230

A Control Group, B Experimental Group, C Experimental Group, D Experimental Group, SD Standard Deviation

Table-2: Comparison of mean body weight (g) of female rats among various groups at start of experiment.

	Sum of Squares	DF	Mean Square	F	p-value
Between Groups	238.675	03	79.558	0.408	0.748++
Within Groups	7026.100	36	195.169		
Total	7264.775	39			

Based on ANOVA. DF=Degree of Freedom, F=f-test (Ratio of variances) ,++Non significant difference (p>0.05)

Table-3: Group wise comparison of mean body weight (g) of female albino rats among various groups at end of experiment after ribavirin administration

Groups	Group	Mean Difference	STD. Error	p-value
	Group B	31.7	6.31	<0.001*
Group A	Group C	39.0	6.31	<0.001*
	Group D	48.2	6.31	<0.001*
Group B	Group C	7.3	6.31	0.657++
	Group D	16.5	6.31	0.059++
Group C	Group D	9.2	6.31	0.472++

*Based on TUKEY'S Test A Control Group, B Experimental Group, C Experimental Group, D Experimental Group *Significant difference (p<0.05) ++Non significant difference (p>0.05)*

Table-4: Comparison of mean paired ovarian weight (g) of female albino rats among various groups after administration of ribavirin

	Sum of Squares	DF	Mean Square	F	p-value
Between Groups	0.006	03	0.002	6.665	0.001
Within Groups	0.011	36	0.0003		
Total	0.017	39			

*Based on ANOVA , DF=Degree of Freedom F=f-test (Ratio of variances) **Highly significant difference (p<0.01)*

Table-5: Group wise comparison of mean paired ovarian weight (g) of female albino rats among various groups after ribavirin administration.

Groups	Group	Mean Difference	STD. Error	p-value
	Group B	0.035	0.008	<0.001*
Group A	Group C	0.014	0.008	0.301++
	Group D	0.017	0.008	0.155++
Group B	Group C	-0.021	0.008	0.053++
	Group D	-0.018	0.008	0.121++
Group C	Group D	0.3	0.008	0.981++

*Based on TUKEY'S Test A Control Group B Experimental Group C Experimental Group D Experimental Group **Highly significant difference (p<0.01) ++Non significant difference (p>0.05)*

Table-6: Comparison of relative tissue weight index of ovaries of female albino rats among various groups after administration of ribavirin.

	Sum of Squares	DF	Mean Square	F	p-value
Between Groups	0.000951	03	0.000317	9.195	<0.001**
Within Groups	0.001241	36	0.000034		
Total	0.002192	39			

*Based on ANOVA DF=Degree of Freedom F=f-test (Ratio of variances) **Highly significant difference (p<0.01)*

Table-7: Group wise comparison of relative tissue weight index for ovaries of female albino rats among various groups after administration of ribavirin.

Groups	Group	Mean Difference	STD. Error	P-value
	Group B	0.00840	0.0026	0.015*
Group A	Group C	-0.00307	0.0026	0.650++
	Group D	-0.00394	0.0026	0.447++
Group B	Group C	-0.01147	0.0026	0.001*
	Group D	-0.01235	0.0026	<0.001*
Group C	Group D	-0.00088	0.0026	0.987++

*Based on TUKEY'S Test, A Control Group B Experimental Group, C Experimental Group, D Experimental Group, *Significant difference (p<0.05), ++Non significant difference (p>0.05)*

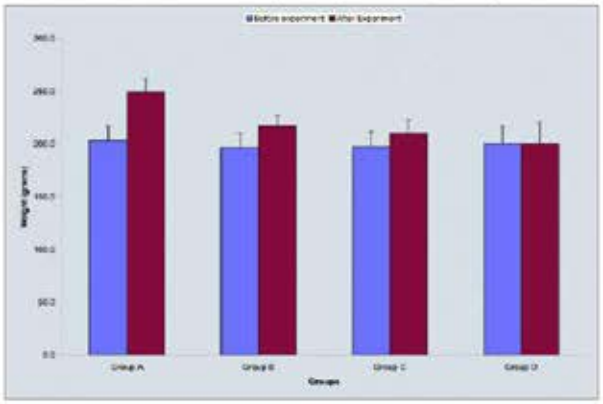

Fig-1: Comparison of mean weight difference of female albino rats-control & experimental groups after ribavirin administration.

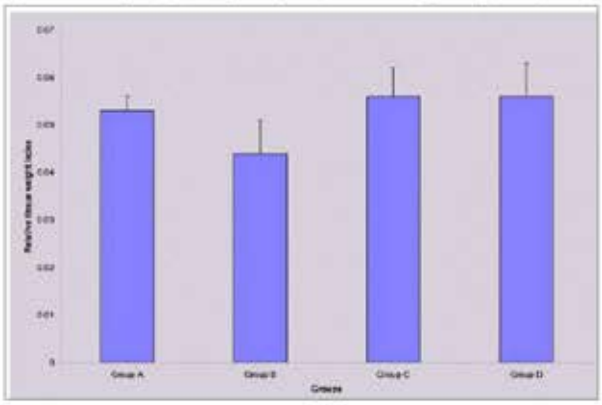

Fig-3: Comparison of relative tissue weight index among control and experimental groups after administration of ribavirin.

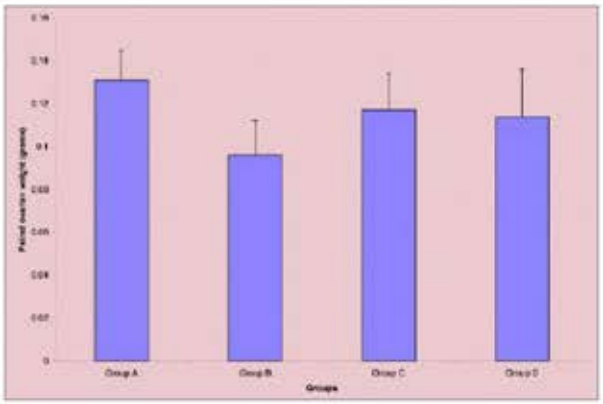

Fig-2: Comparison of mean paired ovarian weight of female albino rats-control & experimental groups.

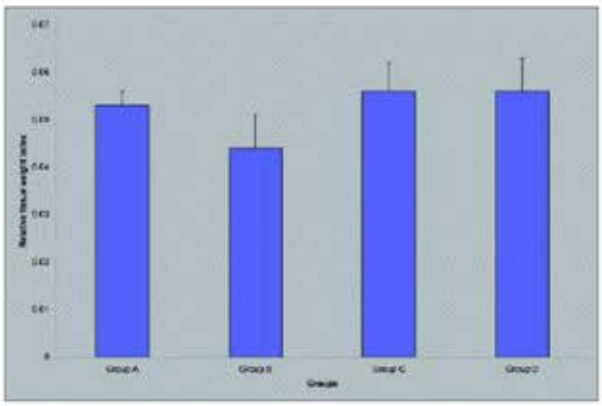

Fig-4: Comparison of relative tissue weight index among control and experimental groups after administration of ribavirin.

In the present research work the body weight of the female albino rats among groups before starting the experiment was statistically non significant (p-value 0.748, **Table 2**). When mean body weights of albino rats were compared after ribavirin administration in doses of 20mg/kg in experimental group B, 100mg/kg in experimental group C and 200mg/kg in experimental group D this showed overall difference among these groups which was statistically significant (p<0.001, **Table 3**). Post hoc analysis revealed that the experimental groups B, C and D all had significantly low weights as compared to control group A (p<0.001). Experimental groups C and D had low weights as compared to experimental group B but statistically insignificant (p=0.657 and 0.059) respectively. The difference between experimental groups C and D was also statistically insignificant (p-value 0.472, **Table 4**).

The results of body weight after ribavirin administration in the present study coincides with the research and study performed by Naryana K et al who also observed decrease in body weight after ribavirin in the experimental animals.[9]

In the present research work there was a decrease in the mean paired ovarian weights after ribavirin administration in the experimental groups B, C and D as compared to the control group A. This may be due to overall decrease in the weights of the rats in these experimental groups. To the best of researcher's knowledge no previous data is available to correlate with our results. When weights were compared among groups the overall difference was statistically significant (p-value 0.001,**Table 4**). Post hoc analysis revealed that after 20mg/kg ribavirin administration in the experimental group B showed statistically significant low ovarian weight as compared to control group A (p-values <0.001). Experimental groups C and D had statistically insignificant low mean paired ovarian weights as compared to control group A (p-values 0.301 and 0.155) respectively. The experimental group C had high ovarian weight as compared to experimental group B but insignificant (p-value 0.053). The difference of experimental groups B and C from experimental group D was also insignificant (p-value 0.121 and 0.981, **Table 5**) respectively. These results coincide when compared with the research and study performed by Naryana K et al in which after ribavirin administration there was decrease in the weights of organs such as seminal vesicles and prostate but no change in the weights of testis was found in experimental animals.[9]

The relative tissue weight index (RTWI) in this study was calculated to see the changes in weights of paired ovaries as compared to the body weights of the animals. Reduction in ovarian weight was accompanied by a relative decrease in the total body weight of the albino rats in the experimental groups after ribavirin administration when compared with the control group A.

When RTWI were compared among groups the overall difference was statistically significant (p-value <0.001, **Table 6**). Post hoc analysis revealed that after 20mg/kg ribavirin administration in experimental group B showed significantly low RTWI as compared to control group A (p-value 0.015) and experimental group C with 100mg/kg ribavirin and experimental group D with 200mg/kg ribavirin showed statistically insignificant results with high RTWI (p-values 0.650 and 0.447) respectively when compared with control group A. Experimental group B when compared to experi-mental groups C and D showed statistically signi-ficant results (p-value <0.001). Experimental groups C and D showed insignificant results when compared with each other (**Table 7**). These results showed that experimental group B when given ribavirin in doses of 20mg/kg showed decrease in RTWI as compared to other experimental groups C and D. Ribavirin in high doses causes pulmonary edema[12] and also damages blood vessel endothelium[13,14] and similar effects like interstitial edema may be responsible for slight reduction in RTWI in female albino rats which were exposed to high doses i.e 100mg/kg and 200mg/kg of ribavirin in experimental groups C and D respectively. In these groups interstitial edema in ovaries was also observed.

Conclusion

This study showed that ribavirin when given for shorter duration of time has a negative impact on the morphology of rat ovaries. The results of present research has shown deleterious effects on weight of ovaries in doses of 20,100 and 200mg/kg of ribavirin. This may increase the risk of infertility in those females who are taking this anti viral drug during their reproductive age group. Further research work is also required regarding its effects on the hormonal assays in females of reproductive age group and to correlate this with the adverse effects on ovarian structure.

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Original Article

Efficacy of Periarticular Sodium Hyaluronate Injection in Lateral Epicondylitis of Humerus

Imran Shabir Mughal, Rana Dilawaiz Nadeem and Omer Iqbal Cheema

Objective: To determine the efficacy of periarticular sodium hyaluronate injection in terms of improvement of pain in lateral epicondylitis of humerus.

Material & Methods: This descriptive case series study was conducted over a period of 6 months at Department of Orthopaedic Surgery at Services Hospital, Lahore. Through non-probability consecutive sampling, one hundred patients fulfilling the inclusion/exclusion criteria were selected from outpatient department and informed consent was taken. Sodium hyaluronate injection was administered into the subcutaneous tissue and muscle 1 cm from the lateral epicondyle of humerus towards the primary point of pain using a two dimensional fanning technique. Patients were followed up in outpatients department for assessment of improvement in pain utilizing Visual Analog Scale (VAS) at an interval of 4 weeks. Treatment was declared effective when the VAS was found to be 0-3 i.e. no or mild pain. Paired sample t-test was used for pain comparison at baseline and at 4 weeks and 5% level of significance was used.

Results: 48 females and 52 males were enrolled who were diagnosed with lateral epicondylitis. Mean age of patients was 42.16±6.78 years with minim and maximum age of patients was 31 and 58 years respectively. Periarticular sodium hyaluronate injection was effective in 75 (75%) patients.

Conclusion: Periarticular sodium hyaluronate injection is an effective method of treatment in lateral epicondylitis of humerus.

Keywords: Hyaluronate, two dimensional fanning technique, lateral epicondylitis, tennis elbow.

Introduction

Lateral epicondylitis of humerus (Tennis elbow) is soreness, pain or inflammation on the outside or lateral side of the upper arm.[1] It is a term frequently used for a condition caused by overuse of arm and forearm muscles that results in elbow pain. This is a significant problem in tennis players.[2] Incidence of lateral epicondylitis is reported to be 0.6% in general population & 9% in tennis players. Currently there is no consensus on the treatment but the non-surgical treatment is the mainstay of treatment. Multiple options are available including RICE (rest, ice, compression and elevation), oral or topical NSAIDs, bracing and physical therapy, [3] and local modalities like extracorporeal shock wave therapy (ESWT).[4] Previous studies with NSAIDs and botulinum toxin injection were associated with adverse effects like rash, mild gastric upset, digital paresis and weakness of finger extension.[5,6] Surgery is mainly reserved for the resistant cases and options include open, percutaneous and arthroscopic procedures. Multiple procedures are described which include Boyd Mcleod procedure, Nirschl procedure, knife and fork day case surgery, open release of common extensor origin, fractional lengthening of forearm extensors, open and percutaneous tenotomy and excision, release and repair of common extensor origin & extensor carpi radialis brevis debridement.[7-14] This study was designed to determine the efficacy of periarticular sodium hyaluronate injection in lateral epicondylitis of humerus. Importance of the study lies in the fact that no local data is available regarding the use of hyaluronate injection in this condition. Study compares and highlights the efficacy of hyaluronate injection which is also virtually free from the side effects common among the other modalities being utilized.

Material & Methods

This descriptive case series was conducted at Department of Orthopaedic Surgery, Services Hospital, Lahore. Non probability consecutive sampling technique was used. Patients, both males and females, age between 30 to 60 years having symptoms for more than 6 months, with the diagnosis of lateral epicondylitis were included in this study with Visual Analoge Score (VAS) ≥ 4. Patients having coexisting arthralgia or arthritis of elbow or radio-

ulnar joint, radial tunnel syndrome, varus instability of elbow, coexisting medial epicondylitis all assessed clinically, or any history of use of any medicine or product containing corticosteroids in past 30 days on available medical record were not included in this study.

One hundred patients were selected after informed consent. A detailed history was taken. Demographic data including age, gender, address, occupation and hand dominance was recorded. A detailed examination was carried out and baseline pain score was noted using VAS. Under aseptic conditions, 20mg/2ml sodium hyaluronate injection was administered into the subcutaneous tissue and muscle 1 cm from the lateral epicondyle of humerus towards the primary point of pain using two dimensional fanning technique. Patients were treated on out patient basis and were advised to avoid any activities that aggravate symptoms. Patients were followed up in outpatients department at 4 weeks and were assessed for pain intensity using VAS.

Data collected was entered and analyzed by using SPSS version 17. Variables were analyzed using simple descriptive statistics, calculating mean ± standard deviation for numerical values like age. Frequencies and percentages were calculated for qualitative variables like gender and efficacy. Paired sample t-test was used to see the effectiveness of treatment at 5% level of significance.

Results

Mean age of patients was 42.16±6.78 years with minimum and maximum age of patients being 31 and 58 years respectively. Mean age of male and female patients was 42.48±6.75 and 41.81±6.86 years respectively. Minimum and maximum age of male patients was 32 and 58 years and of female patients was 31 and 58 years respectively. Gender distribution of patients shows that there were 48 (48%) female and 52 (52%) male patients. There were 93 patients whose right side was effected and in only 7 patients left side was effected. Pain intensity was assessed by using visual analogue scale (VAS) scale. Pain intensity was assessed at two intervals i.e. on base line and at 4 week after the procedure. Mean pain intensity at base line was 7.75±1.35 and at 4th week it was 2.12±1.97. Using paired sample t-test it was concluded that there was a significant mean pain reduction at 4th week as compared to baseline pain, i.e. p-value = 0.000 (**Table 1**). Periarticular sodium hyaluronate injection was effective among 75 (75%) patients (**Fig. 1**). Among male patients effectiveness was observed in 35 (67.3%) and in female patients effectiveness was observed in 40 (83.3%) patients at 4th week post procedure.

Table-1: Descriptive statistics for age (years).

	Gender		
	Male	Female	Total
N	52	48	100
Mean	42.48	41.81	42.16
Std. Deviation	6.75	6.86	6.78
Minimum	32.00	31.00	31
Maximum	58.00	58.00	58

Table-2: Descriptive statistics for pain intensity.

	Baseline	4th week
N	100	100
Mean	7.75	2.12
Std. Deviation	1.35	1.97
Minimum	5.00	.00
Maximum	10.00	7.00
p- value	0.000 (significant)	

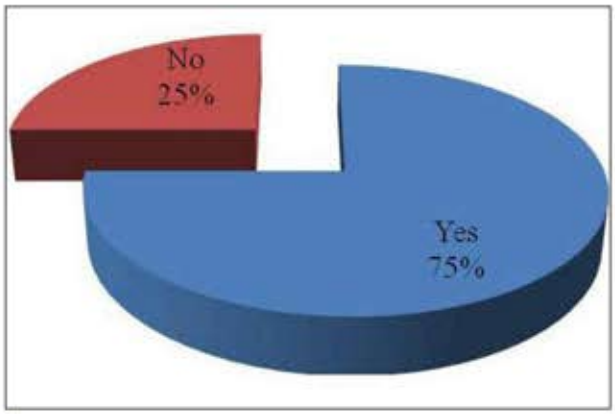

Figure-1: Effectiveness of treatment.

Discussion

Tennis elbow is a benign self-limiting condition which improves in 70% to 80% of patients with or without treatment within 12 months but it is a long time for a patient to wait not only in terms of pain and disability, but also results in loss of economic productivity. What patients often require is a safe, minimally invasive procedure that will enable them to return to their daily activities as soon as possible.[15]

In this study, mean age of patients was 42.16±6.78 years which is in accordance with the other studies where mean age is in the fifth decade of life.[3,16] Present study also showed a male dominance as it had 52 males as compared to 48 female patients, which is not in accordance with some of the studies which have shown this condition to be more prevalent in the female gender.[16]

This study shows that 93 patients had right sided involvement as compared to only 7 patients with left sided involvement. Right side involvement showed predominance over left side. This right sided dominance has already been shown in a study conducted by Shiri R et al.[17]

Study demonstrated the effectiveness of the modality in 35 (67.3%) males & 40 (83.3%) females. This is probably due to the fact that hyaluronate injection produces pain relief and this is followed by immediate resumption of heavy activities by men leading to recurrence of symptoms.

Many treatments have been proposed leading to a number of trials and reviews including several recent meta-analyses but have led to no conclusion as to which is the best. The NSAIDs and botulinum toxin injection were associated with adverse effects like rash, mild gastric upset, digital paresis and weakness of finger extension.[5,6] Smidt et al reviewed literature on physical therapy and found no evidence of effect, with the exception of ultrasound, where a minor effect was shown.[18] Bisset et al published a meta-analysis of 28 randomized studies of different physical therapies for lateral epicondylitis; most studies had a small number of subjects and only eight had long term follow-up of effect of therapy. They found effectiveness of corticosteroid injections or physiotherapy over the modality of wait and see. However, the benefits of corticosteroid injection were short term and were paradoxically reversed after six weeks.[19] Some authors noted that patients who received ESWT had improved symptoms.[4] Side effects from this treatment included transient reddening of the skin, pain at the site of the treatment, small hematomas, migraines, and syncope.[20] Hyaluronate is a naturally occurring biological agent and is relatively free of these side effects.[21] A recently carried out study has shown an effectiveness in 89% of patients after two injections.[3] However, present study has a success rate of 75% with single injection of sodium hyaluronate.

Conclusion

Results of this study confirmed the efficacy of periarticular sodium hyaluronate injection in lateral epicondylitis of humerus. Now we can recommend that periarticular sodium hyaluronate injection is an effective way of management in patients with lateral epicondylitis.

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Original Article

HISTOMORPHOLOGICAL STUDY OF MINOR SALIVARY GLANDS IN ADVANCING AGE

Ashiq Hussain, Muhammad Amin and Atiya Khalid

Objective: To study age related morphological changes in the minor salivary glands
Material & Methods: Thirty five specimens of minor salivary glands were collected from dead bodies of adults, both male and female, brought to the Forensic Department of King Edward Medical University Lahore for autopsy within twenty four hours of unnatural death. Dead bodies with surgical scars on body, lymphadenopathy & enlarged salivary glands were excluded from the study. Autopsy specimens were placed in five groups according to estimated age. Samples were collected, processed and stained with Hematoxylin and Eosin for histomorphometric studies under light microscope for diameter of acini, number of acini, number of intercalated ducts and number of interlobular blood vessels.
Results: Statistically significant changes in diameter of acini and mean number of acini were observed in different study groups with advancing age (p value = 0.000) while no significant change was observed in mean number of intercalated ducts and interlobular blood vessels.
Conclusion: With increasing age mean diameter and number of acini decreases while number of intercalated ducts and interlobular blood vessels remains unchanged in minor salivary glands.
Key words: Minor salivary glands, acini, intercalated ducts, interlobular blood vessels.

Introduction

Minor salivary glands (MSG) are located just beneath the mucosal surface of tongue, lips, palate and cheeks and open in the oral cavity by means of short ducts.[1] These glands are not present in gums and anterior hard palate. They are 450-750 in number and classified into four groups depending upon their location:[2,3]

- Lingual (anterior & posterior; von Ebner's glands)
- Palatal
- Labial
- Buccal

Anatomically there is no true capsule of glands and connective tissue septa separates glandular masses into lobules. Supporting connective tissue blends with that of adjacent connective tissue. The acini are made up of truncated pyramidal-shaped mucus cells, and some include serous cell components arranged as occasional demilunes. The lingual serous (Von Ebner's) glands are the only exceptions which are located in the posterior part of the tongue.[3-5]

The secretory cells contain basally located, oval nuclei having denser chromatin material & secretory granules lie apically. Rough endoplasmic reticulum is present in the basal cytoplasm and Golgi complexes are apical in location or lateral to the nucleus. Mucin is the main product of these cells; few other macromolecules are also secreted but rate of flow is much lower than serous cells.[4]

Intercalated ducts are the first ducts into which saliva drains from secretory elements. These ducts are lined with cuboidal cells, which have round, centrally located nuclei. Myoepithelial cells may be located at the basal side of the intercalated ducts. These cells are usually fusiform in shape and are oriented lengthwise along ducts.[5] Presumably, they support the acinar and ductal cells and help them in expelling actively the ductal contents.[6] The main excretory ducts are lined with pseudostratified columnar epithelium until their opening into oral cavity.[7]

The people with hyposalivation may have problem in eating dry foods, denture retention and develop denture sores. The patients also complain of tongue sticking to the palate, halitosis, a chronic burning sensation and intolerance to spicy foods.[8]

The incidence of age related diseases increases rapidly throughout the world in elderly individuals. Among the common problems that have a significant impact on their quality of life are xerostomia, salivary gland hypofunction and edentulism.[9,10]

Dry mouth or Xerostomia the subjective feeling of oral dryness, is a major complaint of many elderly individuals; although they seek medical help, it usually provides no adequate relief.[11] Complaints of xerostomia are more common at night because salivary production is at its lowest circadian level during sleep; the problem may be compounded by mouth breathing.[8]

With regard to salivary gland morphology and composition of saliva, age-related changes have been

reported in healthy individuals. In the salivary glands of the elderly, regardless of disease or medications, there is a substantial age-related replacement of the functional parenchymal tissue by non functioning adipose and fibrous tissue, while the proportional volume of acini is reduced, accompanied by a reduction in the salivary flow rates and in composition.[12,13]

Age related changes in the salivary glands are under the control of genes which are either up regulated or down regulated. Although sufficient literature is available regarding age related changes in major salivary glands yet meager research work has been performed to identify changes in minor salivary gland with age.

This study was aimed to establish the histomorphological changes that occur in the parenchyma of minor salivary glands with advancing age in the absence of any factor which predisposes to their atrophy.

Material & Methods

This descriptive study was conducted in Anatomy & Forensic Departments of King Edward Medical University, Lahore. Thirty five specimens of labial minor salivary gland were collected from dead bodies of adults, both male and female, brought to the Forensic Department of KEMU Lahore for autopsy within twenty four hours of unnatural death. Dead bodies with surgical scars on body, lymphadenopathy and enlarged salivary glands were excluded from the study. Autopsy specimens were placed in five groups A, B, C, D and E according to estimated age. **(Table-1)**

Table-1: Age distribution.

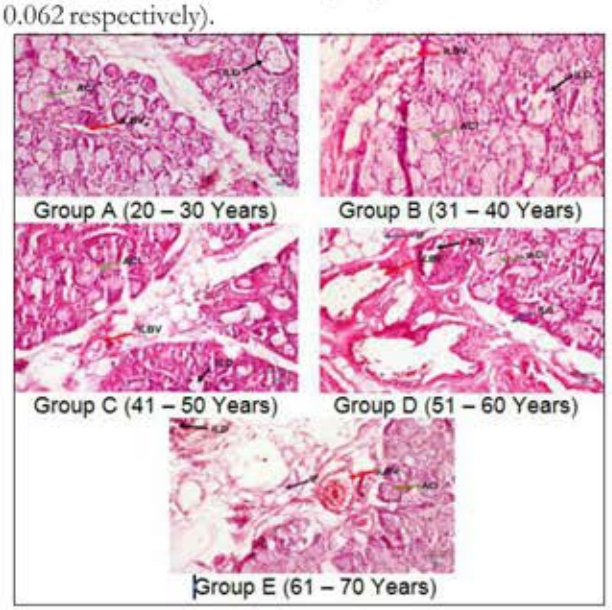
Group	Age Range
A	20- 30
B	31- 40
C	41- 50
D	51 - 60
E	61 - 70

Specimens from labial glands were collected from mucosal surface of middle of the lower lip by giving horizontal incision parallel to red area of lower lip. Collected samples were processed and stained with Hematoxylin and Eosin for histomorphometric studies under light microscope and at random non overlapping fields of each slide were studied for diameter of acini, number of acini, number of intercalated ducts and number of interlobular blood vessels.

Results

The mean diameter of acini with SD in labial glands in group A, B, C, D and E was 75.00±2.24, 72.14±5.67, 64.28 ±6.87, 36.96±1.89 and 28.46±4.18 respectively. The mean number of acini in labial glands with SD in group A, B, C, D, and E were 30±1.63, 26.43±1.72, 18.29±0.76, 14.29±0.73 and 10.86±0.69 respectively. A statistically significant difference in mean diameter of acini and mean number of acini in labial glands of different groups was observed with advancing age (p-value=0.000).

The mean number of intercalated ducts in labial glands in group A, B, C, D, and E were 3.14±0.89, 3.10±0.57, 2.99±0.53, 3.00±0.53 and 2.89±0.48 respectively. The mean number of interlobular blood vessels with SD in labial glands of groups A, B, C, D and E were 3.04±1.00, 3.00±0.53, 2.99±0.69, 3.00±0.95 and 2.98±0.48 respectively. The mean number of intercalated ducts and mean number of blood vessels in labial glands did not show any statistically significant change (p-value=0.074 and 0.062 respectively).



Discussion

Age related morphological changes in parenchymal and stromal components of labial minor salivary glands were examined under the light microscope. A substantial decrease in total mean diameter of acini of labial glands with advancing age was seen in present study which could be due to different and independent behavior of acini with ageing which

can be explained in terms of reduced secretions from the minor salivary glands in old age.

Our findings were consistent with the findings of Azevedo et al, who also documented age related decrease in size of acini of MSG whereas Vered et al, studied age related histomorphometric changes in labial salivary glands with special reference to acinar components also showed similar changes in acini with advancing age. Statistically significant variations were noted in mean diameter of acini among different study groups (p-value=0.000) and the trend was decreasing as age was increasing.[14,15]

The mean number of acini in labial gland showed a decreasing pattern with increasing age. It also showed statistically significant decrease in number of acini. (p-value = 0.000). The number of acini showed a general trend of age related decrease which might be due to infiltration of fibrous tissue as well as adipose tissue replacing the acini. Our findings were in accordance with the findings of Dayan, who also noted that the mean number of acini decreased with ageing. He observed that there was 48% decrease in number of acini in minor salivary glands in old age group which was due to replacement of parenchyma by fibrous tissue and lymphocytic infiltrate.[16] Similarly Drummond & Chisholm[17] and Scott in separate studies observed 44% decrease in number of acini in minor salivary glands.[14-17]

Although the adipose tissue increased significantly with age, the increase was less than that of connective tissue. Vered et al (2000) stated that there was more increase in adipose tissue in minor salivary glands in aged people than fibrous tissue.

In present study the acinar tissue in minor salivary glands in ageing was mainly replaced by fibrous tissue and less by adipose tissue which was in accordance with the findings of De Wilde et al & Syrjanen.

Dayan reported an increase in infiltration of the lymphocytes in the ageing glands. Wilde et al in 1986 found no significant increase in lymphocytic infiltrate in minor salivary glands of elderly people while Drummond & Chisholm, Syrjanen claimed different severity of age related increase of lymphocytic infiltrate. In 1986 Nair and Schroeder explained this infiltrate as a part of mucosa associated lymphoid tissue in the oral cavity or as an age related autoimmune process.[14-20]

Among the stromal components, no age related changes in the intercalated ducts and vessels were observed. Generally, the total of mean numbers of intercalated ducts in different study groups of labial glands were 3.20±0.83. The intercalated ducts in these glands did not show any statistically significant change with advancing age as the p values were > 0.05 (p-value=0.074). In present study results of this parameter were in accordance with Buchner et al who observed no significant increase in mean number of ducts with aging while Wilde et al in his research reported that in areas with ageing of minor salivary glands number as well as diameter of the intercalated ducts were increased which was contrary to the findings of this study.

Drummond and Chisholm also found out that increase in ductal volume is a result of mild ductal dilatation and partly due to ductal hyperplasia whereas Wilde et al observed that increased ductal volume is not due to ductal dilatation rather it's a result of increased number of ducts and he supported observation by increased length density of interlobular ducts. The presence of duct like structures were visualized in the glands of elderly individuals by Azevedo LR and many research workers who labeled them as apparent or relative ducts. These were labeled as apparent because doubt existed about whether these structures were duct remnants or atrophic acini.[14]

The total mean number of interlobular blood vessels in different study groups showed no statistically significant change with passage of time (p-value= 0.062). The findings of current study were consistent with the results of Vered et al who observed striking changes in the stromal component of the minor salivary glands with ageing like adipose tissue and lymphocytic infiltrate but no change in the number blood vessels while Azevedo et al noted that congested blood vessels increased with age. Drummond and Chisholm also found no change in number of blood vessels in minor salivary glands with ageing.[18-21]

Conclusion

With increasing age mean diameter and number of acini decrease while number of intercalated ducts and interlobular blood vessels remain unchanged in minor salivary glands.

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Original Article

Gender Perceptions and Barriers Towards Their Practice in Underserved Areas Among Medical Students: A Multicentre Study

Abhishek Singh, Pankaj Chikkara, Yogesh Kumar, Diganth C Divya, Shwetank Goel, Shelesh Goel, Anu Bhardwaj and Nand Kishore Singh

Objective: To understand & explore the perceptions of medical students towards their intention to practice in underserved areas and to identify the barriers restricting them to take up rural service.

Material & Methods: I2738 medical undergraduate students from five different medical institutions were requested to fill questionnaires during May 2012 to April 2013. SPSS version 17 was used for analysis. Unpaired t test and Chi-square (χ^2) test were applied.

Results: Majority 1719 (64.6%) were not willing to practice in rural area. Rural-background students were more likely to indicate willingness for rural practice ($p<0.001$). 'Easy/stress free life' and 'being respected as a doctor' were gender wise statistically significant potential benefits of working in a rural area. Connectivity problems, absenteeism of support staff, available living facilities, distant hometown, social life, low recognition of work, prestige of the job, sense of fulfilment, lack of good physical work environment, security problems and lack of recreational facilities were found to be statistically significant ($p<0.001$). Seventy seven percent of males and almost seventy five percent of females identified "low salary" as an underlying factor.

Conclusion: In spite of having positive view towards the importance of rural health care, certain aforementioned barriers prevent medical students to serve in rural areas. The findings can be utilized to design or modify the specific strategies to tackle the crisis of doctors in rural India.

Key words: Medical students, rural service, barriers.

Introduction

The global problem of the uneven distribution of the health workforce between cities and villages, with its severe consequences on health outcomes in rural areas, is also marked in India. Despite more than a half century of proclamations on primary healthcare, most rural facilities in India continue to lack enough providers. Addressing the scarcity of medical practitioners in rural India is fundamental to achieving universal health care in the country.[1]

The paucity of qualified health workers in rural areas is a critical challenge for India's health sector.[2] There is no doubt that the imbalance of doctors in rural and urban areas needs correction. Density of doctors in India is 6 for a population of 10,000.[3] India finds itself ranked 52 of the 57 countries facing Human Resources for Health crisis.[4]

Medical professionals also have an essential ethical obligation to help distribute equitably the life-enhancing opportunities affordable by healthcare.[5] Today's medical student is tomorrow's health care provider either in urban or rural setup. Therefore it is very essential to understand and explore the perceptions of medical students towards their intention to practice in underserved areas and to identify the barriers restricting them to take up rural service.

Material & Methods

The present cross sectional study was carried out during May 2012 to April 2013 among medical undergraduate students from five different medical institutions situated in four different states (UCMS-Delhi, PGIMS & MMIMSR- Haryana, SKIMS- J&K, KMC- Karnataka) using self-administered questionnaire.

The study population consisted of MBBS students who were currently studying in respective medical colleges. At the time of study there were 4 batches (first year to final part 2) of medical students with varying numbers depending on institutional admission capacity in each medical college. All these students formed the study population. Those students who could not be contacted after three attempts were excluded from the study. Informed consent was taken and complete confidentiality was ensured. Students were explained about the nature

and purpose of study and requested to fill the questionnaires which were distributed by authors in the classrooms just after the completion of lectures. The time allocated for the completion of the questionnaire was 15 minutes. Out of total 2738 students approached, 2660 returned the completed questionnaires. The questionnaire was framed for the purpose of recording socio-demographic profile including the personal characteristics of the study participants. Educational level of parents, residential status, family background, familiarity with rural areas and other relevant data was captured. Potential benefits or drawbacks of working in rural setup were probed in great detail.

The collected data was entered in Microsoft Excel. Coding of the variables was done. SPSS version 17 was used for analysis. Interpretation of the collected data was done by using appropriate statistical methods. Unpaired t test was used to assess the significance of difference between the mean age of respondents and their willingness to practice in rural areas. Chi-square (χ^2) test was applied to test the statistical difference in gender perceptions.

Results

Of the 2738 students approached, 57 students refused to participate. The response rate was 97.9%. Out of total, 21 questionnaires were discarded during data analysis because of incomplete information. Out of total 2660 students, 1629 (61.2%) were males. Respondents' mean age was 22.4 ± 1.1 years.

Differences in the characteristics of those willing and those unwilling to practice in rural areas were explored. Out of total 2660 students, majority 1719 (64.6%) were not willing to practice in rural area. Students whose parents were educationally well qualified were significantly less likely to serve in rural areas. Rural-background students were more likely to indicate willingness for rural practice. ($p<0.001$) **(Table 1)**

Table-1: Profile of study subjects and their willingness to practice in rural areas

Characteristic Variables		Willingness to Practice in Rural Area Yes n (%) 941 (35.4)	No n (%) 1719 (64.6)	p value
Age (years) - mean		22.2	22.6	0.17
Gender	Male	524 (32.2)	1105 (67.8)	0.000**
	Female	417 (40.4)	614 (59.6)	
Location of medical college	Delhi	92 (22.9)	310 (77.1)	
	Haryana	422 (35.5)	768 (64.5)	0.000**
	Jammu & Kashmir	51 (29.8)	120(70.2)	
	Karnataka	376 (41.9)	521 (58.1)	
Father's education level	≤Graduate	591 (39.0)	925 (61.0)	0.000**
	≥Post graduate	350 (30.6)	794 (69.4)	
Mother's education level	≤Graduate	693 (40.1)	1036 (59.9)	0.000**
	≥Post graduate	248 (26.6)	683 (73.4)	
Residential status	Living with parents	364 (33.4)	726 (66.6)	0.07
	Living away from parents	577(36.8)	993 (63.2)	
Family background	Rural	314 (53.6)	272 (46.4)	0.000**
	Urban	627 (30.2)	1447 (69.8)	
Familiarity with rural context	Familiar	379 (33.9)	738 (66.1)	
	Not well familiar	562 (36.4)	981 (63.6)	0.18
Perception of current status of	Satisfactory	139 (32.8)	285 (67.2)	
rural health services in India	Unsatisfactory	802 (35.9)	1432 (64.1)	0.22

*$p<0.05$, **$p<0.001$*

Table-2: Potential benefits of working in a rural area as perceived by the students willing to practice in such areas.

Gender perceptions	Male n (%)	Female n (%)	p value
Health services for the poor	367 (62.8)	215 (71.0)	0.015*
Feeling of serving the nation	315 (53.9)	189 (62.4)	0.016*
Gain knowledge about rural people and diseases	212 (36.3)	136 (44.9)	0.013*
Easy / stress free life	423 (72.4)	181 (59.7)	0.000**
Being respected as a doctor	396 (67.8)	168 (55.4)	0.000**
Less competition so career opportunities are more	193 (33.0)	124 (40.9)	0.020*
Others	10 (1.7)	7 (2.3)	0.537

*Males (n=584), Females (n=303), *p<0.05, **p<0.001*

Table-2: Potential drawbacks of working in a rural area as perceived by the students not willing to practice in such areas

Gender perceptions	Male n (%)	Female n (%)	p value
Infrastructure facilities are grossly lacking	810 (65.8)	382 (70.5)	0.053
Opportunities for development of children (availability of good schooling, extra activities, future opportunities)	705 (57.3)	342 (63.1)	0.021*
Lesser opportunities for career growth (opportunities regarding learning, training, research and higher education)	559 (45.4)	276 (50.9)	0.032*
Financial attributes (low salary)	948 (77.0)	403 (74.4)	0.226
Connectivity (transport availability, sense of isolation)	707 (57.4)	234 (43.2	0.000**
Family's well-being and comfort (spouse job availability, spouse career growth, support to parents)	741 (60.2)	293 (54.1)	0.015*
Absenteeism of support staff (helping hands for working)	530 (43.1)	174 (32.1)	0.000**
Limited professional contacts and experience	702 (57.0)	275 (50.7)	0.014*
Living facilities (lack of hygiene and sanitation, housing, electricity, water)	892 (72.5)	349 (64.4)	0.000**
Have to live away from family (distant hometown)	923 (74.9)	293 (54.1)	0.000**
Social life (entertainment facilities, social circle)	700 (56.9)	234 (43.2)	0.000**
Low recognition of work, prestige of the job, sense of fulfilment	467 (37.9)	127 (23.4)	0.000**
Lack of good physical work environment (furniture, toilet etc.)	718 (58.3)	244 (45.0)	0.000**
Security (possibility of problems at night, afraid of working alone)	582 (47.3)	301 (55.5)	0.001**
Low standard of living, limited technology	765 (62.1)	296 (54.6)	0.002*
Lack of recreational facilities	744 (60.4)	248 (45.8)	0.000**
Others	17 (1.4)	11 (2.0)	0.312

*Males (n=1231), Females (n=542), *p<0.05, **p<0.001*

Discussion

The provision of rural healthcare services through trained doctors is a real threat and challenge for India. Others[6] also have observed an acute deficiency of health workers in rural areas, particularly the physicians. Doctors believe that not only does rural medical service fail to improve access to healthcare in these areas, it also requires personal sacrifice. And they ask: why are medical students expected to make greater sacrifices than other professionals?[5]

The present study showed that the majority (64.6%) of medical students were not willing to serve in rural or underserved areas. Similar results were observed by Saini NK et al.[7] Most (84.1%) of respondents believed that the current status of rural health services was unsatisfactory.

It was observed in the present study that female doctors were found more motivated (40.4%) to serve in rural areas than the male counterparts (32.2%). It comes in contrast with the findings of Khan AR from Malaysia.[8] Both female and male students were equally motivated to work in rural communities in his study.

Not surprisingly our study shows that rural background students were more likely to be willing to practice in rural areas than those from urban areas. Similar findings have been observed by previous studies among medical students.[9,10] Doctors who feel aggrieved at being forced to serve in rural areas are unlikely to fulfil their obligations to the people there.[5]

Classroom or bedside teaching does not inculcate empathy or compassion among students. Nor does it sensitize them to the real needs of the rural community. Young doctors posted in rural areas often lack insight into the socioeconomic determinants of diseases and do not know how to treat these diseases economically and effectively. Students fear that the time taken by rural postings hinders their efforts to achieve their career goals.[5]

On being asked about the potential disadvantages of working in a rural area, the commonest mentioned were 'lack of infrastructural facilities', 'less salary','low standard of living', and 'limited exposure as a doctor'. Wilson NW[11] suggested similar factors in order to redress the inequitable distribution of healthcare professionals to rural and remote areas.

77% of males and almost 75% of females identified "low salary" as an underlying factor which prevented them from taking up rural positions. Others[12] also concluded the same. Most students admitted to the medical colleges especially to private medical colleges are required to personally finance their expensive education. Can one really expect students who have made what is essentially an investment to forget about money and think of their professional ethics and social obligations?[5] Another study by Shankar PR from Nepal[12], on attracting and retaining doctors in rural service concluded that the government should invest in improving working conditions in rural areas.

Conclusion

The findings of the present study highlight the positive view regarding the importance of rural health service among surveyed medical students. However, certain factors such as lack of infrastructure and low salary were perceived as potential barriers to take-up rural service. The findings can be utilized to design or modify the specific strategies to tackle the crisis of doctors in rural India.

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Original Article

Pathological Complete Response Of Neo-adjuvant Chemotherapy (NACT) Doxorubicin Plus Cyclophosphamide In Patients With Locally Advanced Breast Cancer at Jinnah Hospital Lahore

Saleha Kanwal, Sara Saeed and Muhammad Akram

Objective: To determine the frequency of pathological complete response (pCR) with neo adjuvant doxorubicin and cyclophosphamide in locally advanced breast cancer (LABC).

Material & Methods: This prospective study was conducted in 92 patients of locally advanced breast cancer. Pathological response was evaluated on modified radical mastectomy (MRM) samples that was performed after 4 cycles of neo adjuvant doxorubicin and cyclophosphamide.

Results: The mean age of the study population was 45.63 years. Among these 38 patients (41.3%) had stage T3 lesion, 53 (57.6%) had T4 and only one patient (1.1%) had T1 at presentation. Ninety patients (97.82%) were with grade 2 and grade 3 tumors. Post anthracycline based NACT 8 (8.7%) patients had pathological complete response, 50 (54.3%) had partial response, 34 (37%) had stable disease. Overall 58 (63%) patients had responded to this treatment.

Conclusion: Anthracycline based NACT is a good option for patients with locally advanced breast cancer in developing countries. The results are not comparable with developed countries but better results can be achieved if these patients present at an early stage or taxanes based NACT is used to improve the response.

Key words: Breast cancer, Anthracycline based NACT, Pathological complete response

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females with estimated new cases of 229,060 in United States in 2012.[1] About half of the breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries.[2,3]

Approximately one in every nine of Pakistani women will suffer from breast cancer at some point in their lives.[4] Breast cancer incidence in Pakistan is the highest reported in any South-Central Asian country. It accounts for 38.5% of all female cancers and about half (43.7%) of all breast cancers are locally advanced.[5] In women diagnosed with LABC in Pakistan, patients with lower socioeconomic status (SES) have larger, more aggressive tumors with worsened survival outcomes.[6]

Locally advanced breast cancer (LABC) includes non-metastatic tumours more than 5 cm in size or involving skin/chest wall. It may be associated with fixed axillary lymph nodes, ipsilateral supra-clavicular, infra-clavicular or internal mammary nodal involvement.[7]

The pCR is defined as absence of the invasive carcinoma on the pathological examination of the breast tissue and axillary lymph nodes.[21] Complete response to chemotherapy is associated with longer disease-free survival (DFS), overall survival and surrogate marker of long term prognosis when compared to non-responders.[9,22,23]

The established data shows Clinical Complete Response (CR) in the range of 7 to 65% and pathological CR (pCR) in 4 to 29%.[8-14] The variation in pathological response is linked with receptors status, grade and tumor characteristics.[15-20]

Material & Methods

It was a descriptive case series, conducted in Department of Oncology, Jinnah Hospital, Lahore. After non probability purposive sampling a total of 92 cases over a period of one year were enrolled. The sample size was calculated with 95% confidence level, 10% margin of error and taking expected percentage of pathologic complete response i.e: 13% of neo-adjuvant chemotherapy with Doxorubicin plus Cyclophosphamide in locally advanced breast cancer.

Women between 20-70 years of age with locally advanced breast cancer, who were having no co-morbidities or previous history of treatment were included. They were given 4 cycles of neo adjuvant chemotherapy doxorubicin 60mg/m^2 and cyclophosphamide 600mg/m^2 q 3 weekly. MRM with axillary clearance was done 6 weeks after the last cycle of chemotherapy.

Data collected on proforma was entered into the Statistical Package for Social Sciences (SPSS) version 10 software. Quantitative variables like age were presented as mean and standard deviation. Qualitative variables like gender, disease response (complete, partial, or no response) were taken as frequency and percentage.

Results

Total 92 patients were enrolled in the study. In 8 resected specimens, no viable residual tumor cells could be identified so the tumor was staged pathologic (p) T0N0 and pathological complete response pCR was concluded as 8.7% (**Table 4**). The overall pathological response was seen in 58 (63%) patients (complete and partial), while 34 (37%) patients did not respond to treatment.

Patients were categorized as pre and post menopausal groups (age <45yr and ≥45yr).

Out of 49 (53.1%) pre-menopausal women 37 (75.5%) showed response to treatment .There were 43 (46.6%) post-menopausal women, 21 (48.8%) of them had response to therapy. Pre-treatment staging of the tumor showed that 53 (57.6%) patients were having T4 lesion and 48 (52.2%) patients were N2. Out of 8 resected samples with no viable malignant tissue 7 were T4 lesions and 1 was T3 lesion (p= 0.05).

Response rates in receptor positive group were significantly higher than receptor negative group (49.9% vs 12.9% p=0.02).Triple negative patients were 39 (42.3%). 21(22.7%) of them showed complete /partial pathological response while 18 (19.5%) patient did not show any response.

Majority of the patients enrolled in study were

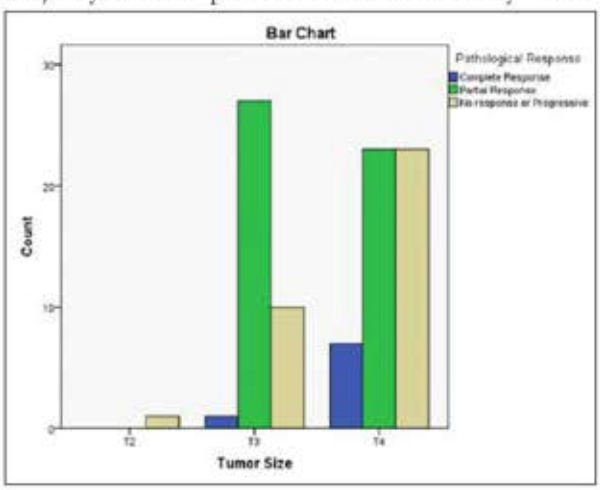

Fig-1: Pathological responses in various tumor sizes.

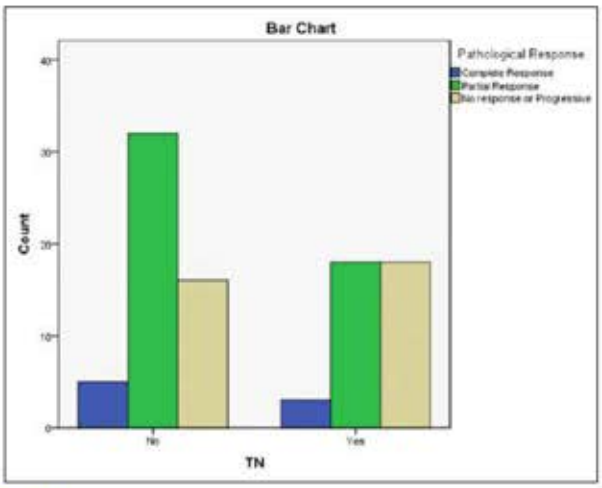

Fig-2: Pathological responses in triple negative breast cancer.

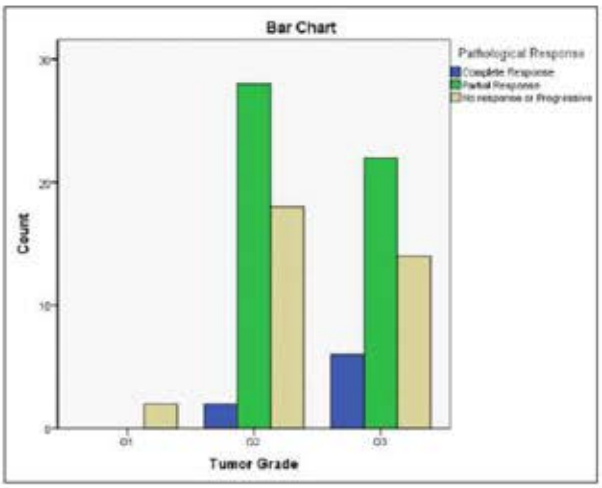

Fig-3: Pathological responses in various tumor grades.

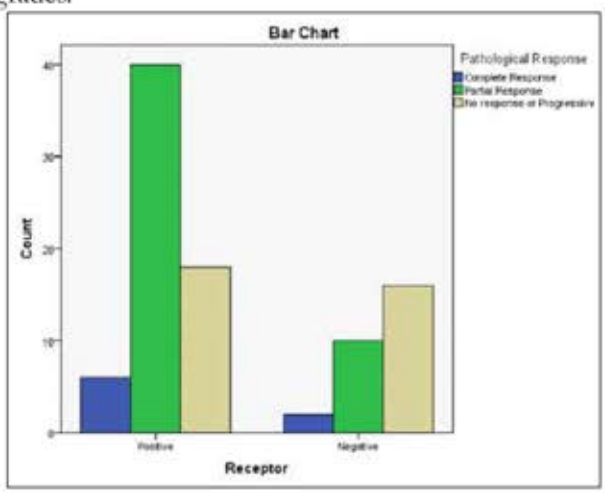

Fig-4: Pathological responses in receptor positive and negative tumors.

clinically N1,N2. Fifty eight (63%) patients with N1/N2 showed response (complete or partial) (p= 0.3) to the treatment. There was only one patient with N0 (**Table 1**).

Most of the patients who responded to treatment were having grade 2 and grade 3 tumor. Eight (8.6%) patients showed pathological complete response to treatment were having grade 2/3 tumor. Fifty (54.3%) patients showed partial response who were with grade 2/3 tumor (**Fig. 3**).

Table-1: Response rates according to demographic and baseline tumor characteristics.

Response		pCR[a]	pCR[b]	PD[c]/SD[d]	p Value
Age (Median)	<45 years (n=49)	3.2% (3)	36.9% (34)	13% (12)	
	≥45 years (n=43)	5.4% (5)	17.3% (16)	23.9% (22)	
Receptor Status	Receptor status +ve	6.5% (6)	43.4% (40)	19.5% (18)	0.02
	Receptor status -ve	2.1% (2)	10.8% (10)	17.3% (16)	
	TND[e] (n=39)	3.2% (3)	19.5% (18)	19.5% (18)	
Tumor Size	T3 (n=38)	1.08% (1)	29.3% (27)	10.8% (10)	0.05
	T4 (n=53)	7.6% (7)	25% (23)	25% (23)	
Nodal status	N0 (n=1)	0	0	1.08%(1)	0.30
	N1 (n=41)	3.2% (3)	28.2% (26)	44.5% (41)	
	N2 (n=48)	5.4% (5)	26% (24)	52% (48)	
	N3 (n=2)	0	0	2.1% (2)	
Grade	G1 (n=2)	0	0	2.1% (2)	0.30
	G2 (n=48)	2.1% (2)	30.4% (28)	19.5% (18)	
	G3 (n=42)	6.5% (6)	23.9% (22)	15.2% (14)	

A. pCR Pathological Complete Response B. pPR Pathological Partial Response C. PD Progressive Disease D. SD Stable Disease TND Triple Negative Disease

Discussion

LABC accounts for 40-60% of all breast cancers in developing countries.[4,5] Pakistan has highest prevalence of breast cancer in Asia especially in young women.[2,3] 10 years data of local cancer hospital in Karachi showed that 58% patients present with locally advanced breast cancer.[12]

In India, between 50% and 70% of patients have locally advanced or metastatic disease at diagnosis. This proportion is high compared with developed countries, where 38% of European and 30% of US breast cancer cases were reported to be either locally advanced at diagnosis or lymph-node positive. 50% of patients with breast cancer in Egypt are reported to be diagnosed with invasive tumors that are larger than 4·5 cm.[22] In USA 40% of invasive breast cancers are diagnosed when tumors are smaller than 1 cm.[4,5] In current study 53 (57.6%) patients were with T4 and there were 38 (41.3%)with T3 lesion.

Clinical and pathological response of breast cancer to NACT is a short-term marker for a long term outcome.[15] LABC is usually inoperable and neo-adjuvant systemic therapy (NACT) generally is nearly always indicated. The goal of neoadjuvant systemic therapy is to induce tumor response and facilitate local control through surgical resection and radiation therapy.[24] NACT also provides the earliest possible treatment of micro metastases and thus improves survival.[25]

Nodal involvement at presentation is associated with a higher risk of a locoregional recurrence (HR 1.61, 95% CI 1.28-2.02).[14] In current study there were 41(44.57%) patients with N1, 48 (52.2%) with N2 and 2 (2.2%) with N3 disease. In contrast to western world where they detect breast cancer early due to screening and better health facilities and in most studies more than 50% patients have no nodal involvement at presentation,[15] this high percentage of nodal involvement in current study indicates the need for screening and early detection.[4,5] Tumor receptor status (ER, PR) in our patients was more or less similar to the western data available.[24]

In current study pCR and pPR was seen in 8.7% and 54.3% patients respectively with AC regimen, but in

studies done in western world the CR and PR rates are 13% (pCR) but 40% (pPR) with the same regimen.[13,14] This observation shows that many of our patients responded to treatment in terms of partial response. The reason for high response rates in these studies was that patients were having early stage disease with N0, N1 status.

The pathological response is better when taxanes are added into it as NACT. The cPR improves to 26% with taxanes.[13] The pCR rate with carboplatin and paclitaxel is up to 31% in patients who were HER2-positive and 67% in ER-negative patients.[2,3] In contrast, in patients with ER-positive, HER2-negative disease, the pCR rate was 12%.[2] AC regimen proved effective in this study in terms total pathological response though the cPR was inferior to the published data. For unknown reasons the response rates were high in post menopausal and receptor positive groups. Hormone receptor status seems to be predictive of relative chemoresistance; multiple trials have shown that the probability of achieving a pCR is significantly inferior in tumors expressing hormone receptors.[26] The previous available data for response in different receptor groups is controversial.[15-17] It is the level of expression of ER and progesterone receptor that might be correlated with the probability of response to neoadjuvant chemotherapy.[27] So for any receptor status response to NACT is difficult to comment on.

High-grade tumors are more responsive to chemotherapy.[18-20] Similar trends were observed in the current study. 63%[18] of Grade II & Grade III tumors showed pathological response (complete/ partial) to NACT.

Conclusion

It is concluded from this study that Anthracycline based NACT is good option for patients with locally advanced breast cancer in developing countries. Though the results are not comparable with developed countries, better results can be achieved if these patients present at an early stage. Moreover taxanes can be incorporated in NACT to improve the response. Further research with other available options is also needed. We should focus more on screening and prevention modalities so as to deal with this problem at very first step.

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Case Report

LIGNOCAINE TOXICITY IN BURN CASES

Muhammad Asim and Saeed Ashraf Cheema

Abstract: Pain is one of the constant features of burn injuries. Many protocols are utilized to alleviate pain in these patients. Lignocaine containing ointment and fusidic acid in combination with hydrocortisone cream are routinely used as topical application in the wound care of burn patients in our setup. During a period of nine years, five patients experienced adverse effects of lignocaine, active ingredient of ointment, which were immediately controlled once this drug was discontinued. All these patients, who were being treated for burn injury involving more than 20% BSA (Body Surface Area), responded well on excluding the lignocaine from treatment medication and did not require any anticonvulsant to treat these adverse effects.

Key words: Burns, Toxicity, Lignocaine toxicity.

Introduction

Pain is a constant element of partial thickness burn injuries.[1] Initial pain is the result of massive nerve stimulation in the affected area and inflammatory mediators like bradykinin, histamine, substance P lead to continuous stimulation of nerve endings.[2]

Mechanical interventions in the area in the form of debridement, change of dressing, change of position and physiotherapy cause amplification of painful and normally non painful stimuli mediated by peripheral and central mechanisms. Additional factors, such as anxiety and depression in burn cases are inevitable and further lower pain threshold. Burn dressings, along with being a barrier against infection, can provide some wound relief. Honey has been described to reduce the element of pain when used as a dressing.[3] Commercially available dressings like hydrocolloids, calcium alginate and semi synthetic skin substitutes also definitely reduce pain.[4]

Although intravenous narcotics remain the main analgesic element, we have been using a combination of cream (containing fusidic acid and hydrocortisone acetate, available with different names) and an ointment (containing neomycin, zinc bacitracin, polymyxin B sulphate and lignocaine also available in the market with registered name) to dress the burn wounds. Whereas, the hydrocortisone content of the cream is classified as a mild corticosteroid and helps decrease the inflammatory response in the skin, ointment has a combination of three antibiotics and the lignocaine content acts as a topical anaesthetic agent. Combination of cream and ointment also helps this application to stay longer on the surface which would be difficult if only cream and gel form of the products are utilized. This combination not only helps reduce the element of pain and thus use of narcotic analgesics, but also helps granulation and epithelialization of the area smoothly and rapidly due to its decreased inflammatory response. This paper details our experience with this dressing and also lignocaine toxicity in five of our cases.

Methodology

Burn cases are admitted under the care of plastic surgeon. After routine first aid and initial resuscitation measures the wound is completely examined and washed with saline and pyodine scrub. As routine the blisters are left undisturbed. As a policy, burns of the non vital areas which are less than 20% BSA are treated on outdoor basis. These cases are treated at home with close follow ups. Also these patients are advised to contact on phone and daily out patients clinic in case of any query. The standard treatment, for both the indoor or outpatients, during the summer season, consists of one daily wash with pyodine scrub and frequent applications of lignocaine containing ointment and fusidic acid and hydrocotisone cream.

The patient attendants are advised to clean any portion of the application which, in due course of time, turns yellow or changes its color, wiping it gently with antiseptic wipes, and re-applying fresh combination of ointment and cream.

Most of the fresh burn cases have been treated successfully with this regimen over the past nine years. This study involved no conflict of interest and was not funded by institute, pharmaceutical or any other body. Written consent of patients was taken for treatment. During this period, however, we have come across five cases of lignocaine toxicity. These cases are being discussed individually.

Case No 1.

About two years ago, the first case of neurotoxicity was identified in a child of five years of age. This

patient had 30% BSA scalds involving both lower limbs, buttocks and lower back. On the second day, he experienced generalised fits of mild to moderate degree. He experienced fits twice after fresh application. Immediate lab tests were performed to rule out the possible cause of these fits. Case was discussed in detail with pediatrician who is a routine member of the team. After detailed history and thorough examination all other known causes of such fits were excluded. The only factor remained was the toxicity caused by lignocaine. Case was provisionally diagnosed as lignocaine toxicity. The application regimen was changed to an ointment not containing the said drug, and no other anti-convulsant medicine was added. Immediately after this change patient stopped having the fits which further supported provisional diagnosis. Finding made us cautious and we were on the watch-out for any such happening. Lignocaine, however, did remain the primary choice because of the local anaesthetic effect.

To date five patients in total have had such fits, and all except one child had no history of any kinds of fits in the past. The one child had a history of febrile fits.

Case No 2.

Second patient was again a child of five years of age with mostly second degree scalds of 30% BSA. Areas involved included back, right lower limb and left buttock. Patient was on routine medication and local application. He experienced three fits with an interval of three hours between the fits. As we had the experience with first case, the lignocaine containing ointment was omitted from the treatment protocol and fits subsided. Rest of the course of the disease was uneventful with eventual complete recovery.

Case No 3.

Third patient was a male, 28 yrs of age, with caustic burns over 45% BSA. Areas involved both lower limbs, and parts of both forearms and abdomen. He was a known drug addict. He also developed fits on third day of treatment. Lignocaine was omitted from the treatment and he did not experience any more fits.

Case No 4.

Fourth patient was a 58 years old female, grossly obese, with flame burns of over 60% BSA. She had involvement of front of chest and abdomen, thighs and legs, face, perineum, and parts of back of trunk and thighs. She developed generalized fits on second day of treatment.

Case No 5.

Fifth patient was a 2 years old baby girl. She presented with scalds over 25% BSA involving the whole right lower limb, right buttock and right side of trunk. She developed fits on second day of treatment which disappeared after the lignocaine was removed from the medication. In all these patients, switching to a non lignocaine containing medication took care of fits.

Discussion

Burns are quite common injury in our society. Most of them are accidental, partly due to lack of caution while handling hot objects, and especially so, near children. These injuries are very painful. Prime goals to treat these injuries include alleviating pain, to stop the wound from progressing in size and depth, to hasten the granulation and restore early skin cover. Whereas the criteria for first aid, empirical use of antibiotics and pain killers are quite clear universally, different approaches exist for wound care. List of local applicants for these wounds is quite long one, starting with silver containing preparations to steroids, papaya, honey, amnion, meshed potato, synthetic and semi synthetic dressings etc.[5,6]

Every one of these applications has its own advantages and disadvantages. Although in the initial learning curve we have regularly been using silver sulphadiazine for local application but for last nine years we have changed our wound dressing and local application protocols all together. Although role of steroids has always been controversial but, during experience of nine years, we have witnessed that it does reduce the inflammation of the area and helps in smooth and earlier granulation of the involved areas. We are treating the burn injuries with combination of fusidic acid & hydrocortisone cream and an ointment containing lignocaine with combination of antibiotics. Cream with these components is available with different names and lignocaine ointment with this combination of antibiotics is also freely available in the market. Whereas lignocaine takes good care of the element of pain, steroid element leads to healthy flat firm red granulation and has a good anti inflammatory effect too. However, present study focuses on the toxicity of lignocaine only.

Lignocaine acts as local anaesthetic agent and thus reduces the element of pain. Intravenous lignocaine has also been recommended for treating the burn pain[7] but studies mention that it has not been

accepted widely because of the feared adverse effects which mainly include excitation of the central nervous system causing nervousness, nausea and convulsions.[8]

Literature mentions that local side effects of lignocaine include bruising and temporary sensation of stinging or burning. Similarly the symptoms of systemic toxicity may include severe numbness or tingling, dizziness and drowsiness, tinnitus (ringing in the ears), slurred speech, metallic taste in mouth, mental status change, muscle twitching and convulsions. It has also been reported to be cardiotoxic.

In present series a total of five cases of one side effect i.e., generalized fits have been reported. These side effects were experienced both by males and females and both in children as well adults. However, as it is obvious, these were reported only in cases with burn injury exceeding 20% BSA. In the first case we had to rule out other causes of generalized fits and only then treatment plan was revisited but, once happened, we were ready for any subsequent event. It is also clear that we needed to stop local application of lignocaine alone and immediately the fits stopped. No anticonvulsant medication was given in all these cases.

Based on experience gained during last nine years and witnessing the fits in five of the cases it can be safely concluded that whereas lignocaine may be used for treatment of burn wounds, one has to be very careful once the open wound area exceeds 20% of the body surface area. In case this adverse effect is observed, immediate measure to be taken is to wipe out the ointment from the wounds and immediately stop further use of this medicine, while patient is being observed conservatively. In our series we did not use any anticonvulsant to reverse the adverse effects of the medication.

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