PMDC No. IP0042

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

Volume 12, Issue 03, July-September 2016

Esculapio

Journal of Services Institute of Medical Sciences, Lahore.

Mutational Analysis of HCV Gene Encoding E1 Glycoprotein

Outcome of Paediatric Kidney Transplant : An Experience From Lahore

Prediction of Oesophageal Varices in Cirrhotic Patients by Prothrombin Time As A Non-invasive Marker

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ESCULAPIO

JOURNAL OF SERVICES INSTITUTE OF MEDICAL SCIENCES, LAHORE.

VOLUME. 12 JULY - SEPTEMBER 2016 ISSUE. 03

PUBLISHED BY

SERVICES INSTITUTE OF MEDICAL SCIENCES, LAHORE.

ONLINE EDITION

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Review Article

SQUINT PUBLICATION IN OPHTHALMIC JOURNALS FROM PAKISTAN: A 10 YEAR REVIEW OF LITERATURE

Imran Khalid, Amtul Mussawar Sami and Khalid Waheed

Objective: To analyze the publication rate of squint, the most frequent study design used, nature of published articles and yearly breakdown of articles in two ophthalmic journals of Pakistan from 2006-2015.

Methods: All editorial, original articles, review articles, case reports published and available on the website of Pakistan Journal of Ophthalmology, Al-Shifa journal of Ophthalmology from 2006 to 2015 was analyzed to determine the publication rate of squint and study design used in different articles.

Results: Overwhelmingly four hundred and twenty seven (427) original articles published. Eighty six (86) case reports, fifty five (55) editorials, and no review article published on squint. Nine original articles (n= 9) 2.1%, one case report (n=1)1.2%, one editorial (n=1) 1.8% published on squint. In five different studies prospective study design was used, cross sectional, retrospective, case and control used in three different studies.

Conclusion: These findings revealed that publications on squint are less prevalent. Most frequent study design used in published articles on squint was prospective and the nature of articles mostly described the surgical aspects of squint.

Keywords: Squint, editorial, case report, original articles, Pakistan Journal of Ophthalmology (PJO). Al-Shifa journal of Ophthalmology (ASJO)

Introduction

Squint (strabismus) is commonly defined as misalignment of the visual axis. It may occur in horizontal and vertical forms or some time occurred in combined form (horizontal and vertical). It is more frequently affected one eye and visual loss may results in amblyopia (lazy eye). Earlier the treatment begun, shorten will be the duration. So that vision can be saved in earlier ages of life. Bile ZK et al studied that squint is second common disorder (545/3289, 17%) in children.[1] A study conducted at Bahawal victoria hospital showed that the rate of squint is 12.4% (124/1000)[2]. Khan A et al (1996) observed that out of 130 million, 7.02 million has squint (5.4%).[3] Squint prevalence is 2% in south India.[4] Shafique et al (2007) reported 59.9% amblyopia and density 23.6%.[5] Khalid et al (2012) reported the incidence of strabismic amblyopia is 26% and density 74%.[6] The squint rate is 17%, 12.4%,5.4%,2%[1-4] incidence is 59.9%,[5] density was23.6%, 74%[5,6] but there is paucity of scientific publications on squint. To our best knowledge, no study in Pakistan discussed the publication rate of squint ophthalmic journals. This study was conducted to find the publication rate of squint in two local ophthalmic journals to highlight the publication deficit of squint and to find the most frequent study design used nature of published articles in Pakistan journal of Ophthalmology (PJO) and Al Shifa journal of Ophthalmology (ASJO) are recognized by Pakistan medical and dental council[7] and Higher Education commission, were selected as the data easily accessible from their respective websites as per inclusion criteria from 2006 to 2015.PJO is an official quarterly journal of Ophthalmological society of Pakistan (OSP) and has X category.[8] ASJO is an official biannual journal of Al-Shifa trust eye hospital Rawalpindi has z category.[9]

Methods

Electronic research strategy was designed to retrieved the data from websites of Pakistan journal of Ophthal- mology and Al-Shifa journal of Ophthalmology www.pjo.com.pk, www.alshifajournal.org respectively from2006 to 2015.All editorial, original article, review article and case report were investigated to analyze the publication rate of squint. The case report and original articles related to squint were studied to elaborate the most frequent study design used in published articles. The nature of articles examined to see the publication trend of authors to find or investigate the medical or surgical aspects of squint. Yearly breakdown of articles published in both journals was noticed to find the interval in published literature.

Table-1: Distribution of Editorials, Articles and Case report in PJO.

Year	Editorials	Original Articles	Case Reports	Review Articles
2006	02	20	06	02
2007	04	34	08	-
2008	04	33	07	-
2009	03	35	04	01
2010	04	32	05	01
2011	04	37	04	01
2012	04	38	08	-
2 013	04	36	09	01
2014	04	39	08	-
2015	04	34	10	-

Table-1: Distribution of Editorials, Articles and Case report in ASJO.

Year	Editorials	Original Articles	Case Reports
2006	01	06	04
2007	02	07	02
2008	02	07	003
2009	02	10	-
2010	02	09	01
2011	02	10	02
2012	02	10	02
2 013	02	12	-
2014	02	12	02
2015	01	06	01

Table-3: Published Data on Squint in PJO and ASJO.

Pubished date	PJO	ASJO	Total	Squint Date Percentage
Editorials	37	18	55	1/55=1.8%
Original articles	338	89	427	9/427=2.1
Case Reports	69	17	86	1/86=1.2%

Table-4: Yearly breakdown of articles and case reports in PJO.

Year	Volume	Issue	Topic
2007	23	1	Incidence of amblyopia in strabismus population
2007	23	3	Recovery of post traumatic Brown syndrome (case report)
2008	24	1	Modified limbal incision, an easy and safe window for extra ocular muscle surgery
2009	25	3	Risk factors of strabismus in southwestern Nigeria
2012	28	3	Graded recession for primary inferior oblique overaction
2013	29	4	Adjustable suture in constant exotropia

Table-5: Yearly breakdown of articles and case reports in ASJO.

Year	Volume	Issue	Topic
2009	5	2	Adjustable suture strabismus surgery: Procedure of choice(editorial)
2009	5	2	The out comes of horizontal surgery
2013	5	2	Visual outcome in strabismic and anisometropic amblyopia after patching therapy
2013	09	1	Frequency of esotropia and exotropia among patients between 3 to 25 years of age
2015	11	1	Exotropia and relationship of orthoptic assessment of exotropia with its surgical outcome

Discussion

Four ophthalmic journals published in Pakistan; they are the Pakistan journal of Ophthalmology, Al Shifa journal of Ophthalmology, Ophthalmology update and Ophthalmology Pakistan. The PJO and ASJO was chosen for this study as their data met the inclusion criteria from 2006 to 2015 (10 years) available, easily accessible and can be retrieved from their websites. PJO has an open access to all editorials, abstracts and full length articles. Thirty nine (39) issues published in PJO, three issues in 2006 and four issues published every year till December 2015. Al-Shifa journal of Ophthalmology has an open access to all abstracts; full length article does not updated. Editorials had no access to be opened. Total fifty five (55) editorials published (thirty seven (37) in PJO and eighteen (18) in ASJO).Four hundred and twenty seven (427) original articles published three hundred and thirty eight (338) in PJO and eighty nine (89) in ASJO, eighty six (86) case reports (sixty nine (69) in PJO and seventeen (17) in ASJO), six (6) review articles in PJO but no review article published on squint.

One editorial published in ASJO but it had no access to be electronically retrieved.[10] One case report reported the acquired Brown syndrome in seven (7) year old male after trauma by donkey's hoof and complete recovery seen after three months.[11]

Nine original articles published on squint, five (5) published in PJO and four (4) in ASJO. Five articles described the surgical aspects of squint. Shafique MM et al described that modified limbal incision, an easy and safe window for extra ocular muscle surgery as compared to the muscle over approach.[12] Asim AA et al reported that Flink's method is an effective procedure for primary inferior oblique overaction.[13] Shakir M et al elaborated that adjustable suture is an easy, tolerable and effective in patients has constant exotropia.[14] Sikder MA et al described that62.9% successful Outcome of horizontal strabismus surgery.[15] Mahreen et al concluded that good surgical results will be obtained if surgery done in accordance with orthoptic assessment.[16]

A case and control study done in southwestern Nigeria showed that the hypermetropia was a significant risk factor of strabismus.[17] A hospital based study showed the incidence of amblyopia was 59.9% in strabismus population. Patching therapy was done on 50 subjects between 3 to 8 yearsin strabismic and anisometropic amblyopia and visual outcome was seventy eight percent (78%)[18].A cross sectional study showed that the frequency of esotropia andexotropia was 63% and27 %r espectively in 3 to 25 years.[19]

Yearly breakdown of articles in ASJO that in 2009 one editorial and two original articles published in a single issue[10,15,18]. With four years interval in 2009 third article[19] published. In 2015 fourth article published after a gap of two years[16].Yearly breakdown of articles in PJO was that the one original article[5] and one case report published in 2007.[11] With one year interval, second[12]articlespublished in 2008, third[17] article in 2009.With three years interval fourth article [13] published in 2012 and fifth article published in2013 after one year[14].In 2014 and 2015, eight issues were published but no article published on squint. Shafique MM was the leading principal author of two articles and co-author of one case report.[5,11,12]

In five different studies prospective study design.[5,12,15,16,18] was used, cross sectional[19], retrospective.[14] case and control[17]design used in three different studies. No study design was mentioned[13] in one article. The most frequent study design used was prospective. Randomized clinical trial was not used in the literature published in PJO and ASJO.

The rate of squint published in Pakistan Journal of Ophthalmology was1.5% (5/338) and Al-Shifa Journal of Ophthalmology was 4.5% (4/89). The resultant publication rate in two ophthalmic journals on squint is 2.1%. Kumar A et al in 2011, selected seven (07) top ophthalmic journals from 2005 to 2009 and analyzed that the sub specialty of squint is 2.3% (282/12426)[20]. This study was intended to inform the clinician about paucity of squint publications in ophthalmic journals. So, the result of this study is similar to Kumar A et al published in Ophthalmolgy

recognized journal of American academy of ophthalmology.

Conclusion

The publication rate of squint is 2.1%. There is need to increase publications on this topic. The authors must be encouraged to publish more articles that may help to find new horizons for clinicians to understand better diagnostic and therapeutic approach to manage the squint and prevent the child from strabismic amblyopia.

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References

1. Bile ZK, Ahmed S, Khan AA. Spectrum of ophthalmic diseases in children at a referral hospital. Pak J Ophthalmol 2007;23(1):
2. Farrukh S, Latif MA, KlasraAH, Ali M. Pattern of pediatric eye diseases. Pak J Ophthalmol 2015;31(3):146-49
3. Abbas M, RH, Butt AI, Ghani N. Prevalence and mode of presentation of vertical deviation in squint Pakistan. RMJ 2005; 30(2)79-81
4. Meundi AD, Athavale AV, Suruliraman SM, Anjan S, Gururaj MS, Dhabadi BB, Rekha R. Prevalence of ocular morbidities among school children in a rural area of south India. South American Journal of Medicine. 2014;2(2):.
5. Shafique MM. Ullah N, Butt NH, Khalil M,Gul T. Incidence of amblyopia in strabismus population. Pak J Ophthalmol 2007;23(1):1-6.
6. Khalid I, Tayyib M. Advanced analysis of strabismus amblyopia. Ophthalmolgy Pakistan 2012;2(3): 25-28.
7. Retrieved form: http://www.pmdc.org.pk/Home/IndexedPakistanofJournals/tabid/148/Default.aspx , on 02/03/2016.
8. Ahmed M. Evaluation of "Pakistan Journal of Ophthalmology" (ISSN: 0886-3067). DD (QA)/HEC/ EHI/2015/508, on 08/09/2016.
9. Retrieved from: http://www.hec.gov.pk/InsideHEC/Divisions/QALI/QADivision/Documents/Health%20Sciences%20Journal_July.pdf , on 02/03/2016.
10. Siddiqui NS. Adjustable suture strabismus surgery: Procedure of choice. Al-shifa J Ophthalmol 2009;5 (2)
11. Khalil M, Malik TG. Shafique MM. Moin M, RanaMK. One case report Recovery of post traumatic Brown syndrome. Pak J Ophthalmol 2007;23(3)
12. Shafique MM. Butt NH, Khalil M,Gul T. Modified limbal incision, an easy and safe window for extra ocular muscle surgery. Pak J Ophthalmol 2008;24(1):2-6
13. Asim AA, Hashmani S, jamil MA, Zaheer CM. Graded recession for primary inferior oblique overaction. Pak J Ophthalmol 2012;28(3):122-26
14. Shakir M, kamil Z, Zafar S, Bokhari SA, Rizvi F. Adjustable suture in constant exotropia Pak J Ophthalmol 2013;25(4):192-96
15. Sikder MA, Alam DM. The out comes of horizontal surgeryAl-Shifa J Ophthalmol 2009;5 (2) 63-66
16. Mahreen, Altaf S. Exotropia and relationship of orthoptic assessment of exotropia with its surgical outcome. Al-Shifa J Ophthalmol 2015;11 (1)29-33
17. Azonobi IR, Adido J, Olatunji FO, Bello A, Mahmoud AO. Risk factors of strabismus in southwestern Nigeria. Pak J Ophthalmol 2009;25(3):129-34
18. Ansar M, Jalis M. Butt AI. Visual outcome in strabismic and anisometropic amblyopia after patching therapy.Al-Shifa J Ophthalmol 2009;5(2) 71-78
19. Malik AM, HabibaUE, Frequency of esotropia and exotropia among patients between 3 to 25 years of age. Al-Shifa J Ophthalmol 2013;9(1) 34-38
20. Kumar A, Cheeseman R, Durnian JM. Sub specialization of the ophthalmic literature. Ophthalmology 2011;118(6):1211-14.

Original Article

MUTATIONAL ANALYSIS OF HCV GENE ENCODING E1 GLYCOPROTEIN

Muhammad Saad Janjua, Rehman Shahzad, Ghazala Jaffery, Faria Malik , Aneeq Waqar and Hina Bukhari

Objective: To evaluate the variations in HCV glycoprotein E1 gene and to map epitopes in the variable E1 regions informing the development of an effective vaccine against HCV.
Methods: To isolate the E1 gene, RNA extraction was done by using the kit method and then it was converted to the cDNA. Confirmation of HCV presence in the collected samples was done through highly conserved core primers. This was then followed by PCR amplificationfor E1 gene. The sequenced E1 genes were translated in silico into proteinsequences.
Results: A These proteins sequences were then analyzed for the presence of B-cell and T-cell epitopes; two B-cell epitopes (CSLYPGHLSGHRMAWD, TASIRSHVDLLVGAAT) and one T-cell epitope (QAFTFRPRR) were found useful. These could be helpful in the formation of a proper vaccine against HCV.
Conclusion: We found 2 B-cell epitopes and 1 T-cell epitope conserved in 3a genotype that may help in vaccine development.
Keywords: Hepatitis C Virus, PCR, E1 Glycoprotein, B-cell Epitopes.

Introduction

The most indigenous pathological agent of non-A and non-B hepatitis is Hepatitis C virus. It was discovered by Choo and Chiron co-operation group in 1989.[1] HCV causes acute and chronic liver disease, cirrhosis and hepatocellular carcinoma.

Approximately 130 to 170 million population of the world are suffering from hepatitis C virus infection. The epidemiology of transmission of this infection has changed from primarily blood route to currently injection abuse commonly in young adults. Other routes are piercing, tattooing, mother to child and sexual transmission.[2]

Hepatitis C virus transmission progresses slowly and asymptomatically in the acute phase of infection. Of the total population infected only 20 percent is capable of clearing the infection and 80 percent progress to chronic liver disease.[3] Hepatitis C virus belongs to family flaviviridae, genus hepacivirus that has six different phylogenetic groups that further have numerous clades. The most frequently found genus in Pakistan is 3a.[4]

By using cell culture, electron microscopy and other scientific procedures we can study the ultrastructure of Hepatitis C virus. The negative stain electron microscopic study of the virus has shown spherical particles of 45 to 70nm in diameter with a bilayer membrane called envelope consisting of E1 and E2 proteins, viral proteases, nonstructural proteins and apolipoprotein E. They also have internal structure presumably the capsid, single positive stranded RNA of 9500 bases in genome.[5] **(Fig 1).**

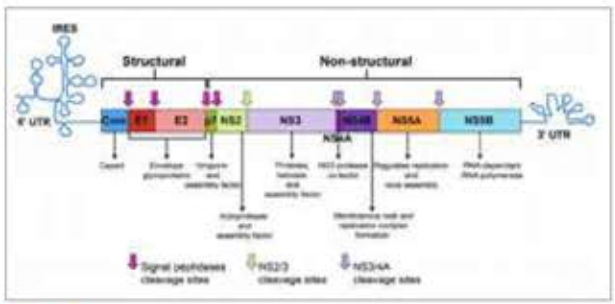

Fig-1: Structure of HCV genome.

The nature of immune response required to clear the hepatitis C infection is still under consideration, but its protein evokes both humoral and cellular immunity.[6] RNA binding protein is a core protein so it is supposed to form the viral nucleocapsid. This protein receives signals from the host and converts them to the mature form that acts as the membrane protein. This core protein is involved in influencing multiple host cell functions, apoptosis, cell signaling, carcinogenesis, and lipid metabolism. This protein has two domains D1 (at N-terminal consists of two-third hydrophilic domain) and D2 (at C-terminal consists of one-third hydrophobic domain). D2 domain is required for membrane characteristics of core.[7] The E1 and E2 envelope proteins play a key role in the entry of virus into host cells. E1 is a transmembrane glycoprotein, with C-terminal liable for association and membrane permeability. It also contains 4-5 N-linked glycans. Most importantly

these glycans are involved inproper folding of HCV glycoprotein and in virus entry[8] E2 also plays a key role in viral breakout from the immune system, as Hyper Variable Regions (HVRs) are found in E2 protein. Mainly two HVRs are responsible in escape of virus. HVR1 plays role in modulating virus entry and HVR2 modulates receptor binding of E2. These envelope proteins E1 and E2 form the heterodimer which appears at the surface of HCV and is surely a contender ligand for cellular receptors like CD81, tetraspanin, heparin sulphate, mannose binding lectins, scavenger receptor class B type I and others. When E2 binds to CD81, natural killer cell surface, it inhibits the cytotoxicity and cytokine production by these cells. It is therefore necessary to kill envelope proteins to inhibit the viral entry. Junction of p7 protein, is present between structural and non-structural proteins, it is small polytypic membrane protein, whose proper function is unknown. Studies have shown that the heterodimerization of glycoproteins E1 and E2 are liable for the virus entry and it is believed that glycans on the heavy glycosylated E1 and E2 are involved in the proper folding of heterodimerization separately; they are non-infectious.[9] Other regions of E2 like HVR1 are responsible for virus interaction with receptors and evasion of immune system due to high rate of variations. However, deletion of HVR1 does not decrease the infectivity of virus. The conserved glycosylation sites specially 4 in E1 and 11 in E2 have been shown to play a role in HCV life cycle, because the deletion of these sites lessen the infectivity of HCV. After attachment to receptors, HCV interacts with occluding or CLDN-1, which facilitates its cellular uptake. HCV entry is mediated by clathrin-dependent endocytosis. Due to acidification, viral envelope fuses with the early endo some membrane which releases the viral nucleocapsid into the cytoplasm. During virion assembly and replication HCV shows unique character, both these processes depend upon the fatty acid pathway and cholesterol metabolism in host cell. Apolipoproteins are also effective regulators of HCV infectivity. Lipoproteins also play imperative role for the HCV entry and initiation of infection. In other words infection starts when virus particles associated with lipoprotein interact with lipoprotein receptors.[10-12]

According to epidemiological studies related to environment and geographical conditions HCV has 11 major genotypes and over 100 subtypes. Six of these genotypes are the major types which are further classified into many subtypes. 3a genotype is the most common in Pakistan and infects 49.5%HCV patients whereas 3b infects 17%, 1a infects 8.35%, 2a is 7.52% and other genotypes are very rare.[13]

These changes in genomes, within the single host, are responsible for evasion of HCV from the immune system.[14]

Treatment of the chronic HCV has improved during the past few years. In HCV infected patients, it persists in about sixty to eighty percent. Peg-interferon (alfa 2a, 2b) with Ribavirin and interferon (alfa 2a, 2b) have been used widely. Currently several other FDA approved treatments are available. For chronic HCV infection, cirrhosis and liver dysfunction the last option is liver transplantation. However all these treatment options are expensive, therefore there is a need of development of a vaccine or a cheaper treatment. Development of HCV vaccine is challenging as the virus exists in the form of Quasispecies. Reason behind this is too much diversification of HCV due to the lack of proofreading mechanism in RNA polymerase of virus.[14] The current study was undertaken to conduct mutational analysis of HCV gene encoding E1 glycoprotein that could be a candidate for HCV vaccine.

Methods

Research work of the study was conducted in the Molecular Biology and Genomics Laboratory of the Institute of Biochemistry & Biotechnology, University of Veterinary and Animal Sciences, Lahore. Sample Collection: Blood samples from 120 HCV PCR positive patients with 3a genotype were collected from Shalimar Hospital and Nawaz Sharif Hospital, Lahore. Five ml of blood sample was collected aseptically from each patient after taking informed consent. Serum was separated by centrifugation and stored at -20 °C for further processing.

RNA Extraction: RNA was extracted from frozen serum samples after thawing them using AJ Roboscreen Innuprep kit (AJ Roboscreen GmBH, Leipzig, Germany). Extraction method was followed according to the manufacturer's instructions.

RNA Quantification: The extracted RNA was quantified using Nano Drop (Thermo Scientific, Dubuque, USA). The values were in the range of 5 ng/µl to 50 ng/µl.

Primer designing: About 53 sequences of HCV 3a genotype from NCBI (http://www.ncbi.nlm.nih.gov/) were retrieved. Sequences were aligned by using MEGA 6 software. Conserved regions were

observed and primers were picked up from those regions.

Primers were chosen by keeping in mind that for good primers GC content should be 40% - 60%, and temperature should be 50°C to 60°C. After selecting the primer sequences **Table 1**, these primers were checked through online software Oligo Tm calculator (http://www.basic.northwestern. Edu/biotools/oligocalc.html). Complementarity, hairpin formation, self-annealing sites were checked and synthesized from Macrogen Company.

CDNA Synthesis:

From RNA, CDNA was synthesized by using Thermo Scientific cDNA preparation kit (Thermo Scientific, Dubuque, USA) following manufacturer's instructions.

Quantification of cDNA: After the formation of cDNA, quantification was done using Nano Drop (Thermo Scientific, Dubuque, USA). As cDNA is single-stranded, so the option for ssDNA was selected on Nano Drop. Quantitative values were in the range of 1300 to 1900 ng/µl.

Confirmation of HCV in collected samples:

To confirm the cDNA of HCV virus, we used the primers of core region and 5' UTR region of HCV virus (Primers sequences are given in **Table 2**).

Table-1: Sequences of primers used in the study.

Primers	Sized bp	Sequence	Tm (°C)
E1P1-F	27	GGGAGGTCTCGTAGACCGTGCACCATG	57
E1P1-R	31	GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC	57
E1P1-F	27	AGACCGTGCACCATGAGCAC	57
E1P1-R	20	TACGCCGGGGGTCA(TG)T(GA)GGGCCCCA	57

Table-2: Core primers sequences for HCV detection.

Primers	Sized bp	Sequence	Tm (°C)
P1-F	27	GGGAGGTCTCGTAGACCGTGCACCATG	57
P1-R	31	GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC	57
P1-F	20	AGACCGTGCACCATGAGCAC	57
P1-R	27	TACGCCGGGGGTCA(TG)T(GA)GGGCCCCA	57

For this nested PCR was done in which two rounds were performed. Reaction mixture of 20 µl was prepared.

Agarose Gel Electrophoresis: After completing the two rounds of the PCR, amplicons were checked by running on the 3% agarose gel. Presence of sharp bands concluded that the samples used were HCV positive.

Amplification of HCV E1 gene: As double round PCR confirmed the presence of HCV in samples, different recipes and PCR conditions were applied to amplify 5 out of 120 HCV positive samples for E1 gene.

Agarose Gel Electrophoresis: After PCR from new primers (Table 1), PCR product was run on 1.2% agarose gelalong with a 1 kb ladder. Amplicon size was around 576 BP as it is the size of the required gene E1.

PCR product purification: PCR product was then purified for further processing. The purified product was run on 1.2% gel to check the presence of required gene. After confirmation of presence of bands, it was sent for sequencing.

Sequencing: The PCR purified product was sent to Centre of Excellence in Molecular Biology (CAMB), Punjab University, Lahore, for sequencing.

Bioinformatics Analysis: After sequencing, bioinformatics analysis was done on the sequenced samples.

Conservancy Analysis: This was done by aligning our sequences with other sequences downloaded from NCBI by using BLAST and Immune Epitope Database (IEDB) analysis tool (http://tools.Immuneepitope.org/main/).

Phylogenetic Analysis: Phylogenetic analysis was done by Mega 6 or CLC sequence analyzer to study the relation of the genomic sequence with the others.Protein Modeling: Protein modeling was performed with I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) online server and quality of the model was assessed with Ramchandran plot and Z-score.

Fig-2: Amplification of cDNA through Core primers

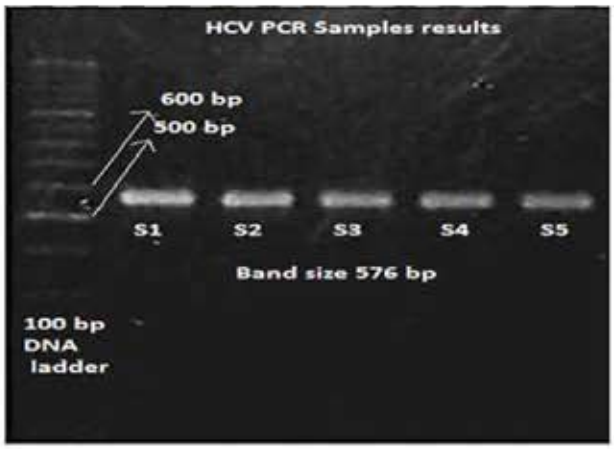

Fig-3: Amplification of HCV E1genes.

Epitope Mapping Epitope mapping and conservancy analysis was done by Immune Epitope Database (IEDB) analysis tool (http://tools.immuneepitope.org/main/).

Results

Confirmation of HCV samples:

To confirm the presence of hepatitis C virus, RNA was extracted from collected serum samples and amplified through nested PCR by using the Core region and 5' UTR region primers. The product of PCR was then run on 3% agarose gel. Bands were present showing the presence of HCV in the samples as seen in Figure 2.As double round PCR confirmed the presence of HCV in samples, different recipes and PCR conditions were applied to amplify E1 gene as seen in **Fig 3.**

After confirming the presence of HCV, the samples were amplified using the E1 primers designed from the Pakistani genomic sequence. PCR were run multiple times, with different recipes and different PCR conditions.

Discussion

HCV is a global health problem and is difficult to treat because of high variations in its genome. HCV is spreading rapidly and it is alarming that there is no vaccine available for its prevention. About 170 million people are affected by HCV worldwide and in Pakistan 3% to 6% population has HCV infection.[15] HCV causes several problems that eventually lead to chronic liver disease, cirrhosis, and hepatocellular

Table-3: Comparison of sequences with standard sequence.

Sr. No	Sequences	Similarity	Gaps	E-value	Score
1	Sequence 1	93%	3%	0.0	837 bits
2	Sequence 2	88%	3%	1e-175	599 bits
3	Sequence 3	91%	2%	0.0	658 bits
4	Sequence 4	90%	2%	1e-180	616 bits
5	Sequence 5	90%	3%	3e-176	610 bits

Table-4: B-Cell epitopes conservancy analysis.

Epitope Name	Epitope Sequence	Epitope lenght	% of protein sequence matches at identity =100%
33	CSLYPGHLSGHRMAWD	16	35.00% (14/40)
31	TASHIRSHVDLLVGAAT	16	32.50% (13/40)

Table-5: T-cell epitopes conservancy analysis

Epitope Name	Epitope Sequence	Epitope lenght	% of protein sequence matches at identity =100%
8	QAFTFRPRR	9	46.15% (18/39)

Carcinoma. Treatment is very complicated due to high mutation rate in HCV virus. There are multiple genotypes in which there are 6 major genotypes. Some genotypes are common in specific regions. 3a genotype is the most common genotype in Pakistan. A serious step is needed to be taken to control its spread. Numerous researches have been conducted on different genes of HCV to control it and to understand how it evades the human immune system. Genes responsible for escaping from immune system are considered to be very important in treatment. E1 is one of these important genes which play a role in the escape of HCV from human immune system. E1 is an enveloped glycoprotein involved in epitope formation. Hyper variable regions are present in E1 gene; these regions help in the escape of HCV from human immune response.[8]

To study variations in E1 gene, serum samples from HCV positive patients were taken and RNA was extracted from them and converted to cDNA which was followed by amplification of the target gene. Amplified PCR products were sequenced and then protein analysis was performed on these sequences.

When nucleotide sequences were obtained, they were converted to protein using ExPaSy translation tool. Primary structure analysis was performed using ProtParam online server and to compare the results, Pakistani HCV E1 gene sequence was used as standard, but the primary structure of all the sequences was almost the same, means no structural changes were detected at the primary level.

To study more about the structures of sample Sequences, secondary structures were made using Phyre 2 online server, changes were noticed in the alpha helices and beta sheets, but they were very minor changes at this level. To check the transmembrane activity of the sequences the TMHMM online server was used and it was confirmed that they are present outside the membrane which means that these genes are involved in the epitope formation or may act as an epitope.

To find out the B-cell and T-cell epitopes, ABCpred and Epijen online servers were used respectively. The maximum epitopes were predicted which were then checked for the antigenicity through Vexijen, and antigenic epitopes were analyzed against 40 E1 gene sequences obtained from NCBI, using IEDB database. So in the results, two B-cell epitopes(CSLYPGHLSGHRMAWD, TASIRSHVDLLVGAAT) and one T-cell epitopes (QAFTFRPRR) were found conserved as they are presentin mostly half of the sequences, which means these epitopes could be of lot of use for the purpose of development of vaccine as these two B-cell epitopes could be used to target the E1 gene of HCV. Development of an effective vaccine against the hepatitis C virus (HCV) has long been defined as a difficult challenge due to remarkable variability of this RNA virus and it has been observed that humans and chimpanzees could be re-infected after re-exposure. On the other hand, progress in the understanding of antiviral immune responses in patients with viral clearance has elucidated key mechanisms playing a role in the control of viral infection. Studies investigating prophylactic vaccine approaches in chimpanzees have confirmed that the induction and maintenance of strong helper and cytotoxic T-cell immune responses against multiple viral epitopes is necessary for protection against viral clearance and chronic infection. A multi-specific B-cell response, resulting in rapid induction of cross-neutralizing antibodies may assist cellular responses. Therapeutic vaccine formulations currently being evaluated in clinical trials are facing the fact that the immune system of chronic carriers is impaired and needs the restoration of T-cell functions to enhance their efficacy.[16] The result of current study would help in identifying the candidate protein of 3a genotypes for HCV vaccine development.

Conclusion

We have found two B-cell epitopes and one T-cell epitope conserved in 3a genotype.

CSLYPGHLSGHRMAWD, a B-cell epitope was 35% similar in 40 sequences of 3a genotypes.

TASIRSHVDLLVGAAT, a B-cell epitope had 32.50% similarity in 40 sequences of 3a genotype.

QAFTFRPRR, a T-cell epitope had 46.15% similarity in 39 sequences of 3a genotype.

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References

1. Khan UH, Ali A, Akram M, Khattak AM. SEROPREVALENCE OF HEPATITIS C; AMONG HEALTHY BLOOD DONORS IN BLOOD BANKS OF KHYBER PAKHTUNKHWA. Professional Medical Journal. 2014;21(4).
2. Baldo V, Baldovin T, Trivello R, Floreani A. Epidemiology of HCV infection. Current pharmaceutical design. 2008;14(17):1646-54.
3. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. Nature Reviews Gastroenterology and Hepatology. 2013;10(9):553-62.
4. Catanese MT, Uryu K, Kopp M, Edwards TJ, Andrus L, Rice WJ, et al. Ultrastructural analysis of hepatitis C virus particles. Proceed- ings of the National Academy of Sciences. 2013;110(23):9505-10.
5. Gastaminza P, Dryden KA, Boyd B, Wood MR, Law M, Yeager M, et al. Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. Journal of virology. 2010;84(21):10999-1009.
6. Grollo L, Torresi J, Drummer H, Zeng W, Williamson N, Jackson DC. Exploiting information inherent in binding sites of virus-specific antibodies: design of an HCV vaccine candidate cross-reactive with multiple genotypes. Antiviral therapy. 2006;11(8):1005.
7. Dubuisson J. Hepatitis C virus proteins. World Journal of Gastroenterology. 2007;13(17):2406.
8. Goffard A, Callens N, Bartosch B, Wychowski C, Cosset F-L, Montpellier C, et al. Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. Journal of virology. 2005;79(13):8400-9.
9. Owsianka AM, Timms JM, Tarr AW, Brown RJ, Hickling TP, Szwejk A, et al. Identification of conserved residues in the E2 envelope glycoprotein of the hepatitis C virus that are critical for CD81 binding. Journal of virology. 2006;80(17):8695-704.
10. Burlone ME, Budkowska A. Hepatitis C virus cell entry: role of lipoproteins and cellular receptors. Journal of General Virology. 2009;90(5):1055-70.
11. von Hahn T, Rice CM. Hepatitis C virus entry. Journal of Biological Chemistry. 2008;283(7):3689-93.
12. Zeisel MB, Felmlee DJ, Baumert TF. Hepatitis C virus entry. Hepatitis C Virus: From Molecular Virology to Antiviral Therapy: Springer; 2013. p. 87-112.
13. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J. 2011;8(1):433.
14. Keck Z-y, Xia J, Wang Y, Wang W, Krey T, Prentoe J, et al. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. PLoS pathogens. 2012;8(4):e1002653.
15. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World J Gastroenterol. 2016;22(4):1684-700.
16. Stoll-Keller F, Barth H, Fafi-Kremer S, Zeisel MB, Baumert TF. Development of hepatitis C virus vaccines: challenges and progress. 2009.

Original Article

Clinical and Laboratory Parameters Monitored in Children Extubated from Ambu Bag and Endotracheal Tube

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Objective: To study the clinical and laboratory parameters of children extubated from ambu bag and endotracheal tube after being manually ventilated for at least more than 24 hours.

Methods: Various clinical and laboratory parameters were evaluated initially at the time of intubation and then at the time of extubation. The clinical parameters evaluated included the heart rate & respiratory rate, Glasgow coma scale, spontaneous respiratory effort, respiratory distress and pupillary reaction. The Laboratory parameters evaluated included TLC, CRP, arterial blood pH, HCO3, PO2 & PCO2, CXR, flow rate of oxygen required to maintain oxygen saturation and the dose of cardiac support in the form of dopamine infusion.

Results: Total 24 patients were included in the study, 11(46%) male and 13(54%) female. Age range was from 0 to 36 months with mean of 6.5 months. The mean values of Laboratory parameters at the time of intubation included a pH of 7.13, HCO3 17, O2 sat 64, PCO2 52, and rate of oxygen flow 3.5 liters/min. The mean values of same parameters at the time of successful weaning were, pH 7.36, HCO3 18, O2 sat 94, PCO2 29, and rate of flow of oxygen 3.5 liters/min. Regarding clinical parameters the mean value for GCS at intubation was 5 which later improved to 13 at extubation. Similarly the pupillary reaction at intubation showed constriction of pupils in 6(25%) and mid-dilated with sluggish reaction in remaining 18(75%), while almost 95% cases had reactive pupils at extubation.

Conclusion: Ambu-bagging though crude but is a successful tool for respiratory support in the absence of ventilator. Clinical and lab parameters can predict the outcome in children who are solely intubated and ventilated by ambu bag.

Keywords: Ambu-bagging, Endotracheal tube, Wean-off, Extubation

Introduction

The Ambu bag is an important emergency tool which offers the basic airway management for the ventilation and oxygenation of the patients until the other definitive airway is established.[1] Although ventilators are the recommended tool for mechanical ventilation, but are not necessarily life savers. Short of very few well equipped hospitals in most of the hospitals, being on ventilator is a ritual before inevitable death...a horrible truth. Due to the lack of adequate number of mechanical ventilators in many tertiary care hospitals all around the country, ambo bagging still remains an alternate arrangement in order to ventilate children with respiratory failure. Although majority of the time this bagging is done by untrained patient's attendants but children including newborns are still successfully weaned off from this crude ventilation support. Weaning is a tricky process even in mechanical ventilation by a ventilator. When the acute phase of the disease subsides, noted by a decrease in the mean airway pressure required, weaning begins. The end of weaning can be defined as the time at which the patient's spontaneous breathing alone can provide effective gas exchange.[3] Predicting extubation failure (EF) is one of the most challenging aspects of critical care medicine.[4] For over two decades, physicians have attempted to define the best methods of discontinuing mechanical ventilation in patients recovering from respiratory failure. An early study of weaning noted that the clinical decision to discontinue mechanical ventilation is often arbitrary, based on "judgment and experience".[5] Difficulty in discontinuation from ventilation is in part attributed to inadequate understanding of the mechanisms responsible for successful outcome and a lack of optimal guidelines to wean off from mechanical ventilation.[6] Generally in case of mechanical ventilation it is desirable to have predictive indexes that can be easily measured and widely applied. A number of indexes, such as vital capacity, maximal inspiratory pressure (PImax), and minute ventilation (VE), have been proposed as accurate predictors of the outcome of weaning.[7]

Many clinicians believe that, for an infant or young child, respiring through a small ETT is akin to

breathing through a straw thereby imposing an unacceptable work of breathing. This notion is contrary to both clinical observation and physiology. Data from Keidan and colleagues show the work of breathing through an ETT to be half the effort required for the mask and oro-pharyngeal airway. They also found that breathing spontaneously with a face mask in place required even more work than when there was no oro-pharyngeal airway. A 3 kg infant accepts a 3.0 mm ETT whereas an adult of 60 kg can tolerate a 9.0 mm ID ETT -- a 20 times increase in body size but only a 3 times increase in ETT size. The sub-glottic area of the infant is also 20 times greater in proportion to body size than that of an adult. Nonetheless, the inverse 4th power relationship of airflow resistance to radius dictates that the infant ETT has a much higher "resting" resistance, but it is irrelevant because of the shorter ETT and low flows generated by the infant compared to the adult (*vide infra*). The net effect is that the infant is breathing through a hose rather than a straw when compared to the adult.[3]

Methods

This study was carried out in Department of Pediatrics Jinnah Hospital, Lahore for a period of 07 months from Oct 2012 to April 2013. It was a prospective collection of data. Children including neonates (more than 35 weeks gestation) who were ventilated by ambu bagging and endotracheal tube with oxygen flow due to non availability/occupancy of ventilators and later successfully weaned off were included in the study. Various clinical and laboratory parameters of children at intubation, and then at extubation were studied. The rationale of study was based on the fact that due to inadequate number of mechanical ventilators for children, all over the country, ambu bagging still remains the tool of ventilation, at times, even for days.

Therefore a study of available clinical data of children who were successfully weaned off will keep the hope alive in such children even without the available facility of mechanical ventilators. 24 successive children who were weaned off were enrolled in the study. The Inclusion criteria was: All neonates(gestational age, 35 weeks and above) and children who were mechanically ventilated using only ambu bag with endotracheal tube for at least 24 hrs, due to non-availability/occupancy of mechanical ventilators and later successfully extubated. The Exclusion criteria included: Neonates < or = 35 wks gestation and children with neuromuscular disease, children partially ventilated mechanically by a ventilator children with fixed dilated pupils and absence dolls eye movement at the time of intubation children with congenital anomalies, neuromuscular diseases and children intubated for less than 24 hours.

Neonates <35 weeks have other contributing factors which make it difficult to wean off from any type of ventilation, therefore were excluded. As an operational definition, extubation as a success was defined as spontaneous maintenance of O_2 Sat without ambu bagging for more than 48 hours after extubation. Similarly a failure was defined as re-intubation within 48 hrs of extubation in the absence of upper airway obstruction.

The list of underlying diseases leading to intubation with percentages was tabulated in order of frequency. The clinical parameters evaluated included the heart rate & respiratory rate standardized to age, conscious status by Glasgow coma scale, spontaneous respiratory effort, respiratory distress in the form of sub-costal, inter-costal recessions and pupillary reaction. The Laboratory parameters evaluated included the TLC, CRP, arterial blood pH, HCO_3, PO_2 & PCO_2, CXR, flow rate of oxygen required to maintain oxygen saturation spontaneously and lastly, the dose of cardiac support in the form of dopamine infusion. Mean, ranges and percentages of the different parameters were calculated. A list of underlying diseases was also tabulated with percentages.

A comparative analysis of various parameters at start of ambu bagging and at the time of extubation was done. During intubation the ambu bagging was done by doctor on duty and patient's attendants with an oxygen flow of 4-6 Lit/min. Regular assessment of vital signs and a regular arterial blood gas monitoring was being done. Fluid management was done as per standard protocols and all patients required a positive ionotropic support in the form of dopamine infusion throughout the bagging.

Weaning-off plan was undertaken on clinical judgment based on improvement of ABGs, conscious status, spontaneous breathing effort by the patient with minimum distress and improvement in the primary underlying disease. 3 doses of intravenous steroids in the form of dexamethasone were given 6 hours prior, at extubation and 6 hours later to avoid laryngeal edema. There was an extubation failure in 5 patients who were re-intubated within 48 hours of extubation due to different reasons, including hospital acquired sepsis, DIC and

probable laryngeal edema.

Results

A total 24 successively intubated patients including newborns were enrolled in the study as per inclusion criteria. Age group was from 0 to 36 months with mean of 6.5 months, 11(46%) male and 13(54%) female. The ambu bagging was done by doctor on duty and Patient's attendants with an oxygen flow of 4-6 Lit/min, and the minimum duration of bagging was >

Table-1: Underlying Diagnoses at the time of intubation with frequencies

S. No	Diagnosis	Frequency	Percentage
1	Pyomeningitis with aspiration pneumonia	03	12.5
2	Gastroenteritis with septicemia	03	12.5
3	Asphyxia Neonatorum Stage II	02	8.4
4	Asphyxia Neonatorum Stage II with Early onset of septicemia	02	8.4
5	Bronchopneumonia with morphine poisoning	02	8.4
6	Asphyxia Neonatorum Stage I	01	4.2
7	Post Asphyxial damage with sepsis	01	4.2
8	Bronchopneumonia with sepsis	01	4.2
9	Late onset neonatal sepsis	01	4.2
10	Status Asthmaticus	01	4.2
11	Infantile spasm with sepsis	01	4.2
12	Post Local Anesthesia with Lignocaine for circumcision	01	4.2

Table-2: Clinical and lab parameters at the time of Intubation & extubation.

Parameter		Intubation	Extubation
PH	Range	6.9-7.4	7.28-7.41
	Mean	7.13	7-36
CO 2	Range	22-86	16-42
	Mean	43	29
HCO3	Range	8-32	12-26
	Mean	17	18
O2 SAT	Range	50-86	90-99
	Mean	64	94
Rate of Oxygen	Range	2-5	2-5
	Mean	3.5	3.5
GCS	Range	3-13	13-15
Pupil		Constricted 6	Constricted 1
		Dilated 18	Dilated and reactive 23
Spont resp effort		Absent 1	Present 24
		Gasping 2	
		Poor 14	
		Present 7	
Resp Distress		Present 10	Present 6
		Nil 14	Nil 18
CXR1		Normal 12	Improved 8
		Pathology 12	Normal 14
			Same 2

Table-3: Clinical and lab parameters of patient who recovered after extubation and those who were re-intubated.

Parameter	Recovered	Re-intubated
Total	19	5
Hours of intubation	25	49
TLC	22300	23800
CRP	16	21
Heart rate at intubation	120	123
Heart rate at extubation	124	127
RR at intubation	27	36
RR at extubation	46	52
Oxygen Saturation	63	62
	94	93
Rate of Oxygen	3.5	3.5
	3.3	3.5
GCS	6	4
	14	13
Dopamine Dose	7.6	7.0
	12.6	12
PH	7.1	7.1
	7.5	7.39
Co2	43	26
	30	28
HCO3	16	21
	18.5	18.5

Patient's attendants with an oxygen flow of 4-6 Lit/min, and the minimum duration of bagging was > Patient's attendants with an oxygen flow of 4-6 Lit/min, and the minimum duration of bagging was > 24 hours. Main underlying diagnoses at time of intubation, in order of frequency were, Asphyxia neonatorum Stage II with or without sepsis & meconium aspiration(21%), Prematurity with Respiratory distress syndrome (16.7%), Bronchopneumonia with Sepsis and/or morphine poisoning (12.5%), Pyogenic meningitis (12.5%), gastroenteritis with sepsis (12.5%) and status asthmaticus (4.2%) **Table 1**. All these 24 were patients were weaned-off from manual ambo bagging, on clinical judgment and available lab parameters **Table 2.** Among these 24 extuabted patients, 19 remained normal and 5 were re-intubated. 2 were intubated within <24 hours and 3 after first 24 hours. Probable causes of reintubation were, DIC in 2 cases, Laryngeal edema in 1 case and in the remaining 2 cases, cause of reintubation was unknown. Outcome: 19 patients who were successfully extubated were cured and discharged. Among 5 re-intuabted patients 2 were discharged after extubation later, 2 expired and one patient got LAMA. The clinical parameters of successfully weaned-off (19) cases and those re-intubated were also compared **Table 3.**

Discussion

Availability of mechanical ventilators in adequate numbers fulfilling the need of sick patients has always been an issue in our country. According to a report published in there were only 150 ventilators in the city of Karachi for 18 million people.[8] In another report published in 2013 it was mentioned that teaching hospitals have multidisciplinary ICUs while there are a total of 480 ICU beds in Karachi in different types of Intensive Care Units which include medical, pediatric and neonatal ICUs. Only 15-20% of these ICUs have purpose built facilities, teaching facilities for staff and residents are available in 1-2%, 20-25% had proper ICU beds, 40-50% of these ICUs have working ventilators with new latest equipment, 30% have supporting equipment but just 5-10% have arrangements for In-house drugs delivery, otherwise most of these ICUs ask the patient attendants to arrange for drugs.[9] Similarly a fresh report published in Jan 2016 mentioned a total of 62 ventilators in public sector hospitals in Rawalpindi for a population of 5 million people, out of which 10 ventilators were out of order.[10] We also face a lack of adequate numbers of mechanical ventilators for sick children in our set up. Most of the time the available ventilators are occupied and due to a heavy input of sick children many of them need to be manually ventilated by Ambo bagging with endotracheal tube and an oxygen source. Although it is a crude method of ventilation, as inspiratory pressure, tidal volume, positive end expiratory pressure, I:E ratio and FiO2 cannot be measured, but still a good number of patients are weaned-off successfully by monitoring other available parameters. The mainstay of monitoring these manually ventilated children is a regular arterial blood gas analysis which can at least guide about the pH, HCO3, PaCO2 & PaO2. Other clinical parameters that can be monitored include conscious status, pupillary reaction, spontaneous breathing effort, improvement of primary disease and lowering requirement of oxygen. Another interesting observation in our study was that there was not a single case of pneumothorax despite the fact that pressures couldn't be measured or controlled during manual bagging.

Regarding various clinical parameters of weaning, the IARS (International Anesthesia Research Society) published a report regarding predictors of weaning and a Glasgow coma scale of more than 12 was considered a good predictor.[11] Same was true in our study where children had a mean GCS of 5 at intubation and 13 at extubation. In the same report a PaCO2 at baseline and a PaO2 more than 60 were also considered to be a good predictor of weaning from ventilation in association with other factors.[11] Similar was the observation in our study where a mean PaCO2 of 54 at intubation was followed by a mean PaCO2 of 29 at extubation.

Similarly a mean oxygen saturation of 64 at intubation and 94 at extubation reflected the importance of O2 sat during monitoring of ventilation in our study. A study conducted in Spain regarding the predictors of weaning from ventilation also mentioned the importance of easy wakefulness and an oxygen saturation of more than 90% as good predictors of weaning.[12] We also noted the reaction of pupils at the time of intubation and later at extubation. It was a significant finding as the pupillary reaction was constriction in 25% and mid dilatation with sluggish response in remaining 75% at the start of ventilation. It improved to normally reacting pupils in 96% of the children at extubation. A similar observation was mentioned in a review article published in 2007, where reactive pupils were considered as an important predictor of weaning from ventilation.[13] another study published in Egypt statistically proved

the fact that a lower PaCO2 and a lower HCO3 were present in patient who could be weaned, as compared to those who were difficult to wean. In our study we could not find a significant difference in arterial HCO3 at the time of intubation and extubation, but the children who were re-intubated within 48 hours had a higher HCO3 than those who were successfully extubated.

Conclusion

Ambo bagging with endotracheal tube and oxygen flow can survive patients. Monitoring of such patients can be done by vitals, serial arterial blood gases, oxygen saturation and the flow of oxygen required to maintain saturation. Treatment of primary disease leading to improvement of symptoms and clinical parameters can give a judgment regarding extubation. Arterial blood gas remained the main tool for following such patients.

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References

1. [Internet]. 2016 [cited 30 April 2016]. Available from: Hyperlink http://doctorshub1.blogspot.com/2013/10/ambu-bag-ventilation-indications.html
2. [Internet]. 2016 [cited 30 April 2016]. Available from: Hyperlink http://www.doctorshangout.com/profiles/blogs/faqs-about-cpr-uses-of-Ambu-bag.
3. Newth CJL, Venkataraman S, Willson DF, Meert KL, Harrison R, Dean JM, Pollack M, Zimmerman J, Anand KJS, Carcillo JA, Nicholson CE. Weaning and extubation readiness in pediatric patients*. Pediatric Critical Care Medicine. 2009 Jan;10(1):111.
4. Khan N, Brown A, Venkataraman S. Predictors of extubation success and failure in mechanically ventilated infants and children. Critical care medicine. 1996 Sep 1 [cited 2016 Aug 31];24(9):156879
5. Wesley E, Baker AM, Dunagan DP, Burke HL, Smith AC, Kelly PT, Johnson MM, Browder RW, Bowton DL, Haponik EF. Effect on the duration of mechanical ventilation of identifying patients capable of breathing spontaneously NEJM. 2009 Aug 20 [cited 2016 Aug 31]. Available fromhttp://dx.doi.org/10.1056/NEJM199612193352502 doi: 10.1056/NEJM199612193352502
6. Wittekamp B, Mook van, Tjan D, Zwaveling J, Bergmans D. Clinical review: Post-extubation laryngeal edema and extubation failure in critically ill adult patients. Critical care (London, England). 2009 Dec 19 [cited 2016 Aug 31];13(6). Availablefrom:http://www.ncbi.nlm.nih.gov/pubmed/20017891.
7. Yang KL, Tobin MJ. A prospective study of indexes predicting the outcome of trials of weaning from mechanical ventilation. New England Journal of Medicine. 1991 May 23;324(21):144550.
8. Archive AN. Only 150 ventilators available for 18 million Karachiites. 2006 Dec 27 [cited 2016 jun 30].. Available from: http://aaj.tv/2006/12/only-150-ventilators-available-for-18-million-karachiites/.
9. Zahid. Only 20-25% hospitals in Karachi have proper ICUs and 50% have monitoring facilities prof.Tipu sultan. 2013 [cited 2016 jun 30]. Available from: http://www.pulsepakistan.com/index.php/main-news-feb-1-14/632-only-20-25-hospitals-in-karachi-have-proper-icus-and-50-have-monitoring-facilities-prof-tipu-sultan
10. Online. More by online. 2016 [cited 2016 jun 30]. Available fromhttp://nation.com.pk/national/09-Jan-2016/critical-patients-suffer-as-allied-hospitals-face-shortage-of-ventilators.
11. [place unknown: publisher unknown]. OpenAnesthesia; 2016 [cited 2016 Aug 31]. Available from: https://www.openanesthesia.org/weaning/.In-text citations: (10)
12. Fernando F. MD, Anderas E. MD, PHD: "When to wean from a ventilator: An evidence-based strategy", Cleveland Clinic Journal of Medicine; May 2003, Vol 70. Number 5: 389-400
13. Bronwyn A. Couchman, Sharon M. Wetzig, Flona M. Coyer, Margaret K. Wheeler; "Nursing care of the mechnically ventilated patient: What does the evidence say?, Intensive and Critical Care Nursing 2007: 22: 4-14

Original Article

Outcome of Paediatric Kidney Transplant : An Experience From Lahore

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Objective: To analyze demography, clinical profile, complications & outcome of children after kidney transplant.
Methods: Eleven patients (6 boys & 5 girls) who underwent kidney transplant from September 2008 till September 2013 were included in the study. Retrospective analysis of their record was done.
Results: A significant improvement in growth parameters, hemoglobin level, cardiac function, blood pressure and bone diseases was noted after kidney transplant
Conclusion: Kidney transplant is treatment of choice for patients having irreversible kidney disease progressing toward end stage kidney disease. It is a superior modality offering not only excretory function but also metabolic, endocrine, erythropoitic and acid base and mineral homeostasis.
Keywords: Kidney transplant, Mineral homeostasis.

Introduction

In the setting of chronic kidney disease when GFR declines to < 15 ml/min/1.73m there emerges the definite need for renal replacement therapy, either in any form of dialysis or in the form of renal transplantation.[1] Various modifications of dialysis are in use since more than 3 decades ago but renal transplantation is the modality of choice in patients with End Stage Renal Disease (ESRD) as it offers better quality of life and lower risk of mortality; this risk being 4 times less than with dialysis. The dialysis provides only excretory function and some degree of fluid and electrolytes regulatory function, while the transplanted kidney provides additional physiological functions as well, these being metabolic, endocrine, immunoregulatory and cytokine homeostasis.[2,3]

Attempts at kidney transplant had been going on since beginning of the twentieth century. First remarkable success was kidney transplant between identical twins in 1954 in Boston. This ingenious approach bypassed the risk of immune incompatibility and reinforced the need to address this problem in the majority, that is, non-identical or non-related donors. There was a significant increase in graft survival with Azathioprine and prednisolone. Later improvements were cross match between donor lymphocytes and recipient serum and HLA matching. Finally, introduction of cyclosporine in the late 1970s led to dramatic improvements in graft survival. Though renal transplant is ultimate treatment in ESRD, yet it is far from reality for the majority of population in Pakistan, where 1/3rd population lives in poverty (<1 U$/day) with very low literacy rates along with other social and cultural problems. Health care budget is also negligible (only 0.7%)[4] Pakistan has population of 190 Millions and children comprise 38% of the total Population. Also limited availability of quality care and lack of expertise in Government centers is major barrier; very few patients who can afford high cost of kidney transplant avail this opportunity in private hospitals, while, majority remains deprived.

Reported worldwide CKD incidence has been as low as 4 per million children in Japan to as high as 14.8 per million population in United stated of America. Reported incidence of ESRD in children from western world is 1.53 children per million per year[5] CKD prevalence is probably quite high in Pakistan, owing to poor health facilities , undetected urinary tract infections and higher prevalence of inherited disorders due to frequent cousin marriages in Pakistan. Reported incidence of ESRD from Pakistan is 3.435 pmcp. Around 75%85% of children with ESRD do not go for RRT because of high cost and lack of access to available facility.[6]

Trained pediatric nephrologists in Pakistan are very few. Over the last one decade International Pediatric Nephrology Association (IPNA) has taken a unique initiative of training people from developing countries; so far three pediatric nephrologists from Pakistan have been trained by IPNA program. In Pakistan still there is no provision for Deceased kidney Transplant and only available source is living

donors.

Here we are describing results of our kidney transplant in Lahore, regarding demography, clinical profile and outcome in terms of patient and graft survival, complications including graft failure, infections, cardiovascular morbidity, bone mineral disease/growth and other parameters of Transplant recipient before and after Transplant.

Methods

We performed this retrospective review of medical records of eleven post transplant patients who underwent kidney transplant from September, 2008 till September, 2014 at Surgimed Hospital, Lahore and Lahore General Hospital. A written consent was taken from their parents and study was approved by local ethical committee. All patients were evaluated by same Pediatric Nephrologist before Transplant and also after transplant, for the total period of follow up. After review of medical record, information which was recorded on pre-designed proforma included growth parameters, duration of CKD, aetiological cause, BP, laboratory findings and duration of dialysis, transplant work up including tissue typing, cross match and panel reacting antibody testing was noted. Post transplant parameter were recorded which included growth parameters, Blood pressure, Hb, phosphate levels, iPTH, echocardiography findings. Donor was first degree relative in all patients.. Treatment protocols were same with small modifications for each patient. Both patients with posterior urethral valves had undergone surgical correction in infancy.

Pre-operative immunosuppression was started one day before transplant; Prednisolone 2mg/ kg as single morning dose was given, Mycophenolate mofetil was given in dose of 600mg/m^2 per dose twice a day and Tacrolimus 0.1 mg/Kg was given 6-12 hours before Transplant (max 10 mg).On day of operation Methylprednisolone 10mg/Kg (max 500mg) infused at time of induction. IV Cephazolin was also given before operation, 250 mg for body weight of <10 Kg, 500mg for 10- 30 Kg weight and 1 gm for >30 kg.

Post operatively IV methyl prednisolone was given in dose of 2mg /Kg on day 1, was shifted to oral prednisolone 2mg/Kg on day2, reduced to 1.5mg/Kg on D3- D4, then 1.2 mg /kg on D5 and D6 with further reduction to 1mg/Kg on day D7 . From 1st till 4th week , dose was reduced by 2.5 mg weekly to minimum of 0.3 mg/KG/day or minimum of 5 mg for weight <30 Kg and to dose of 2.5 5 mg weekly to a dose of 0.3mg/Kg /day for weight of >30 kg. from W5- w8 dose was reduced by 1 mg weekly, from w9- w12 by 1mg weekly till total dose of 0.2mg/kg/day or at least 5 mg daily for weight of >30Kg. From 6 months ->1 year for pre-pubertal , for those on Tac protocol and if no rejection after six months change to alternate day regimen at 0.2mg/Kg/EOD. For post pubertal PNL dose was kept at 5 mg OM (once in the morning) After 2 years in post pubertal dose was switched to 0.2mg/Kg/EOD or 10 mg EOD maximum if no rejection and stable renal function.[7]

Mycophenolate mofetil (MMF) was administerd to all patients with dose of 300mg/$m^{2/}$dose every 12 hrs (8 - 12 mgKg/dose) 12 hourly, max dose 500mg 12 hourly either one hour before or 2 hours after meals. This dose was reduced to 150 mg/$m^{2/}$ dose 12 hourly after 6 months. Dose adjustment is required for renal impairment, dose would be omitted if absolute neutrophil count <1.5× 10^6/L.

Tacrolimus was only started , 12 hours after surgery when creatinine would drop <150µmol/L, in dose of 0.15mg/Kg/ dose 12 H aiming at whole blood trough level of 10- 15ng/ml from 0- 1 month, and 8 10 ng/ml from 1 to 6 months post operatively and 5-8 ng/ml after 6 months. Ranitidine was given to all patients 3mg/Kg at bed time.

Oral Valganciclovir was given as prophylaxis for 3 months when donor was negative but recipient was positive or both were positive but 6 months when donor was positive and recipient was negative. Co-trimaxazole oral in dose of 3 mg/Kg /dose on alternate day was given for 3 months from day 4 on ward. IV cephazolin was started 1 day before Transplant and given till removal of Foley's catheter. Oral nystatin at dose of 250,000 units every 8 hourly for one month was given.

CMV immune-surveillance with PCR monitoring was done twice a month for one month, then monthly for 6 months and 3 monthly for one year. Data recorded and analyzed pre and post operatively and on each follow up visit included BP, body weight, height, number of symptomatic culture proven urinary tract infections, number and time point of rejection episodes if any , patient and graft survival, calcium, phosphorus and iPTH level, Hb level and left ventricular function and use of growth hormone. One patient received pre-emptive Transplant at GFR of 16 ml/min/1.73 m^2 while all others received Hemodialysis for mean period of 6.5 months. Acute rejection was defined as decreased urine output, high B P, pyuria or worsening or new proteinuria and rise in serum creatinine and stoppage of urine flow within

first 3 days after Transplant. Chronic rejection will be diagnosed by gradual and asymptomatic slow rise in serum creatinine statistical analysis: Parameters before and after Transplant were compared by paired T test. A p value of less then 0.05 was considered significant.

Results

In our study there were 6 males and 5 females, mean age at time of transplantation was 112.36 month (SD ±16.65), range 84 132 month. Mean follow up period was 28.636 m (SD±19.663), range 5 months 70 months. All patients received Transplant from living- related donors. Nine patients were on HD (mean 6.5 months), two patients received pre-emptive transplant. Regarding diagnosis Primary diagnosis was obstructive uropathy due to posterior uretheral valves associated with vesicoureteral reflux in 2 boys, while 2 siblings having diagnosis of Familial hypercalciuric hypomagnesemic nephrocalcinosis, (FHHNC) , one each with Focal Segmental Glomerulosclerosis (FSGS), Systemic Lupus Erythematoses (SLE), recurrent UTI,s, renal hypoplasia/dysplasia and in three patients cause was not known. There was significant improvement in growth parameters after Transplant, Mean weight before Transplant was 25 ±5.752 (range 20-37), after Transplant mean weight was 41.0±8.19 (range 24- 29) with p value of 0.00001 95% CI (-22.0200 10.161), Mean height before Transplant 126 ± 6.55 (115-133) after transplant 144±7.0(134-156) with p value 0.000195%CI (-22.8-15.18). Echocardiography of all patients was performed by pediatric cardiologist, there was mild to moderate hypertrophy with left ventricular dysfunction in 8 patients and severe dysfunction in one. On serial echocardiography after transplant left ventricular function improved in all 8 but ejection fraction (EF) remained low in one with severe dysfunction, requiring anti cardiac failure treatment. Systolic BP before transplant 125±11.18 (110-150), while after transplant mean systolic BP remained 113±1.94 (110-115) p value of 0.004, mean diastolic BP before transplant was 86±8.60 (70-95). Mean diastolic BP after transplant was 72±9.1 (60-95) with p value of 0.003. Mean Hb before transplant was 8.30 g/dl±1.40(range 5.5-10), after transplant mean Hb was 11.2±1.4(range 9.5-14) with p value of 0.01, Intact parathormone (iPTH) before transplant was 475±175.7 (range200-900) mean iPTH after transplant was 55.27±49.75(15-200) with p value of 0.0002 . All patients had high phosphate levels before transplant with mean of 6.30±1.13 (range 4-7.5), this level dropped to 4.27±0.36 (range3.5-4.5) with p value of 0.0001 after transplant. Two patients received growth hormone therapy after transplant, in one patient attained peek height velocity > 12cm/year leading to height progress from 133 cm before GH to 148 cm 17 months later, and advancement of SMR by 2 stages, Second patient receiving GH is gaining height with peak height velocity of 10cm/year.

Table-1: Demography of subjects.

Patient	Gender	Aetiology leading to ESRD	Age at the at the time of presentation	Follow up Period
1	M	Obstructive uropathy / VUR	120 months	46 months
2	M	Obstructive uropathy / VUR	125 m	42 m
3	M	FHHNC	132 m	70 m
4	M	FSGS	98 m	29 m
5	F	SLE	120 m	36 m
6	F	Idiopathic	96 m	31 m
7	F	FHHNC	132 m	26
8	F	Idiopathic	96 m	10 m
9	M	Idiopathic	84 m	8 m
10	108m	Renal hypoplasia/dysplasia	125 m	12 m
11	108m	Recurrent urinary tract infections	108 m	5 m
		Mean Age= 112.36m (SD + 16.65)	Mean Age= 112.36m (SD + 16.65)	

** FHHNC = Familial Hypomagnesaemia with Hypercalciuria and Nephrocalcinosis*
** FSGS = Focal Segmental Glomerulosclerosis / * SLE = Systemic Lupus Erythematosus*

Table-2: Comparison before and after transplant.

	Before TX	Range	After Tx	Range	P-value
Weight (kg)	25±5.752	20-37	41.00±8.19	24-29	0.00001
Hight (cm)	126±6.55	115-133	144±7.00	134-156	0.0001
Systolic BP (mmHg)	125±11.18	110-150	113±1.94	110-115	0.004
Dystolic BP (mmHg)	86±8.60	70-95	72± 9.15	60-95	0.003
HB (g/dl)	8.30±1.40	5.5-10	11.2±1.400	9.5-14	0.001
IPTH (ng/l)	475±175.7	200-900	55.27± 49.75	15-200	0.002
Phosphate (mg/dl)	6.30± 1.13	4-7.5	55.27± 49.75	3.5-4.5	0.0001

Complications we experienced after transplant were graft loss in one due to renal artery thrombosis (RAT) on 6th post op day, which later underwent transplant nephrectomy. Second patient, case of posterior urethral valves with bilateral VUR had recurrent urinary tract infections that require ureteric re-implantation as well as oral tamsulosin.

Discussion

Pakistan is sixth most populous country; population has increased tremendousley over last 5 decades from 46,673,627 in 1961 to 179, 160,111 in 2012 with population density of 225, having male 51.36% and female 48.63%. Under 18 pediatric population makes considerable proportion.

Medical facilities never grew to match this population explosion, this situation is further compounded by the fact that country is still struggling with economic turmoil as well as infectious disease. So scarce facilities and development is seen in areas like Pediatric Nephrology and definitive therapy like kidney transplant is still a dream for majority.

Although Results of kidney transplant have improved over last few decades owing to better immunosuppressive protocols using CNIs (Cyclosporine and Tacrolimus) and antiproliferative medications like azathioprine or MMF.[8] Reported results by NAPRTCS 2010 as 96.5, 91.5 and 84.3% for living donor recipient and 95.1, 84.1 and 78% for deceased donor recipient.[9]

Factors other than effective immunosuppression also contribute to Outcome of kidney Transplant which includes age of donor and recipient, prolonged cold ischemia time, presence of preformed anti- HLA antibodies, episodes of acute rejection, ethnicity, infections, adherence to medications and bladder function are important determinants.

According to North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) report children who received Transplant kidney under the age of 12 years and with obstructive uropathy performed less well than those who got transplanted after 12 years of age.[10]

LVH is found in 47% of patients with CKD at start of diaysis and 75% of patients of on HD for 10 years [11]. Left ventricular hypertrophy and low ejection fraction are major determinant of cardiovascular morbidity and mortality in patients with ESRD.[12,13]

Regression of left ventricular hypertrophy and improvement in ejection fraction along with control of hypertention after kidney transplant has been documented in various studies.[14,15]

Anemia is an important and independent risk factor for LVH, HF and cardiovascular morbidity and mortality.[16]

Growth hormone has clearly shown benefit in post transplanted, growth retarded patients, provided the other amenable factors including anemia, nutrition, metabolic acidosis, fluid & electrolyte abnormalities and renal osteodystrophy has been addressed properly. Maximum benefit is obtained when started pre-pubertal [17]. Studies have also shown that risk of acute rejection or graft dysfunction does not increase with treatment of growth hormone.[18]

Conclusion

Kidney transplant is an ideal modality for children with ESRD, need to be offered to patients to with ESRD. Risk of complications is very small but benefit in terms of better quality of life, better growth and psychological well being is huge. After transplant children can attend school and live normal active life.

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References

1. National Kidney Foundation. KDOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002; 39:S1
2. Humes HD, Buffington D, Westover A J, Roy S, Fissell WH. The bioartificial kidney: current status and future promise. Pediatr Nephrol (2014) 29: 343- 351
3. McDonald SP, Craig JC, Australian and New Zealand Paediatric Nephrology Association. Long-term survival of children with end-stage renal disease. N Engl J Med 2004; 350:2654.
4. Rizvi AH et al, Kidney International (2003) 63, S96S100; doi:10.1046/j.1523- 1755.63. 83.20. Renal transplantation in developing countries. Sindh Institute of Urology and Transplantation (SIUT), Dow Medical College, Karachi, Pak.
5. Kahaut EC (1999) End stage renal disease, Chap 325. In McMillan JA, Deangelis CD, Feigin RD, Warshaw JB, Oski FA (eds) Oski's pediatric principles and practice. 3rd edn. Mosby, Pennsylvania, p 1572 (IPNA Journal Oct 2006, p 1462
6. Rizvi SA et al, Pediatric kidney transplantation in developing world: challenges and solutions. Am J Transplant 2013 sep; 13 (9):2441- 9
7. Yap HLK. Renal transplantation: Pre- operative/ post- operative care and transplant immunosuppression. In: practical pediatric nephrology. An update of current practices. Ed : Chiu MC, Yap HK. Mrdcom limited 2005; pp314- 325
8. Melter M, Briscoe DM. Challenges after pediatric transplantation. Semin Nephrol 2000; 20:
9. Tejani A, Ho PL, Emmett L, et al. Reduction in acute rejections decreases chronic rejection graft failure in children: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). Am J Transplant 2002; 2:142.
10. The North American Pediatric Renal Trials and Collaborative Studies 2010 Annual Report. Available at: www.naprtcs.org (Accessed on May 04, 2012)
11. Ellis EN, Martz K, Talley L, et al. Factors related to long-term renal transplant function in children. Pediatr Nephrol 2008; 23:1149.
12. Ekardt KU, scerhag A, Macdougall IC, t al. Left ventricular geometry predicts cardiova- scular outcome associated with Anemia correction in CKD. J am Soc Nephrol 2009; 20: 2651
13. Stewart GA, Gansewoort RT, Mark PB etal. Electroc- ardiographic abnormalities and uremic cardiomyopathy. Kidney Int 2005; 67:217
14. Wang AY, Lai KN. Use of Cardiac biomarker in end- stage renal disease. J Am Soc Nephrol 2008; 19: 1643
15. Ferreira SR, Moises VA, Tavares A, Pacheo- silva A. Cardiovascular effect of successful renal transplantation: a 1-year sequential study of left ventricular morphology and function, and 24-hour blood pressure profile. Transplantation 2002; 74: 1580
16. 17-Rigatto C, Foley RN, Kent GM, et al. Long term changes in left ventricular hypertrophy after renal transplantation. Transplantation 200; 70: 570
17. Berard E, Andre JL, Guest G et al. Long term results of rhGH treatment in children with renal failure: experience of the French society of Pediatric Nephrology. Pediatr Nephrol 2008; 23: 2031
18. Hodson EM, Willis NS, Craig JC. Growth hormone for children with chronic kidney disease. Cochrane databae syst rev 2012;2 CD 003264

Medical Guidelines

Original Article

Clinical Spectrum of Dengue Fever in Pediatric Age Group At a Tertiary Care Hospital In Lahore

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Objective: To assess frequency of various clinical presentations of dengue fever in pediatric age group at a tertiary care hospital in Lahore.

Methods: A cross sectional survey was conducted from Dec 2012 to Sep 2013 in pediatrics department of Jinnah hospital Lahore. 150 patients were enrolled as per inclusion criteria. Clinical features were abstracted on a standardized Performa and investigations were done, including complete blood count, hematocrit, anti-dengue IgM (If fever more than 5 days), NS1 antigen (If fever less than 5 days) by Elisa and ultrasound scan of abdomen to document free fluid in abdominal or thoracic cavity. Data was analyzed using SPSS Version 17.

Results: One hundred and fifty patients with mean age of 8.4 years (SD of ± 2.8 years) were enrolled. 86 patients (57.3%) were males with male to female ratio of 1.3: 1. High grade fever was present in all the 150 patients (100%) & Diarrhea in 21(14 %) patients. Other clinical features included: petechiae in 25 (16.3%), epistaxis in 20 (13.3%), hematemesis in 8 (5.3%), hepatomegaly in 33(22%) and splenomegaly in 15 (10%) subjects. The evidence of dengue hemorrhagic fever was seen in 23 patients (15 %), while there was no case of Dengue Shock Syndrome.

Conclusion: Most of dengue fever cases were from 5 to 10 years of age group. High grade fever was the most common clinical manifestation of dengue fever, followed by hepatomegaly, petechiae, diarrhea, epistaxis, splenomegaly and hematemesis. Dengue fever was more common in males as compared to females, and about one in six cases had dengue hemorrhagic fever.

Keywords: Dengue fever.

Introduction

Dengue fever (DF) is an acute infectious disease of antiquity. It is probably the most important arthropod-borne viral disease in terms of human morbidity and mortality, with 100 million infections occurring annually, for which no effective therapy exists.[1] DF is caused by any of the four serotypes of Dengue Virus, a member of Flavivirus family. The principle vector for dengue is Aedes Aegypti, a highly urbanized, daytime biting mosquito that breeds in stored water. The clinical spectrum ranges from a self-limiting infection to more severe forms like dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) with a mortality rate of up to 40 percent.[2] All four dengue serotypes are now endemic around the globe. Pakistan is now hyper endemic showing co-circulation of all four serotypes.[3,4]

Infection with one type of virus imparts life-long immunity against that particular virus and partial transient immunity against the other three types of viruses. The risk of severe disease is much higher in sequential rather than primary dengue infection.

Dengue fever (DF) is usually characterized by fever, headache, myalgias and arthralgias, as some of the prominent symptoms. It may be associated with severe thrombocytopenia and clinically significant bleeding, but it is rarely life-threatening.[5] DHF is an entity characterized by a transient and rapid increase in vascular permeability with hemo-concentration, thrombocytopenia, and in the most severe cases, hypovolemic shock and coagulopathy.[5, 8] The most serious forms include dengue hemorrhagic fever and dengue shock syndrome, having high case fatality rate of up to 5 %.[18] Laboratory investigations usually done include hemoglobin (Hob gm %), total and differential leukocyte count (TLC and DLC), platelet count (PLT count), hematocrit (HCT). Non-structural antigen1 (NS1) and PCR is done to identify the virus and its genotype. Other tests are liver function tests (LFT) including prothrombin time (PT), serum albumin, IgM& IgG antibodies for dengue fever, ultrasound abdomen and chest X ray.[6]

Various studies conducted in Thailand[11], SriLanka[10],

India [9] and Bangladesh[12] on clinical spectrum of dengue fever in children showed that clinical features of dengue fever has different prevalence in different regions.

A few examples of the variable range of signs and symptoms in different population show that bleeding manifestations were seen in 11% of patients in Thailand as compared to 59% in Bangladesh. Fever was reported in almost all cases except for one study conducted in Bangladesh showing only 76 % cases of Dengue Fever with fever. Similarly, variable frequency of other clinical features e.g. diarrhea, bleeding manifestations, hepatomegaly and splenomegaly is also reported across various geographical regions.

The occurrence of dengue fever in Pakistan has shown a rising trend. However local data on various clinical manifestations of dengue infections in children is scarce. We conducted this study to assess frequency of various clinical features and presentations of dengue fever in pediatric age group in a tertiary care hospital of this region in order to compare the variability of clinical signs with other adjacent geographical areas.

Methods

A cross sectional survey was conducted from Dec 2012 to Sept 2013 (nine months) in Pediatrics Department of Jinnah Hospital, Lahore. Taking an expected percentage of hematemesis i.e. 6% (least among all) of various clinical presentations of dengue fever, a confidence interval of 95% and a margin of error of 4%, a sample size of 150 cases was calculated. Patients were enrolled using non-probability / purposive sampling. Patients were included if they fulfilled the following criteria:

Inclusion criteria:

- Confirmed case of dengue fever (as per operational definition)
- Age up to 13 years
- Either gender

(Dengue fever: Clinical features suggestive of dengue fever (as per WHO), plus positive NS1 antigen or positive IgM)

Exclusion Criteria:

- Super added infection e.g. malaria diagnosed by slide positivity.
- Children with any other chronic disease e.g. CCF, Aplastic anemia

After informed consent, 150 subjects, fulfilling the inclusion criteria were recruited for the study and complete demographic profile was taken. History was taken regarding fever, diarrhea, epistaxis, hematemesis and occurrence of dengue cases at the same location. Clinical examination in the form of vital signs and general physical examination was done to document petechiae and fever. Blood sample was taken for hemoglobin, hematocrit and platelets count, anti-dengue IgM (If fever more than 5 days) and NS1 antigen (If fever less than 5 days) by Elisa technique. Ultrasound abdomen and chest was done to document hepatomegaly, splenomegaly and plasma leakage (ascites, pleural effusion).

All information was recorded in a structured questionnaire. Data was entered and analyzed in SPSS version 17.0. Numerical variables like age were presented as mean and SD. Frequency tables and percentages were generated for fever, diarrhea, epistaxis, hematemesis, hepatomegaly, splenomegaly, petechiae for different age and gender strata.

Results: A total of 150 patients were included in this study to document the clinical features of dengue fever during a period of nine months. Age ranged from 1 year to 14 years with a mean age of 8.4 years and standard deviation (SD) of ± 2.8 years. Majority patients were from age group 5-10 years (n=90, 60%), followed by 10-14 years (n=40, 26.7 %) and <5 years (n=20, 13.3%). Most patients were male (n=86, 57.3%) and male to female ratio was 1.3:1. Amongst all 150 subjects 86 (57.3%) were males and 64 (42.7%) females. Male to female was 1.3: 1.

High grade fever was present in all the study cases (100%) and diarrhea in 21(14 %) patients. A number of hemorrhagic manifestations were observed including petechiae in 25 (16.3%) patients, epistaxis in 20 (13.3%) patients and hematemesis in 8 (5.3%) subjects. An evidence of hepatomegaly was found in 33(22%) patients and splenomegaly in 15 (10%) patients. 23 (15%) patients had evidence of plasma leakage and labeled as dengue hemorrhagic fever. There was no case of dengue shock syndrome (DSS).

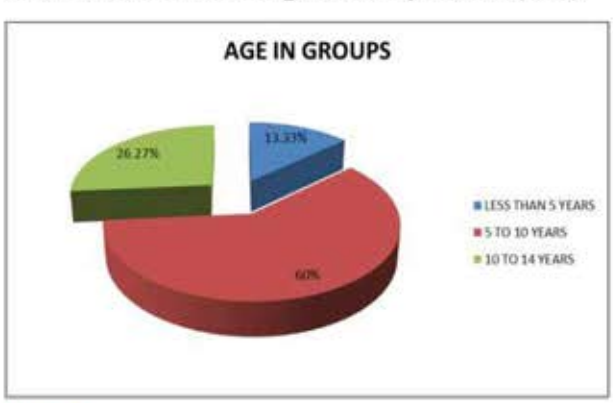

Fig-1: Age Distribution.

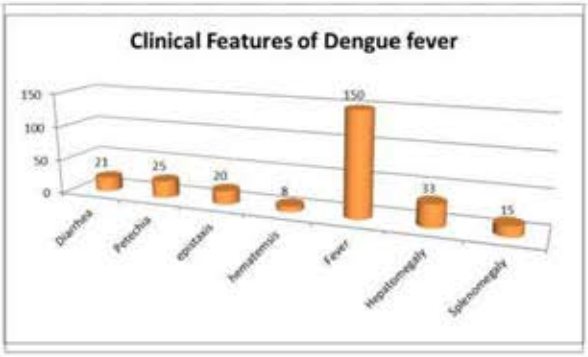

Fig-2: Clinical features of dengue fever.

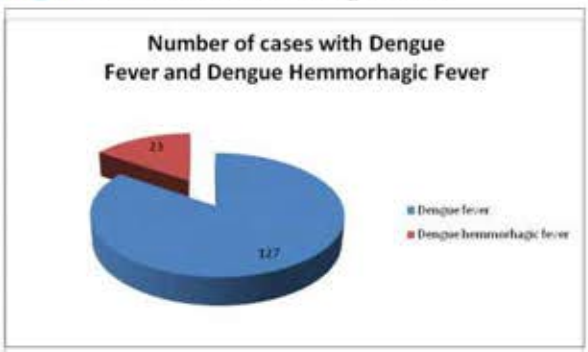

Fig-3: Clinical features of dengue fever.

Discussion

The frequency of various clinical manifestations varies across the region. It can partly be explained on the basis of various dengue virus serotypes and other genetic & environmental factors.[16] Since we only included symptomatic patients, fever being essential criteria of dengue fever by World Health Organization (WHO) was the most common presenting symptom. In our study all cases were having high grade fever in comparison to a study conducted in Bangladesh[12] where only 75.9% of study patients had high grade fever. Dengue viruses can involve almost any organ system. The evidence of gastrointestinal involvement in the form of diarrhea was seen in 14 % patients in our study. This frequency is higher than that reported in other countries like Bangladesh12 (9.3%) and India9 (6.2%). However, an even higher percentage of diarrhea has been reported from other cities of Pakistan e.g. Karachi[7] (25.5%) and Faisalabad (17%). The difference across countries & cities in Pakistan can be partly explained on the basis of circulation of different Dengue virus serotypes, for example, all[4] serotypes were reported from Lahore in 2009, while DENV 2 was responsible for the large outbreak in 2011. Frequency of hemorrhagic manifestations is also variable across different studies. These manifestations are more common in patients with evidence of thrombocytopenia, vascular fragility (Positive Hess test) and evidence of plasma leakage. However, minor bleeding is commonly seen in cases of Dengue Fever and there is no correlation between platelets count and bleeding risk.[15]The most common bleeding manifestation was petechiae (16.7%) followed by epistaxis (13.3%) and hematemesis (5.3%). The observed frequency of petechiae (16.7%) is comparable to that seen in North India[9] where a similar figure of 16.7% was reported but is lower as compared to a study in Thailand[11](36%) and Karachi[7](42%). It was higher than reported in a study conducted in Bangladesh[12] (7.4%) and Srilanka[10] (7.7%). Frequency of epistaxis was 13.3% in our study which is almost similar to that in a study in Philipine[14](11%) and much higher than in a study conducted in India9 (2.5%) and a study in Srilanka[10] (6%). Hematemesis was present in 5.3% of patients in our study which is similar to a study in Srilanka[10] (6%) but higher than that in study in India[9] (1.3%) and lower than that in Bangladesh[12] (13%). Hepatomegaly was present in 22% of our study cases. This frequency was much lower than the study conducted in India[9] (60%) and Bangladesh[12] (31.5%). Splenomegaly was present in 10% of patients in our study and that is almost comparable to the Indian data9 (11.8%) and was much lower than that in another study in North India (60%). On the contrary frequency of splenomegaly in our study was higher than a study in Bangladesh[12] where it was found to be 3.7%. One interesting finding in our study was the number of cases with evidence of dengue hemorrhagic fever e.g. 23 patients (15%) which was much less than reported in a study in Bangladesh having 59 % DHF. In our study no case of dengue shock syndrome was reported. In a study conducted in North India[9] reported percentages were dengue fever (8%), dengue hemorrhagic fever (51%) and dengue shock syndrome (42%).This is again much higher percentage of DHF and dengue shock syndrome as compared to our study. In a study conducted in Sri Lanka[10] number of DF cases was 17.3% while 82.7% had DHF. This difference can be explained by difference strains in different regions and the phenomena of primary vs. secondary infection. In our data no death was reported in pediatric age group, and this was in contrast to adult patients studied in the same hospital in 2011 were the death percentage was 3.5%.[17] Difference in clinical features might be explained by the prevalence of different strains of DENV in different areas and also may be differently causing DF and DHF, but further studies are required to document the clinical features

of dengue fever according to various strains and also clinical features of dengue fever and dengue hemorrhagic fever.

Conclusion:

Most of dengue fever cases were from 5 to 10 years of age group. High grade fever was the most common clinical manifestation of dengue fever followed by hepatomegaly, petechiae and diarrhea. Dengue fever was more common in males as compared to females. Around one in six patients had dengue hemorrhagic fever.

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References

1. Ahmed SI, Khalid MA, Baqai HZ, Ali SF, Ranja ZA. Dengue fever in northern Pakistan: The hepatic implications. J Rawal Med Coll 2009; 13(2):56-59.
2. Chen RF, Yang KD, Wang L, Liu JW, Chiu CC, Chang JT. Different clinical and laboratory manifestations between dengue hemorrhagic fever and dengue fever with bleeding tendency. Trans R Soc Trop Med Hyg 2007; 101: 1106-13.
3. Humayoun MA. Multiple Dengue serotypes and high frequency of Dengue hemorrhagic fever at two tertiary care hospitals In Lahore during the 2008 Dengue virus outbreak In Punjab, Pakistan. Int J Infect Dis 2010; 14(Suppl 3):54-59.
4. Ahmed MM. Clinical profile of dengue fever infection in King Abdul Aziz University Hospital Saudi Arabia. J Infect DevCtries 2010; 4(8):503-510.
5. Capeding RZ, Brion JD, Caponpon M, Gibbons RV, Jarman R G, Yoon I. The Incidence, Characteristics, and Presentation of Dengue Virus Infections during Infancy. Am J Trop Med Hyg 2010; 82(2):330336
6. Chandrakanta, Kumar R, Garima, Agarwal J, Jain A, Nagar R. Changing clinical manifestations of dengue infection in north India. Dengue Bulletin 2008;32;118-125
7. Riaz MM, Mumtaz K, Khan MS, Patel J, Tariq M, Hilal H. Outbreak of Dengue Fever in Karachi 2006: a clinical perspective. J Pak Med Assoc 2009; 59(6):336-44.
8 Kamath S R, Ranjit S :Clinical Features, Complications and Atypical Manifestations of Children with Severe forms of Dengue Hemorrhagic Fever In South India; Indian J Pediatrics 2006; 73 :889-95
9 Mittal H, Faridi MM, Arora SK, Patil R: Clinicohematological Profile and Platelet Trends in Children with Dengue during 2010 Epidemic in North India. Indian J Pediatrics 2012(10):586-7
10 Malavige GN, Ranatunga PK, Velathanthiri VGNS, Fernando S, Karunatilaka D H, Aaskov J, and Seneviratne S L et al: Patterns of disease in Sri Lankan dengue patients; Arch Dis Child 2006; 91(5): 396400
11. Libraty DH, Myint KSA, Murray CK, Gibbons RV, Mammen MP, et al (2007). A comparative study of Leptospirosis and Dengue in Thai children. Plos NegI Trop Dis 2007;1(3): 111
12. Alam ABMS, Sadat SA, Swapan Z, Ahmed AU, Karim MN, Paul HK, Zaman S, et al: Clinical Profile of Dengue Fever in Children; Bangladesh J Child Health 2009; Vol.33(2): 55-58
13. Kumar A, Rao CR, Pandit V, Shetty S, Bammigatti C, MinoliSamarasinghe CM: Clinical Manifestations and Trend of Dengue Cases Admitted in a Tertiary Care Hospital, Udupi District, Karnataka ; Indian J Community Med. Jul 2010; 35(3): 386390.
14. Carlos C, Oishi K et al; Comparison of clinical features and hematologic abnormalities between dengue fever and dengue hemorrhagic fever among children in Philipines; Am. J. Trop. Med. Hyg; 73(2), 2005, pp. 435440
15. Khan Assir MZ 1 , Kamran U, Ahmad HI, Bashir S, Mansoor H, Anees SB, Akram J; Effectiveness of platelet transfusion in dengue Fever: a randomized controlled trial; Transfus Med Hemother. 2013 Oct; 40(5):362-8.
16. Haider Z , Ahmad FZ, Mahmood A, Waseem T, Shafiq I, Raza T, Qazi J, Siddique N, Humayun MA; Dengue fever in Pakistan: a paradigm shift; changing epidemiology and clinical patterns; Perspex Public Health. 2015 Nov; 135(6):294-8.
17. Assir MZ, Ahmad HI, Mason MA, Kamran U, Yusuf NW.; Deaths due to dengue fever at a tertiary care hospital in Lahore, Pakistan; Scand J Infect Dis. 2014 Apr;46(4):303-9.
18. Siddiqui FA, Haider RA, Bhutta ZA; Endemic Dengue Fever: a seldom recognized hazard for Pakistani children; J Infect Dev Ctries 2009; 3(4): 306-12

Original Article

The Effect of Counselling in Preoperative Anxiety Among the Patients Undergoing Third Molar Surgery

Asma Ahmad, Muhammad Mujtaba and Muhammad Ismail Tariq

Objective: To determine the effect of counselling on pre-operative anxiety among the patients undergoing third molar surgery.

Methods: A randomized controlled trial was conducted in the department of maxillofacial surgery, Punjab Dental Hospital and Mayo Hospital, Lahore from September, 2013 to December, 2013. A total of 100 patients planned for third molar surgery by the maxillofacial surgeon on the basis of symptomatology and investigations and also having baseline anxiety above the cutoff point on Hamilton Anxiety Rating Scale (HAM-A) were selected and randomly divided into the control and the experimental groups. The pre- operative anxiety was assessed objectively and subjectively on Hamilton Anxiety Rating Scale (HAM-A) and Dental Anxiety Scale (DAS) (Urdu version) respectively at the time of booking the patient for the operation (session 1) and scoring was done. Detailed counselling was done to the experimental group in addition to providing information as usual which included routine briefing which was given to all the patients of both the groups. Preoperative anxiety was assessed again on the same scales in the same manner in both the groups just before the surgery (session 2) and the results were compared.

Results: A statistically significant difference was found in pre-operative anxiety scores of both the experimental and control groups between session 1 and session 2. In the experimental group, preoperative anxiety was significantly reduced after intervention (p-value=0.00) as compared to the control group in which it was significantly increased (p- value=0.00) just before the surgery.

Conclusion: Counselling is an effective method for reducing preoperative anxiety in patients undergoing third molar surgery.

Keywords: Preoperative anxiety, dental anxiety, third molar surgery, counselling.

Introduction

Third molar surgery is one of the commonest procedures carried out in the department of oral and maxillofacial surgery.[1] Owing to the special anatomical variations in third molars, surgical intervention is usually a traumatic experience.[2] It is associated with immense fear and various intra and postoperative complications leading to pre-operative anxiety in the individuals undergoing surgery. Pre-operative anxiety results in various physiological changes in the body which further worsens these complications.[3] The common complications include excessive bleeding, damage to surrounding tissues, trismus, swelling and pain.[4] About 10-20% of the adult population suffer from severe dental anxiety affecting the attendance in dental clinics adversely. Highly anxious individuals are often caught in a vicious cycle of fear whereby they avoid dental treatment leading to poor dental health. They seek care only in severe pain and so have to undergo extensive treatments.[5] Despite improvements in anesthesia and technical advancements along with public awareness, anxiety is still a major problem.[6] This is evident from the high levels of pre-operative anxiety in local population (62%-73% females and 42% males).[7] Several factors contribute towards high levels of dental anxiety including gender, age, rural or urban living, level of education, socio-economic status and earlier traumatic dental experiences.[8] However, pain is the most important feared factor. Thus, reassurance and adequate pain control have a key role in relieving anxiety. This reassurance should start at the time of booking the patient for theoperation.[9] Preoperative tailored information at the initial visit leads to less anxiety and higher satisfaction.[10] Preoperative anxiety can be managed by the use of different pharmacological[11] and psychological methods.[12] Among these, counselling prior to surgery is an easy and cost effective method to avoid negative outcomes.[12,13] Importance of counselling is commonly overlooked leading to increased patients' suffering and economic burden.[4]

Preoperative visit and provision of information help

to reduce the physiological parameters[14] and preoperative anxiety.[3] This leads to reduced perioperative complications.[15] In literature review, many studies have supported the positive effect of counseling.[12,13,16] However, a few studies could not find any beneficial effect of this intervention.[17,18]

Methods

An interventional study / randomized control trial (RCT) was conducted in the department of maxillofacial surgery, Punjab Dental Hospital, Lahore and the department of maxillofacial surgery, Mayo Hospital, Lahore during a period of 4 months from September, 2013 to December, 2013. Using a nonprobability convenience sampling technique, 100 patients were included in the study. These patients had an age of 18 years and above who presented in the department of maxillofacial surgery for third molar extraction and were also willing to participate in the study on voluntary basis. Patients having previous experience of third molar surgery were not included. These patients were assessed for anxiety by applying Hamilton Anxiety Rating Scale (HAM-A). The patients having anxiety level above the cutoff point were selected to participate in the study in order to maintain the homogeneity of the sample. These patients were randomly divided into two groups with 50 patients in each group. Group A was the experimental and group B was the control group. The patient's demographic characteristics were recorded on a structured proforma. Pre-operative general anxiety was objectively assessed and the findings were recorded on HAM-A. Preoperative dental anxiety was marked by the patient on Corah's Dental Anxiety Scale (DAS) (Urdu version). It is atranslated and adapted form of English version of Corah's Dental Anxiety Scale (DAS), which is a validated scale for the assessment of dental anxiety. Scoring was done on both scales and it was labeled as session 1 which was conducted at the time of booking the patient for surgery. Intervention in the form of detailed counselling was applied to the experimental group as a psychological intervention in an exclusive environment in a single session in addition to information provided as usual before surgery to all the patients of both the groups. On the day of surgery, pre-operative anxiety was assessed just before the operation in the previous manner and again scoring was done on the same scales and it was labeled as session. The difference in scores before and after intervention was documented and compared. Statistical Analysis: Data was entered in to a computer database using IBM SPSS Statistics version 20 (IBM Corporation) and was summarized as a data master sheet. The mean ± SD (standard deviation) was calculated for quantitative variables, i.e., age and score of the both scales. Percentages were calculated for qualitative variables i.e., gender and socioeconomic status. A linear regression was used to compare the groups while taking into consideration the baseline differences. The independent sample t-test was used to compare the means of pre and post- intervention anxiety scores of both scales. A P-value ≤ 0.05 was considered as statistically significant.

Results

The age range of total sample (n=100) was 15-60 years with mean age of 30.30±7.85 years. Mean age of group A (n=50) was 30.34±8.00 years and that of group B (n=50) was 30.26±7.79 years. There were 43 males and 57 females included in the study. The group A consisted of 22 (44%) males and 28 (56%) females while group B included 21 (42%) males and 29 (58%) females. Regarding socio-economic status group A consisted of 28 (56%) subjects from middle and 22 (44%) subjects from lower class while group B included 29 (58%) and 21 (42%) subjects from middle and lower class respectively. No subject in the study belonged to upper socio- economic group. The pre-intervention and post-intervention level of anxiety in group A was compared with group B. The effect of counselling on anxiety scores in DAS and HAM-A were determined by applying linear regression after controlling the difference of anxiety level at baseline. Beta coff. values indicated that there was a change in anxiety scores of session 2 by 0.618 in DAS and 0.715 in HAM-A for a unit change in anxiety scores of session 1 in the experimental group (A). The results showed a positive effect of counselling in the scores of both scales. This effect was found to be statistically significant (p-value<0.05) **Table 1**. The means of pre-intervention and post-intervention anxiety scores were compared in DAS by applying independent sample t-test in both the groups. There was no significant difference between pre-intervention anxiety scores in DAS in both the groups. After intervention, there was a significant difference in DAS score between the two groups (p-value<0.05). In group A, there was a decrease in anxiety to a significant extent after intervention as compared to group B where there was an increase in anxiety just before the surgery **Table 2.** The means of pre-intervention and post-intervention anxiety scores were compared in HAM-A by applying independent

sample t-test in both the groups. The pre-intervention anxiety score in group A was high as compared to group B. After intervention, there was a significant difference in HAM-A score between the two groups (p-value<0.05). In group A, there was a decrease in anxiety to a significant extent after intervention as compared to group B where there was an increase in anxiety just before the surgery **Table 3.** Thus, the scores of preoperative anxiety before and after intervention were statistically analyzed and compared and it was found that preoperative anxiety was significantly decreased and increased in the experimental and the control groups respectively (p-value<0.05).

Table-1: Effect of counselling on preoperative anxiety (n=100).

Scale	Model	Beta Coff.	95% CI		P-value
			Lower	Upper	
DAS	Groups (A)	-5.739	-6.402	-5.076	0.000
	Session 1 (Pre-intervention)	0.618	0.527	0.709	0.000
HAM-A	Groups (A)	-6.020	-7.058	-4.8307	0.000
	Session 1 (Pre-intervention)	0.715	0.601	0.830	0.000

Table-2: Comparison of pre and post-intervention anxiety scores according to DAS (n=100).

Anxiety Scores	Groups	N	Mean Value	SD	P-value
Pre-intervention	A	50	12.62	3.64	0.254
	B	50	11.78	3.67	
Post Intervention	A	50	9.06	2.43	
	B	50	14.28	3.12	0.001

Table-3: Comparison of pre and post-intervention anxiety scores according to HAM-A (n=100).

Anxiety Scores	Groups	N	Mean Value	SD	P-value
Pre-intervention	A	50	13.74	5.32	0.016
	B	50	11.56	3.37	
Post Intervention	A	50	8.74	4.28	
	B	50	13.20	3.84	0.001

Discussion

The results of the current study showed that preoperative anxiety was significantly reduced in the experimental group after intervention as compared to the control group in which it was rather increased significantly in the absence of counselling. Various studies investigated the impact of providing preoperative information in relieving anxiety and found the same results. In Hong Kong, Ng et al. found that preoperative information about the recovery, intra and postoperative periods helped to decrease the anxiety of the patients.[19] Spaulding, in a qualitative, observational study in Britain and indicated that the pre-operative knowledge about the expected outcome reduces anxiety.[20] Sjoling et al. looked for the effect of providing specific preoperative information, in an in terventional study. The treatment group was able to become active in their treatment after the specific information they received, which lead to a significant reduction in their state anxiety.[21] This finding supported that specific information improved patient's self-care capabilities as a whole. Guo indicated a significant decrease in anxiety score in an experimental trial.[16] The results are in accordance with the present study in which preoperative anxiety was increased in the control group just before the operation. It is the role of a surgeon to build a rapport and develop trust by educating and providing standard information to the patients.[22] Ayaz et al. highlighted the importance of preoperative counseling and better doctor- patient communication. It has also been associated with lower levels of anxiety.[12] However, results of some of the studies are not in accordance with the findings

of the current study. In an experimental study by van Wijk and Lindeboom, the effect of standard information in an isolated consultation with an oral and maxillo-facial surgeon one week before operation was tested against the control group in which same information was given just before the extraction of third molars. No difference was found between the anxiety levels of the control and experimental groups. However, this consultation was highly appreciated by the patients.[10] This finding is apparently in contrast to the resultsof present study but due to the appreciation from the patients, this intervention may not be regarded as ineffective. Casap et al. suggested that over detailed history and disclosure before the procedure can increase the stress levels of patients.[17] However, in another study, the high information text was rated more informative by the participants, thus requiring less additional information and ultimately leading to higher satisfaction.[3] Although the results of these studies are contradictory findings indicate the need for tailoring the information
according to the individual's need and providing it in an appropriate format and manner,[23] which indirectly supports the findings of present study. By going through the literature, it may be concluded that managing anxiety is still a challenge in oral and maxillo-facial surgery all over the world irrespective of the technical, pharmacological and surgical advances. Dental patients are mostly operated under local anesthesia. Therefore, they are usually active during surgery and recovery to a large extent depends upon their level of relaxation during the procedure.[24] It has been observed that pre-anesthetic assessment reduces preoperative anxiety.[25] The situation becomes more complicated in this part of the world due to lack of awareness. In general, stress reducing and anxiolytic perioperative psychological techniques are of considerable value both for the patient as well as for the surgeon. This is more relevant to our culture where there are many myths and misconceptions about the use of anxiolytic medicines. It may be said that preoperative counselling of the patients improves quality of care. As only a few studies have been conducted on this very important aspect in local population, it needs further research.

Conclusion

In routine dental practice, surgeons are so busy that they overlook the importance of preoperative counselling which may result in increased patients' suffering and economic burden. The current study has highlighted the importance of this simple behavioralintervention which was found to be highly effective if delivered in an appropriate format and sensitive manner.

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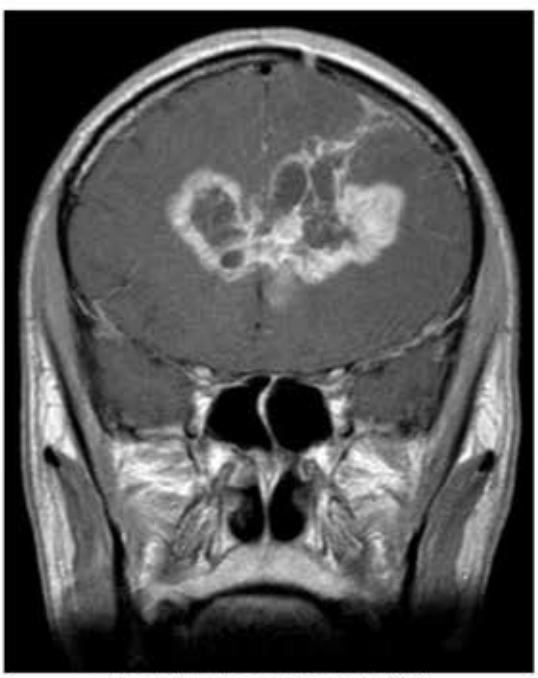
References

1. Beshkar M, Momeni M, Hasheminasab M. Third Molar Surgery: Insights from recent literature. J Craniomax Res. 2014;1(1):2-7.
2. Ahmed HMA. Management of third molar teeth from an endodontic perspective. European Journal of General Dentistry. 2012;1(3):148-160.
3. Brasileiro BF, de Braganca, RMF, Sickels JEV. An evaluation of patient's knowledge about perioperative information for third molar removal. J Oral Maxillofac Surg.2012;70:12-18.
4. Ferris-Torres E, Valmaseda-Castellon E, Berini-Aytes L, Gay-Escoda C. Informed consent in oral surgery: The value of written information. J Oral Maxillofac Surg. 2011;69:54-58.
5. Armfield JM, Stewart JF, Spencer AJ. The vicious cycle of dental fear: exploring the interplay between oral health, service utilization and dental fear. BMC Oral Health.2007;7:1.
6. Hmud R, Walsh LJ. Dental anxiety: causes, complications and management approaches. J Minim Interv Dent. 2009;2(1):67-78.
7. Khan FA, Jafar MF. Frequency of preoperative anxiety in Pakistani surgical patients.JPMA. 2009;59(359):5-0.
8. Malvania EA, Ajithkrishnan CG. Prevalence and socio-demographic correlates of dental anxiety among a group of adult patients attending a dental institution in Vadodara city, Gujrat. India J Dent Res. 2011;22(1):179-80.
9. Ukpong DI.. Assessment of preoperative and postoperative anxiety: A comparison of two measures in elective major surgery patients. Nigerian Journal of Psychiatry. 2010;8(3):29-32.
10. Van Wijk A, Lindeboom J. The effect of a separate consultation on anxiety levels before third molar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.2008;105:303-307.
11. Caroll JK, Cullinan E, Clarke L, Davis NF. The role of anxiolytic premedication in reducing preoperative anxiety. Br J Nurs. 2012;21(8):479-83.
12. Ayaz H, Atta-ur-Rehman, Fahim-ud-Din. Post-operative complications associated with impacted mandibular third molar

removal. Pakistan Oral and Dental Journal.2012; 32(3):389-92.
13. Bradt J, Dileo C, Shim M. Music interventions for preoperative anxiety. Cochrane Database of Systematic Reviews 2013, Issue 6. Doi:10.1002/14651858.CD006908.pub2.
14. Mardi D, Donna M, Naida H, Shane R. Decreasing patient's preoperative anxiety: a literature review. Australian Nursing Journal. 2007; 14(11):35.
15. Zhang CY, Jiang Y, Yin QY, Chen FJ, Ma LL, Wang LX. Impact of nurse-initiated preoperative education on postoperative anxiety symptoms and complications after coronary artery bypass. J Cardiovasc Nurs. 2012; 27(1):84-8.
16. Guo P. A preoperative education intervention to reduce anxiety and improve recovery among Chinese cardiac patients: A randomised control trial. 2012. Ph.D. thesis, University of Nottingham, London. i.17. Casap N, Alterman M, Sharon G, Samuni Y. The effect of informed consent on stress levels associated with extraction of impacted mandibular third molars. J Oral Maxillofac Surg. 2008;66:878-881.
18. van Wijk A, Lindeboom J. The effect of a separate consultation on anxiety levels before third molar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.2008;105:303-307.
19. Ng SKS, Chau AWL, Leung WK. The effect of pre-operative information in relieving anxiety in oral surgery patients. Community Dent Oral Epidemiol. 2004;32(3):227-235.
20. Spaulding NJ. Reducing anxiety by preoperative education: make the future familiar.Occup Ther Int. 2003;10(4):278-293.
21. Sjoling M, Nordahl G, Olofsson N, Asplund K. The impact of preoperative information on state anxiety, postoperative pain and satisfaction with pain management. Patient Educ Couns. 2003;51(2):169-76.
22. de Jongh A, van Wijk AJ, Lindeboom JA. Psychological impact of third molar surgery: A 1 month prospective study. J Oral Maxillofac Surgeons. 2011;69:59-65.
23. Ting KEL, Ng MSS, Siew WF. Patient perception about preoperative information to allay anxiety towards major surgery. IeJSME. 2013;7(1):29-32.
24. Lago-Mendez L, Diniz-Freitas M, Senra-Rivera C, Seoane-Pesqueira G, Gandara-Rey JM, Garcia-Garcia A. Postoperative recovery after removal of a lower third molar: role of trait and dental anxiety. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:855-860.
25. Masood Z, Haider J, Jawaid M, Alam SN. Preoperative anxiety in female patients: The issue needs to be addressed. KUST Med J. 2009;1(2):38-41.

Picture Quiz

Q: What is the name given to this pathology?



See Answer on Page # 138

Original Article

Prediction Of Oesophageal Varices In Cirrhotic Patients By Prothrombin Time As A Non-invasive Marker

Fatima Hamdani, Nasir Abbas ,Tahir Bashir and Sajid Nisar

Objective: The objective of the study was to determine the diagnostic accuracy of prothrombin time for the non invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as Gold Standard.

Methods: The study was conducted in Medical unit 4, Services Hospital Lahore over a period of 6 months. It is a cross-sectional study

Results: In our study, 43.5%(n=87) were between 12-30 years and 56.5%(n=113) were between 31-60 years, mean±sd was calculated as 39.90±12.29 years, 43.5%(n=87) were male and 56.5%(n=113) were females, frequency of esophageal varices keeping upper gastrointestinal endoscopy as gold standard was recorded in 57%(n=114) while 43%(n=86) had no findings of the morbidity, diagnostic accuracy of prothrombin time for the non-invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as gold standard 53%(n=106) true positive, 2%(n=4) false positive, 4%(n=8) false negative and 41%(n=82) as true negative. Whereas specificity, sensitivity, positive predictive value, negative predictive value and diagnostic accuracy was calculated as 92.98%, 95.35%, 96.36%, 91.11%, and 94% respectively.

Conclusion: We concluded that the predictive value of prothrombin time for presence of oesophageeal varices is a higher and it is a useful non-invasive diagnostic modality.

Keywords: Esophageal varices, non-invasive diagnosis, prothrombin time, diagnostic accuracy

Introduction

Cirrhosis is the end stage of every chronic liver disease, resulting in formation of fibrous tissue, disorganization of liver architecture, and nodule formation, which interferes with liver function and results in portal hypertension.[1]

Liver damage from chronic liver can disturb balance between clotting and fibrinolysis. The causes are multiple: quantitative and qualitative platelet defects; decrease production of coagulation factors and inhibitor of coagulation; vitamin K deficiency; synthesis of abnormal clotting factors; decreased clearance of activated factors; hyperfibrinolysis and disseminated intravascular coagulation.[2]These coagulation abnormalities can predispose patients from minor localized bleeding to massive life threatening haemorrhage or thrombus formation.[3]

Oesophageal varices are present at diagnosis in approximately 50% of cirrhotic patients, being more common in Child-Pugh class C. The greatest bleeding risk is seen in large varices classified as being >5mm diameter and is also influenced by liver disease severity as assessed by Child-Pugh score, and by the presence of red wale markings on varices at endoscopy. Therefore, these factors should also be taken into consideration to classify "high-risk varices.[4]

Variceal hemorrhage is a leading cause of morbidity and Mortality in cirrhosis.[5] Therefore, it is recommended that patients with cirrhosis should undergo endoscopic screening for Esophageal Varices (EV) at the time of diagnosis.[6]

In this study, the non invasive marker prothrombin time will be correlated with presence of oesophageal varices in patients with liver cirrhosis as compared to Upper gastrointestinal endoscopy which is Gold Standard. This study didn't evaluate the direct correlation between Prothrombin time and presence of oesophageal varices.[10]

The available data shows variations in results[10,11] so I want to study so that if significant results come then interventions can be made to diagnose varices noninvasively and to assess the predictive value of prothrombin time for presence of oesophageeal varices. Thus we can prevent life threatening variceal bleeding. This will also prove a diagnostic tool for stratification of patients regarding the presence of varices.

Methods

The study was conducted in Medical unit 4, SHL.200 patients was sample size keeping sensitivity 60.4% and specificity 91.7% of Prothrombin time for

diagnosis of oesophageal varices with 95% confidence interval and 10% margin of error with prevalence of varices 50.8%[7,9] by taking endoscopy as gold standard. It was non probability consecutive sampling.It is a cross-sectional study and of chronic liver disease showing coarse liver eco. Patients of 12 60 years of age and both genders. Patients presenting with variceal bleed. Patients taking non selective beta blockers and/or nitrat and patients who have received any therapeutic intervention for their varices like banding or injection sclerotherap. Patients who refuse to undergo upper gastrointestinal Endoscopy were included. 200 Patients with coarse liver ecotexture on abdominal ultrasound were selected from emergency department of SIMS/SHL and Prothrombin time was determined by using blood sample of patient by emergency laboratory, SHL. After taking informed consent these patients were booked for diagnostic upper gastrointestinal endoscopy at a later date. Upper gastrointestinal endoscopy was performed in these selected patients and presence or absence of oesophageal varices was documented. All those patients who did not fulfill inclusion criteria were rejected so that bias could be controlled.

Data analysis:

Data was entered and analyzed using SPSS 15. Quantitative variables like age were expressed by using mean±SD. Qualitative variables like gender, presence or absence of varices and prothrombin time was expressed using frequency and percentages. A 2x2 contingency table was generated to evaluate sensitivity, specificity, PPV, NPV and accuracy of prothrombin time in the prediction of oesophageal varices by taking endoscopy as gold standard.

Results

A total of 200 cases fulfilling the inclusion/exclusion criteria were enrolled to determine the diagnostic accuracy of prothrombin time for the non invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as Gold Standard.

Age distribution of the patients was done which shows that 43.5%(n=87) were between 12-30 years and 56.5%(n=113) were between 31-60 years, mean±sd was calculated as 39.90±12.29 years. **Table No. 1.**Gender distribution of the patients was done which shows that 43.5%(n=87) were male and 56.5%(n=113) were females. **Table No. 2**

Frequency of esophageal varices keeping upper gastrointestinal endoscopy as gold standard was recorded in 57%(n=114) while 43%(n=86) had no findings of the morbidity. **Table No. 3** Diagnostic accuracy of prothrombin time for the non-invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as gold standard 53%(n=106) true positive, 2%(n=4) false positive, 4%(n=8) false negative and 41%(n=82) as true

Table-1: Distribution of patients by age (n=200).

Age (Years)	No. Of Patients	Percentage
12-30	87	43.5
31-60	113	56.5
Total	200	100
Mean±sD	39.90±12.29	

Table-2: Distribution of patients by gender (n=200).

Age (Years)	No. Of Patients	Percentage
Male	87	43.5
Female	113	56.5
Total	200	100

Table-3: Frequency of esophageal varices keeping upper gastrointestinal endoscopy as gold standard (n=200).

Esophageal varices	No. Of Patients	Percentage
Yes	114	57
No	86	43
Total	200	100

Table-4: Diagnostic accuracy of prothrombin time for the non invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as gold standard

Prothrombin Time	Upper gastrointestinal endoscopy Positive	Negative	Total
Positive	True positive (a)	False positive (b)	a+b
	106 (53%)	4 (2%)	110 (55%)
Negative	False negative (c)	True negative (d)	c+d
	8 (4%)	82 (41%)	90 (45%)
Total	a+c 114 (57%)	a+c 86 (43%)	200 (100%)

Sensitivity = 92.98%
Specificity = 95.35%
Positive predictive value = 96.36%
Negative predictive value = 91.11%
Accuracy rate = 94%

Negative. Whereas specificity, sensitivity, positive predictive value, negative predictive value and diagnostic accuracy was calculated as 92.98%, 95.35%, 96.36%, 91.11%, and 94% respectively.
Table No. 4

Discussion

Variceal hemorrhage is a leading cause of morbidity and mortality in cirrhosis.[11] Primary prophylaxis with nonselective beta blockers and endoscopic band ligation may reduce the risk of variceal bleeding.[12] Therefore, it is recommended that patients with cirrhosis should undergo endoscopic screening for esophageal varices (EV) at the time of diagnosis.[13] If no varices are observed on initial endoscopy in patients with compensated cirrhosis, endoscopy should be repeated in 3 years; in decompensated cirrhotic patients, it should be repeated annually.[14] As a result of the cost and invasive nature of endoscopic screening, there is interest in developing a noninvasive predictor of the presence and development of varices that would decrease the number of endoscopies performed.[11] Predicting the presence of esophageal varices by non-invasive means would restrict the performance of endoscopy to those patients with a high probability of having varices.

In our study, the non invasive marker prothrombin time was correlated with presence of oesophageal varices as compared to Upper gastrointestinal endoscopy which is Gold Standard to determine that if significant results come then interventions can be made to diagnose varices noninvasively and to assess the predictive value of prothrombin time for presence of oesophageeal varices.

In our study, 43.5%(n=87) were between 12-30 years and 56.5%(n=113) were between 31-60 years, mean±sd was calculated as 39.90±12.29 years, 43.5%(n=87) were male and 56.5%(n=113) were females, frequency of esophageal varices keeping upper gastrointestinal endoscopy as gold standard was recorded in 57%(n=114) while 43%(n=86) had no findings of the morbidity, diagnostic accuracy of prothrombin time for the non-invasive diagnosis of esophageal varices keeping upper gastrointestinal endoscopy as gold standard 53%(n=106) true positive, 2%(n=4) false positive, 4%(n=8) false negative and 41%(n=82) as true negative. Whereas specificity, sensitivity, positive predictive value, negative predictive value and diagnostic accuracy was calculated as 92.98%, 95.35%, 96.36%, 91.11%, and 94% respectively.

Our findings are in agreement with a study showed Sensitivity of 60.4% and Specificity of 91.7%8 though sensitivity in our study was higher than this study but specificity was closely related to our study, our findings are in contrast with a study showing that prothrombin time was found to have Sensitivity of 61.8% and Specificity of 81.8% in a study done in china.[7] Wan-dong Hong and others revealed that a tree model that was consisted of spleen width, portal vein diameter and prothrombin time was developed by classification and regression tree analysis achieved a diagnostic accuracy of 84% for prediction of large esophageal varices.

Pilette C and co-authors studied the diagnostic accuracy of non-endoscopic means for the diagnosis of esophageal varices and recorded that prothrombin index, diagnosis of large esophageal varices (grades 2±3): diagnostic accuracy was globally 71%, and 72% with 3 variables: platelet count, prothrombin index, spider naevi and concluded that using a few non-endoscopic criteria, esophageal varices can be correctly diagnosed in 81% of patients with chronic liver disease and in 71% of patients with cirrhosis. These results show that the non-invasive screening of patients who are candidates for the primary prevention of variceal bleeding is possible, but should be improved before being used in a clinical setting.

However, considering the abvoe facts, we are of the view that significant accuracy emphases that interventions can be made to diagnose varices noninvasively and the predictive value of prothrombin time for presence of oesophageeal varices is a useful diagnostic modality. Thus we may prevent life threatening variceal bleeding. It also proves to be a diagnostic tool for stratification of patients regarding the presence of varices. However, these findigns are primary in our local setup, further trials should be done to authenticate our findings.

Conclusion

We concluded that the predictive value of prothrombin time for presence of oesophageeal varices is a higher and it is a useful non-invasive diagnostic modality.

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References

1. Garcia-Tsao G, Sanyal AJ, Grace ND and Carey W. "Prevention and management of Gastro-oesophageal varices and variceal haemorrhage in cirrhosis. AASLD Practice Guideline Hepatol 2007;46: 92238.
2. Siddiqui SA, Ahmed M, Ghani MH, Memon MA, Mustafa G, Ghori MA. Coagulation abnormalities in patients with chronic liver disease in Pakistan J Pak Med Assoc 2011;61(4):363-67
3. Peck R M. Review article: coagulation disorders in chronic liver disease. Aliment Pharmacol Ther 2007;26(1):21-8.
4. Rye K, Scott R, Mortimore G, Lawson A, Austin A, Freeman J. Towards Noninvasive Detection of Oesophageal Varices Int J Hepatol 2012;9:1155.
5. Qamar AA, Grace ND, Groszmann RJ, Garcia-Tsao G, Bosch J, Burroughs AK. Platelet count is not a predictor of the presence or development of gastroesophageal varices in cirrhosis. Hepatology 2008;47:153-9.
6. Thomopoulos KC. Non-invasive Prediction of Esophageal Varices: Is It Possible? Saudi J Gastroenterol 2011; 17(1): 13.
7. Hong WD, Dong LM, Jiang ZC, Zhu QH, Jin SQ. Prediction of large esophageal varices in cirrhotic patients using classification and regression tree analysis. Clinics 2011;66(1):119-124.
8. Muhammad SK, Shaikh MA, Shaikh BA. Sensitivity, specificity and predictive values of noninvasive markers of esophageal varices in cirrhosis of liver. Asian J Med Res. 2012; 1(3): 98-102.
9. Kim H, Choi D, Gwak GY, Lee JH, Park MK, Lee H I et al. Evaluation of esophageal varices on liver computed tomography: Receiver operating characteristic analyses of the performance of radiologists and endoscopists. J Gastro Hepatol 2009;24(9):153440.
10. Javed Iqbal Farooqi, Hameed Ahmed, Qazi Ikramullah, Farooq Ahmed, Masood-ur-Rehman. Predictors of esophageal varices in patients of liver cirrhosis. JPMI 2007; 21(01): 60-64.
11. Deficiency of thrombin activatable fibrinolysis inhibitor in cirrhosis is associated with increased plasma fibrinolysis. Hepatology 2003;38:2307.
12. Qamar AA, Grace ND, Groszmann RJ, GarciaTsao G, Bosch J, Burroughs AK, et al. Platelet count is not a predictor of the presence or development of gastroesophageal varices in cirrhosis. Hepatology. 2008;47:1539.
13. Gluud LL, Klingenberg S, Nikolova D, Gluud C. Banding ligation versus betablockers as primary prophylaxis in esophageal varices: systematic review of randomized trials. Am J Gastroenterol. 2007;102:2842-8.
14. GarciaTsao G, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. Hepatology. 2007;46:922-38.

Answer Picture Quiz

"butterfly glioma"
Typical appearance of a butterfly glioma.

Original Article

VACUUM ASSISTED CLOSURE VERSUS CONVENTIONAL DRESSINGS IN TREATMENT OF OPEN UNTIDY WOUNDS

Mansoor Ali Jamali, Sohail Ahmed Memon, Sameena Naz and Tahmida Almani

Objective: To compare the vacuum assisted closure versus conventional dressings of wounds in terms of mean duration of wound healing and change in wound surface area.

Methods: Duration of study was 6 months. It was carried out between April 7, 2012 and October 7, 2012. A total 100 adult patients of either gender with open wounds were admitted and managed as indoor patient. The wounds were initially excised surgically. Patients were divided into two groups. Group 1 includes the patients whose wounds were managed with modified vacuum assisted closure (MVAC) therapy. Group 2 or Gauze group included patients whose wounds were managed with conventional gauze dressings. The variables of study were mean duration of spontaneous healing/ becoming graftable and change in wound surface area in cm^2.

Results: Out of 100 patients, 73 (73%) were males while 27 (27%) were females. Mean duration of wound healing in VAC group was 14.04 ±1.41 whereas mean duration of wound healing in Gauze group was 9.12 ± 2.43. Independent samples t-test was used to compare duration of wound healing in both the groups which was statistically significant (p-value 0.001). Mean change in wound surface in VAC group was 25.66 ± 66.0 whereas mean change in wound surface in Gauze group was 7.2 ± 6.97. Independent samples t-test was used to compare mean change in wound surface which was statistically significant (p-value 0.001) in both the groups.

Conclusion: The study concludes that the modified vacuum assisted closure was more safe and efficacious than moist wound therapy for the treatment of open untidy wounds. Modified Vacuum Assisted Closure therapy of wounds promotes early healing resulting in significant decrease in wound surface area in lesser time period.

Keywords: Wound, Vacuum Assisted Closure, Conventional Dressings.

Introduction

Wounds contribute a major percentage of the patient managed at the Department of plastic surgery. They are often associated with significant morbidity. The management of wounds poses complex and difficult challenge for the plastic surgeon. The patients with problematic wounds constitute a significant workload for health care organizations. Successful management of these wounds require adequate knowledge of the wound etiology, wound bed preparation and the definitive surgical procedures such as grafts and flaps which are employed to resurface the wound.[1,2,3] Wounds are caused by variety of causes like trauma, malignancy, osteomyelitis , burns, diabetes, and vascular diseases. Wounds could be classified into acute and chronic based on duration. But the most practical classification is given by Rank and Wakefield which divide wound into tidy and untidy. Tidy wounds are inflicted by sharp instruments and contain no devitalized tissue. Untidy wound results from crushing, tearing, avulsion, vascular injury, or burns and contain devitalized tissue. The surgeon's main objective is to transform untidy to tidy by removing all infected and necrotic tissue. [4,6] The ideal intrinsic wound healing environment as proposed by winter is moist, uninfected with a good blood supply containing the correct balance of inflammatory mediators. Recently wound treatment is oriented towards creating a wound environment that will enhance blood flow in the wound bed to promote healing and allow surgical intervention to cover the wound.[2,3] The choice of one over another is best made by considering wound characteristics and treatment goals. The goal is clean wounds that are to be closed primarily or are granulating well. In general, hydrogels, films, and composite dressings are best for wounds with light amounts of exudates; hydrocolloids are used for wounds with moderate quantities; and alginates, foams, and NPWT (Negative Pressure Wound Therapy) are best used for wounds with heavier volumes of exudate. [7,9] NPWT is a type of vacuum dressing to promote healing in acute or chronic wounds and it also promotes healing of

second and third degree burns. It was first used by Fleischmann et al in 1993, following successful use of this technique in 15 patients with open fractures. It is also beneficent for diabetic foot ulcers and management of the wound dehiscence after laparotomy.[28,29] Vacuum Assisted Closure (VAC) is a technique in which controlled negative pressure of a vacuum is used so that infectious material and other fluids are sucked out of the wound. A key component to the initiation of healing process is thorough debridement. The use of Vacuum assisted closure therapy in concurrence with debridement of the affected area increases the frequency of healing.[12] But VAC is costly and requires expert personnel.[13,16] Therefore Modified Vacuum Assisted Closure (MVAC) therapy was invented as it is a simple, cheap, having marked clinical benefits and material used in this technique is easily available material in local market .[17,18] There are few studies on comparison of negative pressure wound therapy(NPWT) and moist wound dressing in treatment of open untidy wounds.[19] The present study was designed to compare the efficacy of Modified vacuum assisted closure with the conventional moist wound dressing in treatment of open untidy wounds in terms of duration of wound healing and change in wound surface area and hence evolve actionable evidence base that could guide our wound management strategies in future patients.

Methods

This randomized control trial performed in department of plastic and reconstructive surgery, Pakistan Institute of Medical Sciences (PIMS) Islamabad. Study was conducted between April 7, 2012 and October 7, 2012. Patients with 13-60 years of age having open untidy wound were included in this study. All the patients having evidence of malignancy, osteomyelitis, or presented with exposed bone, tendons, nerve or vessel were excluded from study.

Data Collection Procedure:

All patients were admitted in Plastic and Reconstructive surgery ward from outpatient department (OPD) and emergency department. The study protocol was approved by the hospital ethics committee. Informed consent was taken from each patient. The dressings were applied by a team comprising of the trainee researcher and 4th year resident of same department supervised by consultant. The patients were divided randomly in two groups by lottery method. In Group 1 patients Modified vacuum assisted closure (MVAC) therapy was used for wound dressing while Conventional gauze dressing was used in 2nd or Gauze group. Necessary wound debridement and toilet was done was done for slough or necrotic tissue before the application of dressings. Wound irrigated with normal saline. A swab for culture was taken before wound irrigation with normal saline and surgical debridement. Prior to application of the drape, the peri-wound skin was prepared and mopped dry. Intravenous antibiotics were given empirically and then according to culture and sensitivity report. Wounds were monitored closely during the hospital stay. In both groups the treatment with vacuum assisted closure therapy or Conventional dressings was continued for 03 weeks and wound size reduction and healing documented by gross examination of the wound. Duration of healing was taken in days while wound surface area was measured in cm2. The wounds were subsequently managed with skin grafts.

Modified Vacuum assisted closure (MVAC):

This type of dressing was used in group 1 patients as shown in figure 1. Wound was prepared by irrigating with normal saline and if necessary surgically toilet was done for slough or necrotic tissue. Sterile, open cell-foam dressing which was gently placed into wound cavity. Open-pore, reticulated 5 mm thick foams were used as they are the most effective at transmitting mechanical forces across the wound and provide an even distribution of negative pressure over the entire wound bed to aid in wound healing. A drainage tube was placed in the wound followed by dressing with sterile gauze pieces and application of occlusive transparent film over the whole assembly. The drainage tube was connected to a suction machine. Intermittent negative pressure ranging from 50 to 125 mmHg was applied so that every 15 minutes, the suction was stopped for five minutes. The dressing (foam plus drapes) was changed every 48 hours.

Conventional Dressings:

These type dressings were used in group 2 patients. Wound was washed with Pyodine soaked gauze pieces in initial 48 hours then twice daily dressings of normal saline soaked gauze were applied.

Results

Total 100 patients were included in this study. Gender and age distribution are shown in **Fig 1** and **Table 1**. Sixty two percent patients were in the 3rd and 4th decades of life. Majority of patients were from

Overall the size of wounds reduced in both groups. Baseline mean wound surface area in MVAC group was 56.04±90.10cm^2 and it reduced to 30.38±54.02cm^2 after 3 weeks. Paired samples t-test was used to compare the size difference and it was statistically significant (p-value 0.024). In gauze group initial size was 55.26±90.07 cm^2 and after 3 weeks it becomes 48.06±83.10 cm^2. Similarly paired samples t-test is also applied in this group but it was not significant statistically (p-value 0.454) . Mean change in wound surface in MVAC group was 25.66±66 cm^2 whereas mean change in wound surface in Gauze group was 7.2±6.97 cm^2. Independent samples t-test was used to compare mean change in wound surface area in both groups which was statistically significant (p-value 0.001). Co- amoxiclav was the most frequently instituted antibiotic

In MVAC group, 40% (n=20) patients had graftable/ healed wounds on completion of two weeks treatment while the remainder 60% (n=30) of the patients had graftable wounds at the end of 03 weeks treatment. In contrast to this, in the Gauze group, only 8 %(n=4) patients had graftable/ healed wounds on completion of two weeks treatment while in the remainder 92%(n=46) of the patients the wounds were graftable at the end of 03 weeks treatment. Chi-square test was used to compare time of spontaneous healing at 2nd week and 3rd week in both the groups which was statistically significant (p-value 0.001) in both the groups.

Mean duration of wound healing in MVAC group was 14.04±1.41 whereas mean duration of wound healing in Gauze group was 9.12±2.43. Independent samples t-test was used to compare duration of wound healing in both the groups which was statistically significant (p-value 0.001).

Discussion

Vacuum assisted closure therapy is a novel method of wound healing. It has several advantageous features over conventional treatment.

In our study, we included a spectrum of patients with open untidy wounds including both acute and chronic. Majority of our patients were relatively young males. Males are more frequently involved in outdoor activities and hence more prone to sustain different wounds secondary to road traffic accidents, falls, firearm injuries and blasts etc. Predominant involvement of young males further amplifies the grave implications of such disabling injuries. Male predominance and more frequent involvement of younger population is well documented in the context of trauma in general. With increasing civil violence, we are receiving increasing number of patients with blast injuries as well.[20]

In our study duration of the wound healing in terms of spontaneous closure or becoming graftable was one of our outcome measures. In this context we found that the wound treated with VAC had faster healing as compared to the gauze treated wounds. Others published studies have also shown faster healing with VAC therapy.[21-23] A variety of factors have been described to account for this accelerated wound healing. VAC therapy continually decontaminates the wound and drains the wound surface of exudates, which contain large amounts of proteases. These would normally inhibit fibroblastic division, collagen production, and cell growth. Fluid removal helps with localized edema that otherwise cause an increase in interstitial pressure with consequent occlusion of microvasculature and lymphatics, decreased nutrient, and oxygen delivery. Protein degradation enzyme is released with metabolic waste accumulation and increased bacterial colonization, which causes capillary damage and hypoxia. VAC therapy also provides a moist environment to promote formation of granulation tissue, which allows for a smoother pathway to re-epithelialize the wound surface. Angiogenesis is also stimulated, which improves tissue oxygenation and tissue reconstruction.[24,25] Mechanical forces exerted on wound surface by low pressure suction are also important. This mechanism mimics the stretch-induced cell proliferation typically found in tissue expansion phenomenon observed elsewhere in the body.[26-27]

Change in the wound surface area with the treatment modality employed, was our other main outcome measure. In this regard we found that the wound treated with VAC had significantly greater reduction in wound size than those wounds treated with gauze dressings. Our observation conforms to several published studies.[1,22,23,28-30]

In our study we found VAC therapy to be convenient for both the patients as well as surgical staff. One major advantage of vacuum therapy is the reduction of the number of dressing changes to once every 48 hours instead of twice or more every 24 hours as in conventional therapy. The reduction of dressing changes leads to an improved patient compliance as the patient suffers less often pain and inconvenience. Besides this, less frequent dressing changes, result in reduced nursing time and thus reduced staff costs for vacuum therapy as compared to conventional therapy: also

Results

Total 100 patients were included in this study. Gender and age distribution are shown in **Fig 1** and **Table 1**. Sixty two percent patients were in the 3rd and 4th decades of life. Majority of patients were from the twin cities of Islamabad and Rawalpindi while remaining were from upper Punjab, Khyber Pukhtoonkhwa (KPK) and Gilgit Baltistan. The causes of wounds were road traffic accidents (RTAs) in 68 %, firearm injuries (FAI) in 24 % and fall from height in 8 %. The wounds were observed on different body sites and included feet, thighs, upper limbs, chest and abdomen / back. **Table 2**

The wound surface area ranged from 9 cm^2 to 500 cm^2. Reduction in the size of wounds was statistically significant in the MVAC group patients as determined on gross inspection of the wounds on weekly basis. At the start of the study the mean wound sizes or surface area in the MVAC group and Gauze group were 56.04±90.10 cm^2 and 55.26±90.07cm^2 respectively. At completion of one week treatment the mean sizes in the two groups were 46.66±78.50 cm^2 and 52.70±87.00 cm^2 respectively. At completion of two weeks treatment the mean sizes in the two groups were 38.94±70.43 cm^2 and 50.82±85.11 cm^2 respectively. At completion of three weeks of treatment the mean sizes in the two groups were 30.38±54.02 cm^2 and 48.06±83.10 cm^2 respectively. Independent samples t-test was used to compare size of wounds at baseline and 1st week in both the groups which was statistically not significant (p-value 0.917 and p-value 0.720) respectively as shown in **table III.** Similarly Independent samples t-test was used to compare size of wounds at 2nd week and 3rd week in both the groups which was statistically significant (p-value 0.029 and p-value 0.005) respectively as shown in **Table 3.**

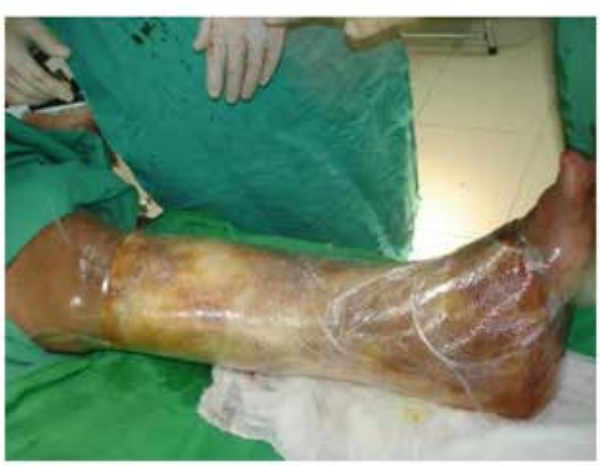

Fig-I: Method of application of MVAC therapy.

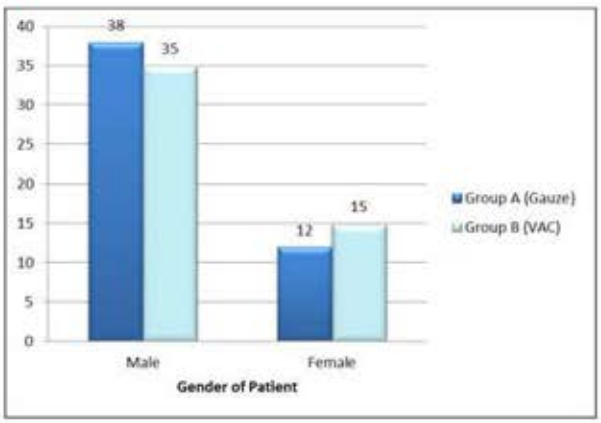

Fig-I: Gender distribution of the patients (n=50 each group)

Table-1: Age distribution among the patients (n=50 each group).

	Age	VAC Group n(%)	Gauze Group n(%)
	13-20 Years	6 (12%)	7 (14%)
	21-30 Years	13 (26%)	13 (26%)
Age Groups	31-40 Years	17 (34%)	18 (36%)
	41-50 Years	13 (26%)	7 (14%)
Total	51-60 Years	1 (2%)	5 (10%)

Table-2: Location wise distribution of the wounds (n=50 each).

	Age	VAC Group n(%)	Gauze Group n(%)
	Feet (left/right)	21 (42%)	22 (44%)
	Thights	6 (12%)	5 (10%)
Site of wounds	Upper limps	11 (22%)	15 (30%)
	Chest	15 (10%)	3 (6%)
	Abdomen/ back	7 (14%)	5 (10%)

Table-3: Reduction in the size of wounds in the two groups (50 patients in each group)

Size of wounds (cm^2)	VAC Group Mean±SD	Gauze Group Mean±SD	P-value
Baseline measures	56.04±90.10	55.26±90.07	0.917
1st week	46.66±78.50	52.70±87.00	0.720
2nd week	38.94±70.43	50.82±85.11	0.029
3 week	30.38±54.02	48.04±83.10	0.005

Hospitalization costs are reduced, due to on average shorter duration of therapy needed for vacuum therapy as compared to conventional therapy.

In our study we found VAC therapy to be more economical. Owing to its low cost, VAC therapy can provide an economical alternative to the other available costly local wound management measures. Such economic implications of wound management are particularly important in the context of our poor patients. Cost effectiveness has also been reported in terms of shortened hospital stays, and decreased overall medical cost in the published literature.[30,32]

In our study we additionally found VAC therapy to be more comfortable for patients as well as the surgical staff. It obviated the need for daily dressing changes. Similar findings have been reported by other studies as well.[32, 33] In our study we observed shorter hospital stay among patients treatment with VAC therapy. A study done by Saziye et al found a particular decrease in the length of hospital stay when compared with the conventional treatment method.[34] However Ko et al[35] did not found similar results with any significant difference in length of stay and treatment duration. Some complications like erosion, eczema and increased body temperature were encountered during vacuum therapy but these are reversible. Erosion of adjacent tissue can be prevented by application of pressure relieving material underneath the tubes. The reaction of the peri-wound area (i.e. maceration and eczema), solved by placement of alginates underneath the adhesive dressing. increased body temperature due to clogging of the system solved by changing the foam dressings.

Pain at wound site during application and removal of foam/gauze occurred with both therapies. It was overcome by analgesics, injection lidocaine underneath the sponge and nonadherent dressing placement at wound base.[36]

Conclusion

Our study concludes that the vacuum assisted closure with the conventional moist wound dressing in treatment of open dirty wounds was more efficacious in terms of duration of wound healing and change in wound surface area. Vacuum Assisted Closure therapy of wounds promotes early healing resulting in significant decrease in wound surface area in lesser time period so that wound is healed or graft may be applied.

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References

1. Vikatmaa P, Juutilainen V, Kuukasjarvi P, Malmivaara A. Negative pressure wound therapy: a systemic Review on effectiveness and safety. Eur J vas Endovasc surg 2008; 36:438-48.
2. Andrabi SI, Ahmed J, Rathore MA, Yousaf M. SI. Vacuum assisted closure of laparostomy wounds "a novel technique". J Ayub Med Coll Abbottabad 2007; 19:89-92.
3. Ichioka S, Watanabe H, Sekiya N, Shibata M, Nakatsuka T. A technique to visualize wound bed microcirculation and the acute effect of negative pressure. Wound Repair Regen 2008; 16: 460-5.
4. Van Bekkum DW. Phylogenetic aspects of tissue regeneration: role of stem cells: a concise overview. Blood Cells Mol Dis. 2004; 32: 1116.
5. Galiano RD, Tepper OM, Pelo CR,. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. Am J Pathol. 2004; 164 (6) : 193547.
6. Earley MJ. Wounds, tissue repair and scars. In: Williams NS,Bulstrode CJK,O' Connell PR eds. Bailey and love's Short practice of surgery. 25th ed. London: Edward Arnold Ltd; 2008: 24-31.
7. Mustoe T. Understanding chronic wounds: a unifying hypothesis on their pathogenesis and implications for therapy. Am J Surg. 2004; 187 : 65.
8. Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg. 2001; 38: 72.
9. Al Fadhli A, Alexander G, Kanjoor JR. Versatile use of vacuum-assisted healing in fifty patients. Indian J Plast Surg 2009; 42: 161-8.
10. Fitzgerald JE, Gupta S, Masterson S, Sigurdsson HH. Laparostomy management using the ABThera™ open abdomen negative pressure therapy system in a grade IV open abdomen secondary to acute pancreatitis. International wound journal. 2013 Apr 1;10(2):138-44.
11. Ubbink DT, Westerbos SJ, Evans D, Land L, Vermeulen H. Topical negative pressure for treating chronic wounds. The Cochrane Library. 2008 Jul.
12. Blume PA, Walters J, Payne W, Ayala J, Lantis J. Comparison of negative pressure wound therapy using vacuum-assisted closure

with advanced moist wound therapy in the treatment of diabetic foot ulcers: A multicenter randomized controlled trial. Diabetes Care 2008; 31:631-6.
13. Fleischmann W, Strecker W, Bombelli M, Kinzl L. [Vacuum sealing as treatment of soft tissue damage in open fractures]. Der Unfallchirurg. 1993 Sep;96(9):488-92.
14. Moues CM, Van Den Bemd GJ, Heule F, Hovius SE. Comparing conventional gauze therapy to vacuum-assisted closure wound therapy: a prospective randomised trial. Journal of plastic, reconstructive & aesthetic surgery. 2007 Jun 30;60(6):672-81.
15. Kaufman MW, Pahl DW. Vacuum-assisted closure therapy: wound care and nursing implications. Dermatology Nursing. 2003 Aug 1;15(4):317.
16. Tauro LF, Ravikrishnan J, Rao BS, Shenoy HD, Shetty SR, Menezes LT. A comparative study of the efficacy of topical negative pressure moist dressings and conventional moist dressings in chronic wounds. Indian Journal of Plastic Surgery. 2007 Jul 1;40(2):133.
17. Flack S, Apelqvist J, Keith M, Trueman P, Williams D. An economic evaluation of VAC therapy compared with wound dressings in the treatment of diabetic foot ulcers. J Wound Care 2008; 17:71-8.
18. Augustin M, Herberger K. Benefits and limitations of vacuum therapy in wounds. Hautarzt 2007; 58:945-51.
19. Etoz A, Kahveci R. Negative pressure wound therapy on diabetic foot ulcers. Wounds 2007; 19 : 250-4. 1.
20. Saaiq M, Shah SA. Thoracic trauma: Presentation and management outcome. J Coll Physicians Surg Pak 2008; 18: 230-3.
21. Braakenburg A, Obdeijin MC, eitz R, van Rooij IA, van Griethuysen AJ, Klinkenbijl JH. The clinical efficacy and cost effectiveness of the vacuum-assisted closure technique in the management of acute and chronic wounds: a randomized controlled trial. Plast Reconstr Surg 2006; 118 : 390-7.
22. Vuerstaek JD, Vainas T, Wuite J, Nelemans P, Neumann MH, Veraart JC. State-of-the-art treatment of chronic leg ulcers: a randomized controlled trial comparing vacuum-assisted closure (V.A.C.) with modern wound dressings. J Vasc Surg. 2006; 44: 102937.
23. Timmers MS, Le Cessie S, Banwell P, Jukema GN. The effects of varying degrees of pressure delivered by negative-pressure wound therapy on skin perfusion. Ann Plast Surg. 2005 ; 55: 665-9
24. Demaria RG, Giovannini UM, Téot L, Frapier JM, Albat B. Topical negative pressure therapy. A very useful new method to treat severe infected vascular approaches in the groin. J Cardiovasc Surg (Torino). 2003; 44 (6):757761.
25. Morris GS, Brueilly KE, Hanzelka H. Negative pressure wound therapy achieved by vacuum-assisted closure: evaluating the assumptions. Ostomy Wound Manage. 2007; 53: 527.y
26 .Saxena V, Hwang CW, Huang S, Eichbaum Q, Ingber D, Orgill DP. Vacuum-assisted closure: microdeformations of wounds and cell proliferation. Plast Reconstr Surg 2004; 114:1086-96.
27. De Filippo RE, Atala A. Stretch and growth: the molecular and physiologic influences of tissue expansion. Plast Reconstr Surg 2002; 109: 2450-62.
28. Gurtner GC. Wound healing: normal and abnormal. In: Thorne CH, Beasley RW, Aston SJ, Bartlett SP, Gurtner GC, Spear SL, eds. Grabb and Smith's Plastic surgery. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2007: 15 - 22
29. Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. Ann Plast Surg 1997; 38:553-62.
30. Wong L K, Nesbit R D, Turner L A, Sargent L A. Management of a Circumferential Lower Extremity Degloving Injury with the Use of Vacuum-assisted Closure. South Med J. 2006 ; 99 : 628 30.
31. Trueman P. Health economics and topical negative pressure therapy. In: Calne S, ed. Position Document. European Wound Management Association. 2007:59.
32. Moues CM, van den Bemd GJ, Meerding WJ, Hovius SE. An economic evaluation of the use of TNP on full-thickness wounds. J Wound Care. 2005; 14(5): 224-7.
33. Jones SM, Banwell PE, Shakespeare PG. Advances in wound healing: topical negative pressure therapy. Postgrad Med J. 2005; 81: 3537.
34. Saziye K, Mustafa C, Ilker U, Afksendyios K. Comparison of vacuum assisted closure device and conservative treatment for fasci- otomy wound healing in ischemia-reperfusion syndrome: pri. Int Wound J. 2011; 8(3): 229-36.
35. Ko YS, Jung SW. Vacuum-assisted close versus conventional treatment for postlarotomy wound dehiscence. Ann Surg Treat. Res. 2014 Nov;87(5):260-4
36. Vuerstaek JD, Vains, Wuite J, Nelemans P, Neumann MH, Veraart JC. State-of-the-art treatment of chronic leg ulcer: A randomized controlled trial comparing vacuumassisted closure (V.A.C) with modern wound dressings. J Vasc Surg. 2006 Nov; 44 (5): 1029-37

Original Article

Frequency Of Complications In meconium Aspiration Syndrome In Hospitalized Babies

Mateen Ishfaq, Naila Jameel and Mansoor Ahmad Mazari

Objective: To assess the frequency of complications in neonates with the diagnosis of meconium aspiration syndrome (MAS).

Methods: A Neonates presented with respiratory distress along with meconium staining of vocal cords and body on 1st day of life was admitted in Neonatology Ward of Services Hospital. Neonates with dysmorphic features, congenital abnormalities of heart & lungs and those having risk factors for sepsis were excluded from the study. A predesigned proforma was used to record clinical data on presentation. This included necessary information like gestational age, weight, gender, signs of post term baby and mode of delivery. The proforma was updated on daily basis to make note of any complications arising during the course of hospital stay till outcome (discharge, leave against medical advice or death).Arterial blood gases (ABGs) were done daily to look for persistent hypoxia and Echocardiography was performed when ABGs and clinical examination suggested development of persistent pulmonary hypertension of newborn (PPHN). Chest x-ray was done at the time of admission and repeated if clinical findings were suggestive of pneumothorax. Other investigations like blood culture were sent when there was clinical suspicion of sepsis. The data was subsequently computed and analyzed using SPSS (Statistical Package for the Social Sciences) version 10 by the authors.

Results: 175 babies with meconium aspiration were included in the study. Complications like pneumothorax was observed in 28babies (16%),persistent pulmonary hypertension of newborn(PPHN) in 35(20%), respiratory failure in 21 (12%) and sepsis in 27 patients(15.4%). 45 patients died (25.7%), 90 were discharged home(51.4%) and 40 were referred to other hospitals (22.8%).

It was observed that commonest risk factorfor MAS was post-maturity, found in 40% with poor APGAR score in 35.4%.There was no significant difference in morbidity between males and females.

Conclusion: It was observed that meconium aspiration syndrome (MAS) is a leading cause of neonatal morbidity and it can be prevented by giving appropriate peri-natal care to high risk pregnancies, vigilance and timely intervention in delivery room.

Keywords: Meconium aspiration, Syndrome, Morbidity, Risk factors, Pneumothorax, Pulmonary Hypertension/persistant, Newborn

Introduction

Meconium aspiration syndrome (MAS) is a medical condition affecting term and post term (>42 weeks of gestation) babies. It occurs when meconium (the first intestinal discharge of new born) is inhaled in lungs before, during or immediately after delivery. The incidence of MAS is 1.7-35% of neonates born through meconium stained amniotic fluid[1](MSAF). Meconium staining of the amniotic fluid occurs in approximately 13% of live births; this percentage increases with increasing gestational age at delivery.[2] Meconium is normally stored in the neonate's intestine until after birth but sometimes during prolonged and difficult deliveries, infant often expels meconium into amniotic fluid. This also causes an interference with the supply of oxygen through placenta; as a result, neonate often initiates vigorous respiratory movements' in-utero. Under these circumstances, the baby may aspirate amniotic fluid/ meconium which is drawn into respiratory tree with considerable respiratory morbidity.

Many perinatal risk factors have been associated with meconium aspiration, including placental insufficiency, maternal hypertension, maternal tobacco use and mode of delivery[2](cesarean section). But, perhaps, the most significant factor is post term delivery. At least one third of infants with MAS require intubations and mechanical ventilation.[3]

MAS is one of the important causes of neonatal respiratory problems eventually leading to increased neonatal morbidity and mortality thus adding to the burden, physical, financial & psychological for doctors, hospitals and parents respectively. Important complications include air leaks (pneumothorax, pneumomediastinum), persistent pulmonary hypertension (PPHN), sepsis and respiratory failure. In this study, the aim is to assess the frequency of complications in early days of life of neonate with MAS and to know the magnitude of this problem, In turn, forming a liaison with Obstetric department to develop better antenatal care for pregnant women with risk factors and proper resuscitation to babies born through MSAF, thus helping to decrease the morbidity.

Methods

This observational (descriptive case series) study was carried out in the Neonatal Unit of Services Hospital, Lahore over a six months period, from December 2009 to May 2010. 175 neonates,based on non-probability purposive sampling, presenting with respiratory distress along with meconium staining of vocal cords and body on 1st day of life were included in the study. All deliveries were attended by specialist pediatrician and babies were resuscitated according to standardized protocols used for these neonates. Neonates who were admitted were shifted to Nursery within 30 minutes of delivery. Neonates presented with respiratory distress who had dysmorphic features on clinical examination, congenital heart diseases (VSD, PDA, TOF) diagnosed clinically and on Echocardiography, congenital lung diseases (congenital pneumonias, diaphragmatic hernias) diagnosed clinically and on chest x-ray and risk factors for sepsis e.g., PV leaking >18 hours, chorioamnionitis, maternal fever >38.8°F assessed on history were excluded from the study. Informed consent from parents was taken and risks and benefits of study were explained. Apart from presenting complaints, necessary information like age & weight of neonate at presentation, gestational age along with signs of post maturity (dry wrinkled skin, overgrown nails, prominent creases in palm and soles) and mode of delivery was noted. On admission clinical status of neonate was assessed and after this they were examined daily and investigated (ABG's, Echocardiography, Blood culture & chest x-rays) for assessment of complications (pneumothorax, PPHN, sepsis) based on clinical suspicion for seven days

Investigations like ABG's and blood culture were taken by standardized sampling techniques and checked on sophisticated equipment in same laboratory to minimize alteration in results. Likewise chest x-ray and echocardiography were done and reported by same radiologist specialized in pediatric imaging and pediatric cardiologist respectively. These investigations were done free of cost in the hospital. Information regarding data of patient and development of complications were entered in a preformed proforma that was updated on daily basis until outcome (discharge, death of left against medical advice, referral). Statistical analysis was carried out using the SPSS 10 programme. Descriptive statistics were applied in the form of frequencies and percentages for qualitative variables andmean &standard deviation for quantitative variables. As study was descriptive case series so no test of significance was applied.

Results

In this study, 175 patients with meconium aspiration syndrome were observed for first 7 days of their life. As far as gender was concerned, 118 babies were male (67.5%) and 57 were female (32.5%). Out of 175 patients, 90 babies survived and discharged home (51.4%), 45 (25.7%) were expired, while 40 were referred to other hospitals (22.8%) **Fig-I**. About 60% of patients were born at term while 40% were post mature. Most of the patients were delivered by cesarean section 91 (52%), 79 babies were delivered by spontaneous vaginal delivery (45%) and 5 by forceps delivery (3%). Commonest risk factor for meconium stained amniotic fluid and meconium aspiration was post maturity (40%) with other risk factors including poor Apgar score (35.4%), maternal hypertension (19%), maternal diabetes mellitus (4%) and in about 0.6% no obvious risk factor was found.

Complications **Table-I** due to MAS were pneumothorax in 28 babies (16%), PPHN in 35 babies (20%). 27 babies developed sepsis proved on blood culture (15.4%). Another complication observed was respiratory failure, in 21 babies (12%). 64 babies developed no complication (36.6%). Major Cause of death was pneumothorax followed by PPHN and sepsis as shown in the **Table-II**. Sepsis was associated with different microorganisms which were isolated on blood culture in these babies are shown in **Fig-II.**

Apart from pneumothorax, serial chest x-rays also showed air trapping and hyper expansion in 52 babies (29.7%), diffuse infiltration in 17 (9.7%) with atelectasis in 8%.

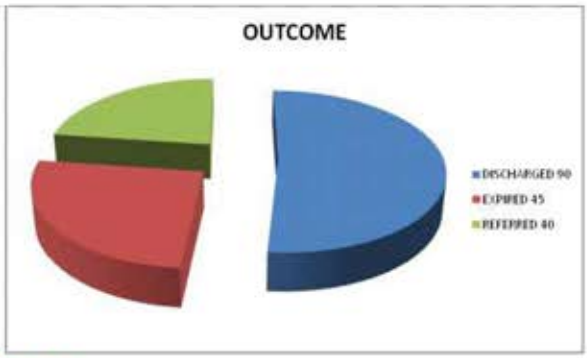

Fig-1: Outcome of MAS.

Table-1: Frequency of complications in MAS.

Complications	No. of Patients (Frequency n=175)	Percentage
PPHN	35	20%
Pneumothorax	28	16%
Sepsis	27	15.4%
Respiratory Failure	21	12%
No complications	64	36.6%

Table-2: Causes of death in newborns with MAS.

Causes of Death	No. of Patients n=45)	Percentage
Pneumothorax	20	11.4%
PPHN	15	8.6%
Sepsis	10	5.7%

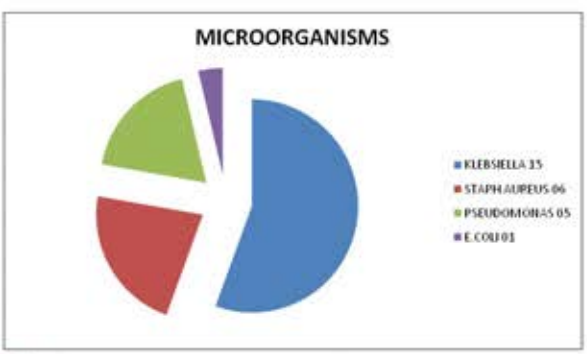

Fig-2: Blood culture pattern.

Discussion

Meconium aspiration syndrome (MAS), as we know it, is a problem found all over the world, irrespective of race and ethnicity. MAS is a major issue regarding respiratory morbidity in neonatal intensive care units (NICU) everywhere in the world but has been efficiently dealt, with proper antenatal obstetrical care and better facilities available for post-natal management of newborns having this condition like extracorporeal membrane oxygenation (ECMO). In Pakistan, it has been a leading cause of admissions in NICU, found to be the 5th in list in a study done by Prakash et al[4] in Karachi after infections, asphyxia, jaundice and prematurity. The disease is characterized by small airway obstruction which produces early onset of respiratory distress in a meconium stained infant with tachypnea, chest retraction, grunting, cyanosis,[5] poor lung compliance andhypoxemia clinically and patchy opacification and hyperinflation radiologically.[6] However, in developing countries like Pakistan, where health facilities are still not enough and limited only to developed cities, there is substantial morbidity and mortality caused by this condition.

In this study, the objective was to assess the pattern of complications, which arise in first 7 days of life in newborns admitted with MAS. Most common complication observed was persistent pulmonary hypertension of newborn (PPHN), in 20% of babies with MAS. Many babies who developed PPHN also had poor APGAR scores at delivery. This showed that hypoxia is an important contributor in the development of PPHN. It was also shown by Bhatt RY et al [7] in India. They observed that 15.5% of babies with MAS developed PPHN.[7]

Pneumothorax was found to be the 2nd most arisen complication, seen in 16% of babies, which is close to another study done by Razzaq[8] in Multan, where it was 13.3%. However in a study done by Green bough et al [9], it was shown to be 15-33% in infants with MAS. Our study differs from this international study, perhaps due to less use of mechanical ventilation, as this is an important risk factor for development of pneumothorax, and our unit does not have enough ventilatory support for these babies. The third major complication was sepsis (secondary bacterial infection), in 15.4% of babies. These babies had no ante-natal risk factors for sepsis like PV leaking, chorioamnionitis etc. Although meconium aspiration is also associated with secondary bacterial pneumonias due to microorganisms like Listeria monocytogenes and Escherichia Coli, these babies developed infection due to other factors. A probable reason for development of bacterial infection was improper handling of these babies in nursery. These babies received multiple pricks for blood sampling and also regular examination of these babies by doctors and staff nurses. Another important reason may be their close proximity to other babies in nursery who actually had sepsis. This was also shown by the blood culture results of these babies which showed growth of microorganisms like Klebsiella and Staphylococcus aureus. This was contrary to the usual pathogens associated with MAS. Only one baby

had growth of Escherichia Coli.

Another complication which was observed was respiratory failure in 12% of babies. Mortality was found to be 25.7% in this study; it is very high as compared to 5% mortality shown by Velaphi et al [10] in their study, and 20% by Razzaq[8] in Multan.In one study, it was found that 9.7% of babies with MAS develop respiratory failure and required assisted ventilation[11].Again it might be due to insufficient facilities for managing these babies, and also due to a large burden of neonates which our nursery is receiving apart from MAS in a very small set up. A large number of patients were referred to other hospitals (22.8%) because proper NICU care was not available. 51.4% babies survived and discharged home. Among these, 36.6% babies developed no complications. Major cause of death was pneumothorax (11.4%) followed by PPHN (8.6%). Pneumothorax is an acute fetal condition, if untreated, and it requires urgent management by needle and chest tube insertion. Increase mortality was due to unavailability of specialist care from pediatric surgery.

This study also highlighted some other important variables like risk factors for MAS. The most common was post maturity, in 40% as in other studies which show an increase incidence of MAS after 40 weeks of gestation [10].It is important to note that avoidance of post mature pregnancy is a preventable factor in MAS. In one prospective study, a decrease in incidence of MAS from 5.8% to 1.5% over an 8 year period was attributed to a reduction in births at more than 41 weeks of gestation.[12] Another factor was poor Apgar score, in 35.4% patients. It has been recognized that this reduction in APGAR might be due to intrapartum suctioning of baby, when head is delivered, by obstetrician who suppresses spontaneous breathing. This intrapartum suctioning is no longer recommended now. This poor APGAR score is also related to complications like PPHN and pneumothorax as discussed above.

In this study we have not assessed the co-relation and association of various obstetrical aspects (duration of labor, type of delivery, indications of cesarean section, and intra partum causes of poor Apgar score) with the development of complications in the neonates with MAS because these important associations should be studied in a large multicentric research to specify obstetrical risk factors related to development of complications.

As far as good outcome is concerned, combined obstetric and pediatric care can lead to prevention and reduced severity of meconium aspiration syndrome with low complication rate and decrease mortality.

Conclusion

It is unlikely that the incidence of meconium passage will decrease substantially. It is important that all health care professionals who attend deliveries should have an understanding of the controversies surrounding the management of meconium-stained amniotic fluid and be well versed in the proper obstetric and neonatal interventions.On the basis of above observations, following recommendations and conclusions can be drawn:

- Proper obstetrical care in the form of identification and monitoring of high risk pregnancies with MSAF, management of pregnancy at 41 weeks' gestation to avoid post-term delivery, decreases the risk of MAS.
- Good postnatal care starting from delivery room to neonatal unit can control much of the morbidity as well as co-morbid conditions like birth asphyxia associated with MAS. The complication should be kept in mind while managing these newborns.
- Newborns with MAS require supportive therapy for the cardiopulmonary system including oxygen supplementation and possibly mechanical ventilation. In this case, staff of NICU, especially doctors, should be well trained in handling ventilators.
- Intensive efforts should be taken for those who develop pneumothorax, alongwith liaison with pediatric surgeons in managing these babies.

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References

1. Gupta V, Bhatia BD, Mishra OP. Meconium stained amniotic fluid: Antenatal, intra-partum and neonatal. Indian Pediatr1996; 33:293.
2. Walsh MC, Faranoff JM. Meconium stained fluid:approach to the mother and baby. Clin Perinatol2007; 34(4):653-65.
3. Bhutani VK, Chima R, Sivieri EM. Innovative neonatal ventilation and meconium aspiration syndrome. Indian J Pediatr2003; 70:421-27.
4. Parkash J, Das N. Pattern of admissions to neonatal unit. Journal of the College of Physicians and Surgeons Pakistan2005;15:341344.
5. Dudell GG Stoll BJ. Respiratory tract disorders. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editots, Nelson Textbook of Pediatrics.18th ed. Philadelphia: Saunders 2007.p.742-43.
6. Cleary GM, Wiswell TE. Meconium staind amniotic fluid and the meconium aspiration syndrome: an update. Pediatr Clin North Am 1998; 45:511-529.
7. Bhat RY, Rao A. Meconium stained amniotic fluid and meconium aspiration syndrome: a prospective study. Ann Trop Pediatr2008; 28(3): 199-203.
8. Razzaq A. Early neonatal morbidity and mortality in meconium aspiration syndrome [dissertation].Multan: Nishtar Medical College; 2007.
9. Greenough A, Pulikot A, Dimitriov G. Prevention and management of meconium aspiration syndrome-assessment of evidence based practice. Eur J Pediatr2005; 164(5): 329-30.
10. Velaphi S, Vidyasagar D. Intra-partum and post delivery management of infants born to mothers with meconium-stained amniotic fluid: evidence-based recommendations. Clin Perinatol2006; 33(1):29-42.
11. Clark RH. The epidemiology of respiratory failure in neonates born at an estimated gestational age of 34 weeks or more. J Perinatol2005; 25: 251-257.
12. Yoder BA, Kirsch EA, Barth WH, Gordon MC. Changing obstetric practices associated with decreasing incidence of meconium aspiration syndrome. ObstetGynecols2002; 99(5pt1):731-9.

Medical News

ANEMIA MAY RAISE RISK OF DEATH FOR STROKE PATIENTS

Older adults who have had a stroke may be at greater risk of death if they have anemia. This is the conclusion of a new study published in the Journal of the American Heart Association.

Stroke affects more than 795,000 Americans each year, and it is a leading cause of long-term disability in the United States. Around 87 percent of all strokes are ischemic, whereby the artery that supplies oxygen-rich blood to the brain becomes blocked. When an artery in the brain leaks or ruptures, this is known as hemorrhagic stroke. According to the research team - including senior author Dr. Phyo Myint of the University of Aberdeen in the United Kingdom - many patients who have experienced stroke have anemia. Anemia is a condition characterized by low levels of circulating red blood cells or hemoglobin, which causes a reduction in the amount of oxygen that is transported to the body's organs and tissues. Signs and symptoms of anemia include fatigue, headache, pale skin, dizziness, shortness of breath, coldness in the hands and feet, and chest pain.

While anemia can affect any age group, pregnant women and older adults are at increased risk for the condition.

For their study, Dr. Myint and colleagues set out to investigate how anemia may impact risk of death following stroke. Twofold risk of death for stroke patients with anemia. The researchers analyzed the data of 8,013 adults of an average age of 77, all of whom had been admitted to the hospital with acute stroke between 2003-2015. The team looked at how participants' levels of hemoglobin - a protein in red blood cells that carries oxygen - and incidence of anemia influenced their risk of death in the year after their stroke. On hospital admission for stroke, the researchers found that around 25 percent of patients had anemia, and this increased their risk of death over the following year. Among patients who had ischemic stroke, the risk of death was increased twofold for those with anemia, compared with ischemic stroke patients who did not have anemia. Hemorrhagic stroke patients who had anemia were at 1.5 times greater risk of death. Furthermore, the researchers found that higher hemoglobin levels among patients were associated with poorer stroke outcomes and increased risk of death, particularly in the first month after stroke. This finding, the team says, suggests that both low and high hemoglobin levels may raise stroke patients' risk of death. The researchers' findings were further confirmed with a systematic review of 20 studies - involving almost 30,000 patients - that looked at the link between anemia and stroke outcomes. Overall, the authors say their research highlights the importance of anemia prevention, diagnosis, and treatment for stroke patients. "One example of an intervention might be treating the underlying causes of anemia, such as iron deficiency, which is common in this age group," says co-author Raphae Barlas, also of the University of Aberdeen.

"As the study has convincingly demonstrated, anemia does worsen the outcome of stroke, so it is very important that we identify at-risk patients and optimize the management."

Courtesy: Medicalnewstoday.com

Original Article

PERINATAL HISTOLOGY OF ENDOCRINE PANCREAS IN ALBINO RAT AN EXPERIMENTAL STUDY

Shaista Arshad Jarral and Muhammad Tahir

Objective: To analyze the process of growth, differentiation and development of pancreatic islet alpha and beta cells during late fetal and early post-natal period.

Methods: An observational experimental study was conducted in the animal research laboratories of University of Health Sciences, Lahore. Adult non-diabetic male and female albino rats were procured from National Institute of Health, Islamabad and kept under standard conditions in animal house. Mating was allowed by keeping female and male rats in the same cage with ratio of 3:1. Pregnancy was confirmed by the observation of vaginal plug. Pregnant rats were divided into 3 groups. First group rats were sacrificed on day 20 of gestation and their foetuses were dissected to procure the pancreatica of Study group A. Pups were born after 22-23 days postcoitum for rest of the pregnant rats. They were divided into two groups B &C, having ten pups each. Group B pups were sacrificed on day 2 postnatal and group C on day 7 postnatal to obtain pancreatic tissue. The pancreatica, so obtained were fixed, processed and sectioned at 4µm thickness. Sections were stained with H&E for light microscopy and with Chrome Alum Hematoxylin-Phloxin stain for differential counts. Observations were made regarding number of islets/section, mean diameter of islets, mean number of total cells /islet, mean number of α cell and β cell/ islet and the mean ratio of β: α cell.

Results: The pancreatic tissue on light microscopy showed both exocrine and endocrine elements; the former predominated later. Islets of Langerhans were observed as clumps of light staining cells in well-developed acinar parenchyma in both fetal and postnatal groups; Both postnatal groups showed strong association of pancreatic tissue with ducts whereas, group A showed mesenchymal tissue in close vicinity of developing islets. Quantitative variables were compared using one way ANOVA. Mean islets per section for group A was 6.3±1, for group B 7.8±1 and for group C 3.1 with significant difference among the groups ($p<0.05$). Mean diameter of an islet was 112±1 for group A, 136±2 for group B, and 171±5 for group C with statistically significant difference among the groups. Total number of cells per islet did not show statistically significant difference ($p>0.05$). Number of β cells per islet was 95±2 for group A, 76±4 for group B and 102±3 for group C, which was statistically significant ($p<0.05$). Number of α cell per islet and the ratio of β and α cell was not statistically significant among the groups.

Conclusion: All the parameters studied showed gradual increase postnatally. Number of islets though decreased but the diameter of islets increased gradually. Differential cell counts showed gradual increase in number with their relative proportion comparable to adult ratios in late postnatal group.

Keywords: Perinatal, Histology, Endocrine, pancreas.

Introduction

The pancreas is a mixed gland, comprising of two different cell populations, exocrine and endocrine. The exocrine component dominates the parenchyma of pancreas and consists of acinar and ductal cells that secrete and transport digestive enzymes respectively into small intestine. Its endocrine cells are segregated in discrete groups; islets of Langerhans, first described by Paul Langerhans. The Islet tissue comprises 1-2% mass of healthy adult human pancreas; there are about one million islets collectively weighing 1-2 grams.[1] Although the adult pancreas is a single organ, it is derived from two growth buds; dorsal and ventral buds. During embryogenesis, the dorsal pancreas arises from foregut tube just ventral to the notochord and caudal to the region of stomach, while the ventral pancreatic bud develops from the endodermal hepatic diverticulumSignals released from both the notochord and endothelial cells are shown to be mandatory for proper regulation of the dorsal pancreas,while signals from the cardiac mesoderm

are found to influence the development of ventral pancreas.[2] Endocrine pancreas comprises of ; alpha (α) cells producing glucagon (15-20%), beta (β) cells producing insulin & amylin (65-80%), delta (δ) cells producing somatostatin (3-10%), PP cells producing pancreatic polypeptide (3-5%), and epsilon cells producing ghrelin, an antisatiety hormone (<1%)[3,4].In adult human pancreas, Islets have a central mass of insulin-secreting β cells and a surrounding mantle of α, δ and PP cells. This relative pattern of cell arrangement may be different amongst species but the beta cells are always clumped together with an adjacent mix of non-beta cells. In experimental studies, rat islets dispersed into single cells, when allowed to re aggregate in tissue culture, the beta cells formed a central core with non-beta cells around it, thus re-establishing the natural pattern which may have a role in normal functioning of the islets.[5] Developmentally, beta cells are believed to be generated from a population of pancreatic progenitor cells. These progenitor cells divide and differentiate into beta cells. Once formed these beta cell are post mitotic. However, replication of differentiated beta cells can lead to addition of new beta cells. High rates of beta cell replication during the early post-natal period results in tremendous increase in the beta cell mass.[6] Both insulin and glucagon are detectable by 8th week of development in humans with insulin positive cells being more prevalent type in early fetal life. The relative delay in hormone biosynthesis and earlier expression of insulin rather than glucagon in human is suggestive of the relative difference of endocrine differentiation programme of human from that of rodents.[7] Perinatal malnutrition impairs neogenesis and beta cell regeneration with preserved beta cell proliferation, thereby, implying an impaired capacity of malnourished animals to neogenesis of beta cell.[8] Postnatal expansion of the pancreatic beta-cell mass is dependent on survivin, a gene critical for cell division and cell survival in cancer cells; it was also implied that survivin had a role in the maintenance of beta cell mass through both replication and anti-apoptotic mechanisms.[9] Perinatal is term used to describe the time around birth originating from two words "peri" means about or around, and "natus" means birth. It is different for different species depending on the length of gestation; in humans, it is considered to range between 28weeks of gestation to 4weeks after birth. In rat, however, it spans between seventeenth day of pregnancy to eleventh day postnatally.[10]

The current study was undertaken to evaluate the process of growth and development of islets of Langerhans during late fetal and early postnatal period using rat as an experimental model.

Methods

20Adult non-diabetic albino rats (15 female, 5 male) were obtained from National Institute of Health, Islamabad and kept under standardized conditions in animal laboratory of University of health sciences. After acclimatization animals were facilitated to mate by keeping female and male adult rats in the same cage in the ratio of 3:1. Pregnancy was confirmed by observation of vaginal plug. Pregnant rats were randomly divided into three groups, A, B and C, each containing five animals. Progression of Pregnancy was monitored by weighing the pregnant animals on alternate days. Group A pregnant rats were sacrificed on 20th day of gestation to extract the fetuses which were dissected to procure fetal pancreatic tissue, while groups B and C females were allowed to continue pregnancy. After 22-23 days of gestation pregnant rats delivered pups which were divided into two groups B and C. Group B pups were sacrificed on day 2 postnatal and group C pups were sacrificed on day 7 postnatal to procure the pancreatic tissue. The pancreatic tissue was dissected out and fixed in 10% formalin for 72 hours. Then it was cut into small pieces and treated in automatic processor, blocks so obtained were cut into 4 μm sections and stained with H&E for light microscopy and Chrome Alum Hematoxylin-Phloxin stain for differential counts of alpha and beta cells of pancreatic islets.

Micrometry

Micrometry was done for diameter of the islets by taking two diameters for each islet at right angle to each other and then a profile diameter was calculated by averaging the two[11]. At least four islets selected at random were so measured on each section and then their average was taken as diameter of islet of that particular section. Four sections of each block were so observed.

Micrometry for cell counts

Cell counting was done at 40X by using the grid on which the islet section was superimposed, cells were counted in all squares of grid superimposing the islet; leaving lower and left lines to avoid double counting..

The total number of cells per islet section and the beta and alpha cells were counted as they differentially stained with chrome alum Hematoxylin-phloxin. Four sections were observed for each tissue and four islets were counted on each section randomly and the average taken. Later on, ratio of beta and alpha cells

One way ANOVA was used for statistical analysis. Multiple comparisons were made using Post Hoc Tuckey test. A p-value of < 0.05 was considered as statistically significant.

Results

The pancreas was identified on dissection as pinkish lobulated mass clamped between duodenum and spleen. Light microscopy was done to observe exocrine and endocrine portions of the pancreas in all three groups on H&E staining. Both exocrine and endocrine tissue were observed in group A pancreatic tissue. Exocrine acini were well-formed but lobulation was less marked **Fig 1**. Most of pancreatic tissue comprised of exocrine parenchyma with rather scanty endocrine tissue; developing islet tissue looked like a bunch of pale staining cells.

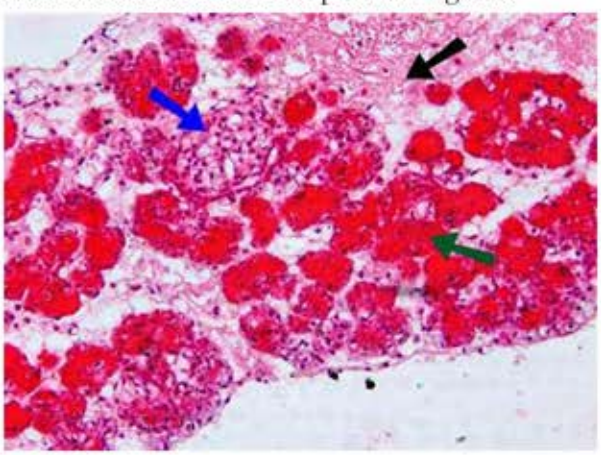

Fig-1: Photomicrograph from group A pancreatic tissue, showing poorly lobulated exocrine acini and islet tissue in scarce amount, with ill-defined capsule around it. Blue arrow showing islets, green arrow (acini) and black arrow (mesenchymal tissue). H&E 200X.

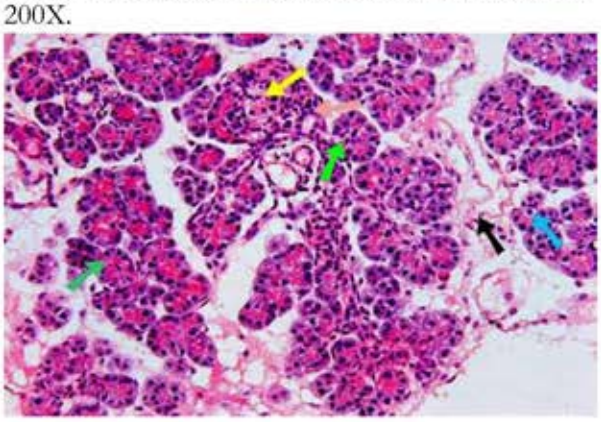

Fig-2: Photomicrograph from pancreatic tissue of group B, showing developing islets (yellow arrow), intercalated ducts (pink arrow), mesenchymal tissue (black arrow), apoptotic body (blue arrow) and exocrine tissue (green arrow). H&E 200X.

Light microscopy of group C tissue showed well differentiated lobes and lobules; islets were well circumscribed with clear demarcation from the surrounding acinar tissue **Fig3.**Islets were independent from ducts and mesenchymal tissue was absent

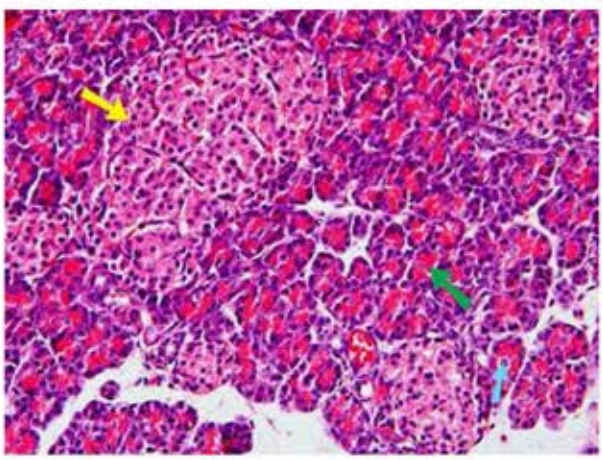

Fig-3: Photomicrograph of a pancreatic tissue preparation from group C, showing pancreatic tissue with well-defined acinar lobules (green arrow) and well-demarcated islets (yellow arrow) in abundance. H&E 200 X.

In group B on light microscopy, islets were in abundance, scattered among exocrine acini, differentiating and branching to form lobes and lobules **Fig -2.** Number of islets per unit field area was counted on random fields, four fields were taken randomly for each slide and then the average was taken. Four slides were studied for each tissue specimen. Mean number of islets per unit field was taken for all groups and then compared **Table1 and Fig 4**.

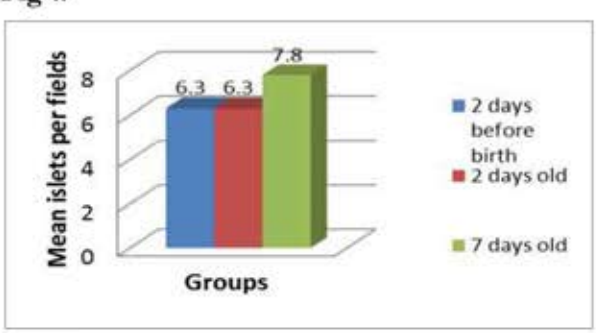

Fig-4: Bar chart showing the mean number of islets per unit field in various groups.

Significant difference was observed in the mean number of islets per field between groups B and C (P=0.05), and C and A (p=0.05) but no significant difference was observed in the mean number of islets per unit field between groups B and A (P>0.05). The mean diameter of islets was taken by measuring maximum and minimum profile dimensions of an islet and then taking average diameter as the islets were of different shapes like oval, round and elliptical. Diameters of four random islets were taken for each slide and four sections were randomly observed for each tissue of each group. Mean diameters were taken and then compared among the groups **Table1 and Fig 5.**

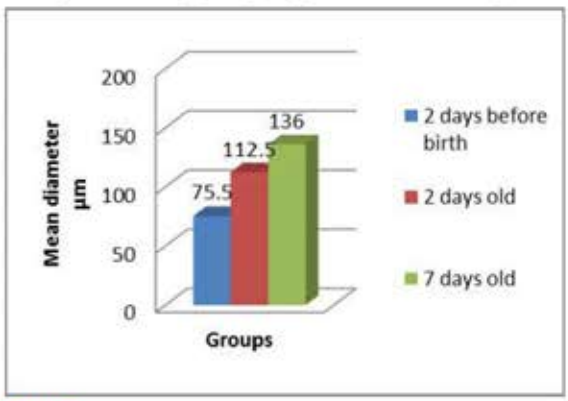

Fig-5: Bar Chart showing the mean diameter of islets in different groups.

Significant difference was observed in the mean diameter of islet between groups A and C (P=0.001). No significant difference was observed in the mean diameter of islet between groups B and A (p-value = 0.07) and groups B and C (P=0.401).

On light microscopy with H & E staining, group A specimens were observed to have two prominent type of cells; the more centrally placed cells were very pale, large and ovoid with basic staining, round nuclei containing prominent nucleoli. Cells were arranged like groups or clump not a particular pattern. In group B specimens the islet cells towards central portion were larger with big, round; prominent nuclei and granular cytoplasm. Cells towards periphery were rather flattened and smaller with small oval or irregular nuclei and finely granular cytoplasm. Islets were observed to have numerous capillaries interspersed among the cells. In group C, the islets were well circumscribed from the surrounding acinar tissue. Cells in the islets were more organized in arrangement than in the previous groups, being arranged in single or double cell cords around well-developed capillaries.Total numbers of cells per islet section were counted for each group; four random islets per observed per section for four of the sections in each tissue of each group. Islets were randomly selected in each section. Mean number of cells per islet were calculated and compared among the groups **Table 1 and Fig 6.**

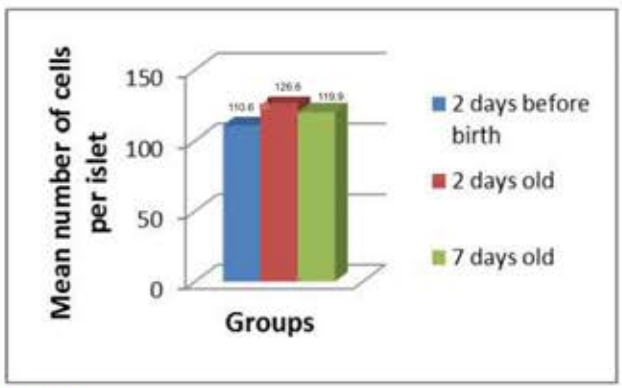

Fig-6: Bar chart showing the mean number of cells per islet in various groups.

No significant difference was observed in the mean number of total cells per islet section on multiple comparisons among the various groups (P > 0.05).

Chrome alum haematoxylin was used to differentiate beta and alpha cells. Beta cell cytoplasm stained bluish while alpha cell cytoplasm stained pinkish while the nuclei stained violet. All the groups showed central concentration of beta cells with an occasional presence of alpha cells in between beta cells. Group A showed at places only few beta cells clumped together **Fig 7.**

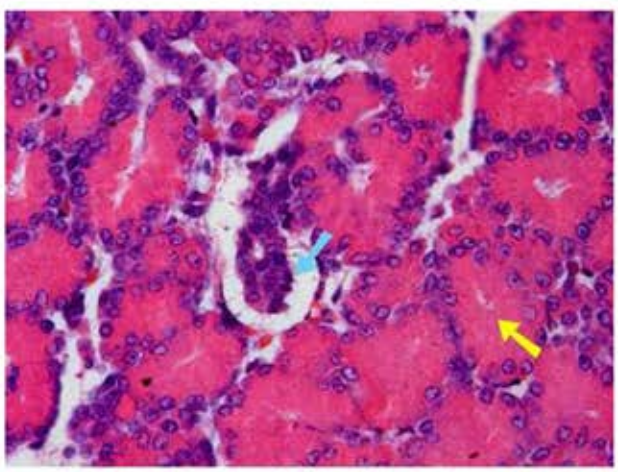

Fig-7: Photomicrograph from pancreatic tissue preparation of group A, showing beta cells stained blue (light blue arrow), these appear like a column of irregularly arranged cells budding out of a duct. Acinar formation of exocrine part of the organ is also being arranged (Yellow arrow). CAH 200 X.

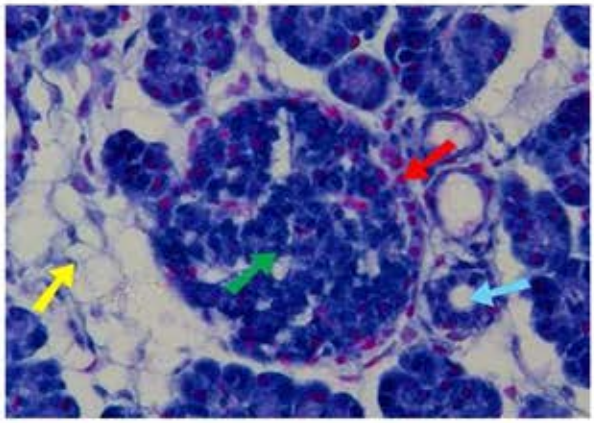

Fig-8: Photomicrograph of group B preparation showing an islet with blue cytoplasmic stain for beta cells (green arrow) , pink staining cytoplasmic granules of alpha cells (red arrow), an intercalated duct bordering islet (light blue arrow) with plenty of mesenchyme (yellow arrow) CAH.400 X.

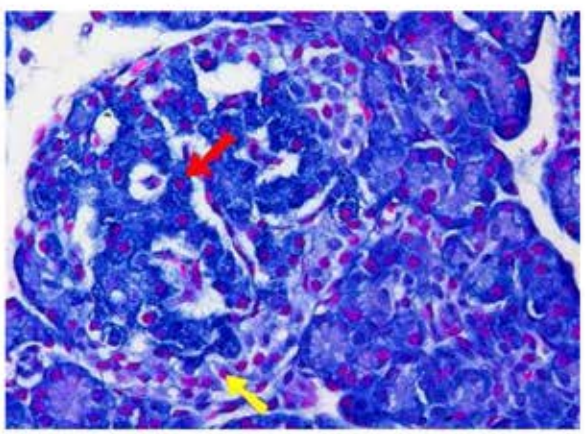

Fig-9: Photomicrograph of group C preparation, showing beta cells in the center staining bluish cytoplasmic granules (red arrow), and pink staining cytoplasmic granules of peripherally arranged alpha cells (Yellow arrow). CAH 400 X.

Group B showed few islets, consisting of only alpha cells, rest of them showed the same pattern (Fig 8). Groups C showed almost similar arrangement of centrally placed beta cells in cordlike arrangement around capillaries and peripherally placed alpha cells (Fig. 9). Mean number of beta cells were calculated for all four groups and compared among the groups **Table and Fig 10.** By multiple comparisons made amongst the different groups, Significant difference was observed in the mean number of beta cells per islet section between groups A and B (p=0.001), groups A and C (p=0.03). No significant difference was observed in the mean number of beta cells per islet section between groups B and C (p=o.497).

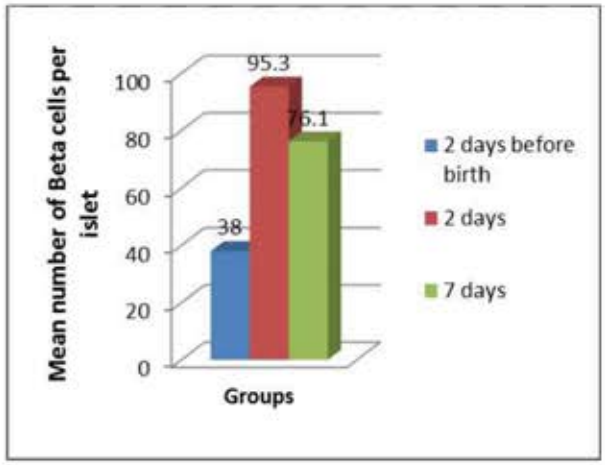

Fig-10: Bar chart showing the mean number of beta cells per islet.

Total numbers of alpha cells per islet section were counted for each experimental group on chrome alum hematoxilin staining. Four random islets were used from each slide and for each tissue of each group four random sections were taken. Mean value was calculated from the total values and then comparison was made between the groups **Table 10 and Fig 11.**

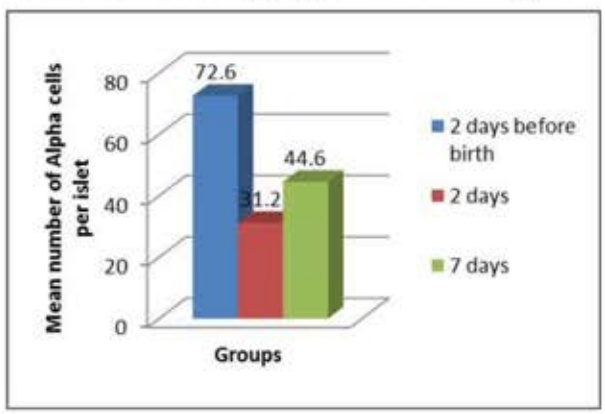

Fig-11: Bar charts showing the mean number of alpha cells per islet section.

ignificant difference was observed in number of alpha cells per islet field in groups with a p value of 0.007. On multiple comparisons significant difference was observed in the mean number of islets per unit section between groups A and B (p=0.009), while no significant difference was observed between groups A and C (p=0.12) and between groups B and C (p=0.69). From the mean number of beta cells per islet field and mean number of alpha cells per islet field the ratio between beta and alpha cells per islet field was calculated for each group and then compared among the groups. **Table and Fig- 12.**

.For group A the ratio ranged from 0.37 to 1.30 with a mean value of 0.58 ± 0.26.The same values were observed to range from 1.30 to 6.50 for group B with a mean of 3.38 ± 1.82 and for group C they were observed to vary in beta alpha ratio from 0.48 to 5.70 with a mean value of 2.81 ± 1.81.

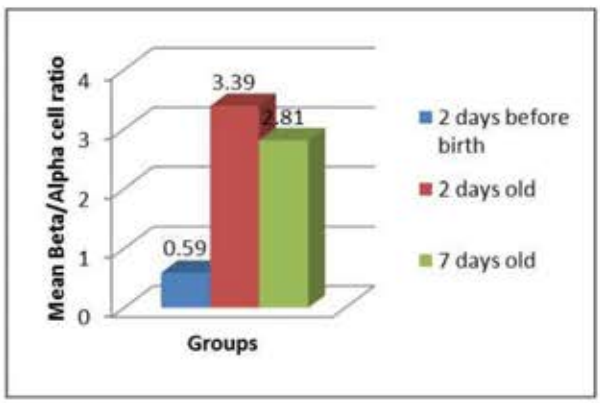

Fig-12: Bar chart showing the mean beta cell /alpha cell ratio in different groups.

Multiple comparisons were made in mean ratio between beta and alpha cells per islet field which showed significant difference in the mean beta cell/alpha cell ratio between groups B and A (p=0.002), between groups A and C (p=0.018).There was no statistically significant difference observed in the mean ratio of beta and alpha cells between groups B and C (p=0.85).

Discussion

Our study observed one fetal and two early post-natal groups and compared the histological structure and quantitative parameters of the endocrine portion of the pancreas among the groups.

Morphometric parameters measured in our study were a total number of islets per section, the mean diameter of islets, the total cell populating islets, differential beta and alpha cells and their relative ratio. The total number of islets was highest in the group C. The mean diameter of islets showed gradual increase towards one-week old pups. The mean number of cells per islet were slightly higher on the 2nd postnatal day with slight fall in next few days, suggesting remodelling by apoptosis or attrition of ill formed cells as observed in literature earlier.[12] During postnatal period beta cells were the main cells populating islet on 2nd postnatal day, followed by some fall in number in one-week old pups. Alpha cells, on the other hand, showed relatively low counts in early postnatal period and slight gain in one-week old age group. However, previous studies showed alpha cells to outnumber beta cells in the late fetal period; implying thereby, alpha cells are the first endocrine cells to differentiate followed by the beta cells or, the alpha cells represent an early stage in the differentiation of beta cells.[13] The relative ratio of beta and alpha cells was seen to be more close to adult ratio in early postnatal day.

Immunohistochemical and morphometric study of the development of new-born rat pancreatic islets found that all the morphometric parameters for the beta cells showed gradual increase during the first four days after birth. The beta cells were well stained and present in the central part of the new-born islets, while the other islet cells were present in the periphery of the islets as seen earlier.[14] We made similar observations regarding the location of different islet cell types, as the beta cells formed the central core with the alpha and other cell types forming the outer mantle around beta cells. This particular organization of the islets may reflect the functional relationships between different cell types. In the rat, the intra-islet microcirculation has been observed to run from the arteriolar entry point outwards, i.e. from the beta cell core to the peripheral endocrine cell mantle[15, 16]. The direction of this microcirculation permits the beta cell core to act on mantle cells.[17] However, the relative distribution of islet cells in the pancreatic may differ

Table-1: The comparative values of various study parameters amongst the three groups.

Parameters	Group A Mean ± SD n=10	Group B Mean ± SD n=10	Group C Mean ± SD n=10	P-value
Islets Per Field	6.3±1.61	6.3±1.41	7.8±1.03	<0.001*
Dia meter of Islet	75.5±22.7	112.5±18.59	136±21.44	<0.001*
Cells per islet	110.60±40.63	126.50±29.93	119.90±56.24	0.588
Number of alpha cells/islet field	72.60±30.45	31.20±16.61	44.60±39.50	0.007*
Beta cells per islet per unit field	38±11.87	95.3±21.99	76.10±42.91	<0.001*
Beta/Alpha ratio	0.58±0.26	3.38±1.82	2.81±1.81	<0.001*

Among various mammalian species. In the buffalo pancreas, the small islets showed alpha cell cords crossing the centre of the islets.[18] Similar findings also described for primates[19]. In the horse and Japanese serow, alpha cells were found located in the centre of the islet[20]. This variation in the organization of islet cells may reflect differences in the interactions between the cells of the islets and their metabolic role.The accelerated rate of growth and development of islet cells in the early postnatal period was also supported by a study in which perinatal development of islet vasculature in rat was investigated; it showed a
pronounced proliferation of the vascular endothelium during the first week after birth which raised the possibility of functional interaction between endothelial and islet cells, contributing to their postnatal maturity.[21]

Conclusion

The endocrine pancreas comprised a large percentage of the pancreatic mass in early rat fetuses. This percentage increased gradually during late embryonic and early postnatal development. All study parameters showed rise in late fetal and early postnatal stage. The proportion of alpha to beta cells was high in late fetal period but later it showed a reversal i.e. the beta cells outnumbered alpha cells in islet which got oriented at the peripheral region of the islet tissue. It is suggested that the whole process of organogenesis of pancreas be studied along with the genetic and epigenetic factors affecting growth and development of pancreas

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References

1. Langerhans, P1869 Contributions to the Microscopic Anatomy of the Pancreas. M.D. Thesis, University of Berlin.
2. Jarikji, Z.H., Horb, L.D., Shariff. Mandato, C.A., Cho, K.W.Y., and Horb, M.E. 2009. The tetraspanin tm4sf3 is localized to the ventral pancreas and regulate fusion of the dorsal and ventral pancreatic buds. Development,136:1791-1800.
3. Elayat, A.A., Al Naggar, M.M., Tahir, M. 1995. An Immunocytochemical and morphometric study of the rat pancreatic islets. J Anat, 186: 629-37.
4. Andraloic, K.M., Mercalli, A., Nowak, K.W., Albarello, L., Calcagno, R., Luzi, L. 2009. Ghrelin-producing epsilon cells in the developing and adult human pancreas. Diabetologica, 52(3): 486-93.
5. Halban, P.A., SI., Powers, K.L., George, K.L., and Bonner-Weir, S. 1987. Spontaneous reasso- ciation of dispersed adult rat pancreatic islet cells into aggregates with 3-dimentional architecture typical of native islets. Diabetes, 36: 783-791.
6. Georgia, S., Bhushan, A. 2004. B-cell replication is the primary mechanism for maintaining postnatal β-cell mass. J Clini Inves, 114: 963-968..
7. Piper K, Brickwood S, Turnpenny L W, Cameron I T, Ball S G, Wilson D I, Hanley Na. 2004. Beta-cell differentiation during early human pancreas development.J of Endocrinol. 181:11-23.available online at http://www.endocrinology .org.
8. Garofano, A., Czernichow, P., Breant, B. 2000. Impaired β-cell regeneration in perinatally malnourished rats: a study with STZ. FASEB J, 14: 2611-2617.
9. Jiang, Y., Nishimura, W., Devor-Henneman, D., Kuewitt, D., Wang, H., Holloway, M.P. 2008. Postnatal expansion of the pancreatic beta-cell mass is dependent on survivin. Diabetes, 57(10): 2718-2727.
10. Sam Kacew. 2010. Drug toxicity and metabolism in pediatrics: chapter 2. Fetal and neonatal drug biotrans- formation; page: 17 by Mont. R Juchau. ISBN-0-8493-4564-2.
11. Morini, S., Braun, M., Onori, P. 2006. Morphological changes of isolated rat pancreatic islets: a structural, ultra structural and morphometric study. J Anat, 209: 381-392.
12. Scalgia, L., Cahill, C.J., Finegood, D.T., Bonner weir, S. 2008. Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. Endocrinology, 138: 1736-1741.
13. Mcevoy, R.C. 1981. Changes in the volumes of the A-, B-, and D-cell populations in the pancreatic islets during the postnatal development of rat. Diabetes, 30(10): 813-7.
14. Badawoud, M.H. 2003. Immunohistochemical and morphometric study of the development of fetal and newborn rat pancreatic islets. Saudi Med J, 24(2):142-6.
15. Bonner-Weir, S., Orci, L. 1982. New perspectives on the microvasculature of the islets of Langerhans in the rat. Diabetes, 31:833-839.
16. Samols, E., Stagner, J.I. 1988. Intraislet regulation. American Journal of Medicine, 85(5A): 31-35.
17. Samols, E., Stagner, J.I. 1991. Intra-islet and islet-acinar portal system and their significance. The Endocrine Pancreas, 93-124. New York: Raven Press.
18. Lucini, C., Castaldo, L., Lai, O., and De Vico, G. 1998. Ontogeny, postnatal development and ageing of endocrine pancreas in Bubalus bubalis. J Anat, 192: 417-424.
19. Bonner-Weir, S. 1991. Anatomy of the islet of Langerhans. The Endocrine Pancreas (ed. Samols E) 15-27. New York: Raven Press.
20. Atoji, Y., Takada, Y., Suzuki, Y., Sujimura, M. 1990. Immunocytochemical identification of four cell types in the pancreatic islets of the Japanese serow capricornis crisp- us. Zoological Science,7: 779-782.
21. Johansson, M., Anderson, A., Carlson, P.O., Jansen, L. 2006. Perinatal development of pancreatic islet microvasculature in rat. J Anat, 208: 191-196.

Case Report

Crimean Congo Haemorrhagic Fever, Lack of Basic Epidemiologic Information and Risks to Public Health

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Abstract: We report a case of Crimean Congo haemorrhagic fever in a health care worker who presented with a history of fever and mild mucosal bleeding. Although Crimean Congo hemorrhagic fever was first reported from Pakistan many decades ago, there is a lack of basic epidemiologic information in non endemic areas such as Lahore. This often precludes the diagnosis, which may prove detrimental for both the patient and health care workers.

Key words: Crimean Congo haemorrhagic fever, Epidemiology, Infection Control

Introduction

Crimean Congo Haemorrhagic Fever (CCHF) is a Viral Haemorragic Fever caused by Nairovirus of the Buyaviridae family. It is transmitted by the Hyalomma tick which feeds on various animals including livestock. The disease can by contracted by a direct tick bite, contact with infected meat or an infected person. The average case fatality rate is 40%.[1] Highly infectious blood from patients has caused several alarming nosocomial outbreaks, particularly when the index case was not suspected.[1-5] Despite the first case report from Pakistan in 1976, resulting in the death of a surgeon,[3] there is a lack of basic epidemiologic data. This results in significant danger to public health in public hospitals. We present a case report which highlights the various impediments faced by healthcare workers in the public sector to diagnose and manage such patients.

Case Report

The patient, a 30 year old female nurse presented to the Services Hospital emergency with presenting complaints of fever for 5 days, blood in the urine for 4 days, bruising all over the body for 4 days and loose stools for 48 hours prior to admission. Her history of present illness revealed that she developed sudden onset high grade fever recorded at 104 F by a thermometer. This was associated with rigors and chills. Some relief was obtained by self prescribed Ceftriaxone and Sulfadoxizine/ pyrimethamine. The fever was also associated with profound weakness and body aches. There was no history of drenching night sweats or any diurnal variation of fever. There was a history of non specific abdominal pain which was mild and generalized, intermittent with no aggravating or relieving factors. There was a history of occasional dry cough. 48 hours later she developed bruises all over her body along with bleeding from her gums, haematuria and menorrhagia. She passed five watery stools in 24 hours. The pertinent review of systems revealed no hemoptysis, upper or lower gastrointestinal bleeding, headaches, joint aches or swelling. She was a resident of Tehsil Samundri, however, she lived in the city of Loralai, Baluchistan, for the past 4 years where she was posted as a nurse in the Obstetrics and Gynaecology department at the district head-quarter hospital. She denied herbal and homeopathy use and there was no history of pets or exposure to any other animals. The patient was married and her last child was born 6 years prior to admission. She was referred from DHQ Loralai to Nishtar Hospital, Multan where she remained admitted in Nishtar Hospital, Multan for 48 hours. Apparently no tests were done and she was discharged. She remained unwell at home. As she had relatives in Lahore, she was brought to the Services Hospital emergency. Her physical examination revealed the patient lying anxiously in bed, Pulse: 74bpm, B.P. 110/65mm of Hg, temp: 98F, R/R 20/min. Scleral Icterus was positive. Petechiae were present on the soft and hard palate. There was mild epigastric tenderness without any visceromegaly. The rest of her examination was within normal limits.

Based on the clinical history and laboratory findings, the following differential diagnosis of Dengue, Malaria, Enteric fever or a Myeloproliferative disorder was generated. She was initially managed on the lines of DHF, Ceftriaxone 2 g i/v once a day was started for diarrhea. Her Dengue NS1 Antigen, IgM and IgG tests were negative. Smear for Malaria was negative. Over the next 36 hours the patient underwent marked clinical improvement along with an increase in platelet counts.

A sudden turn of events was marked by a phone call received by the Services Hospital administration from the Loralai DHQ Hospital Administration. The

patient had an exposure with a confirmed case of Crimean Congo Hemorrhagic Fever in Loralai who had died recently. As no public sector laboratory in Punjab for Crimean Congo Hemorrhagic Fever diagnosis was available, therefore the WHO representative was immediately called for specimen collection. The patient was isolated however; it was a struggle for the staff to obtain Personal Protection Equipment.

Table-1: Lab investigations.

CBC	29/07/2014	01/08/2014	02/08/2014
Hb	13.0	13.6	11.0
TLC	3.1	2.93	3.3
Plt	15	18	31
Retic	1.2%		
SGOT		4389	388
SGPT		819	
Alk.p		851	936
Urea/Creat	18/0.7	12/0.4	
Na/K	140/3.7	141	16/0.6
		3.5	
INR		1.1	

As the patient had rapid and marked clinical improvement she was discharged. After discharge the CCHF PCR report from the National Institutes of Health, Islamabad was positive. The patient was seen in follow up, she and all her close contacts were healthy.

Discussion

Crimean Congo Haemorrhagic Fever (CCHF) has been reported in Pakistan for many decades now. Even now, there is a lack of disease awareness and many health professionals are unaware of the local disease epidemiology. The disease is not endemic in Lahore but trainees in our unit are always given an update prior to Eid ul Azha due to increased risks posed by livestock. Many doctors were unaware that Loralai was in an endemic area of CCHF. Overcrowding and resource limitations made it extremely difficult to implement adequate infection control, namely patient isolation and access to Personal Protection Equipment. There was no local laboratory to diagnose the disease and the sample had to be sent to Islamabad which increased the result reporting time. Clinical experience with Ribavirin supports its use.[6] It is also recommended by the WHO.[1] As the patient rapidly improved and the confirmatory results were obtained after her discharge, Ribavirin was withheld. Public Health departments need to aggressively educate and sensitize health care workers about the disease Epidemiology and management. Government hospitals need to make infection control a priority and lastly, there needs to be more emphasis on the Viral Haemorrhagic Fevers and emerging infections in the FCPS Medicine curriculum.

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References

1. http://www.who.int/media-centre/factsheets/fs208/en
2. Mandell, Douglas and Bennett's: Principle and Practice of Infectious Diseases, 7th Edition
3. 3. Burney, M.I., Ghafoor, A., Saleem, M., Webb, P.A., and Casals, J. Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever-Congo virus in Pakistan, January 1976. Am J Trop Med Hyg. 1980; 29: 941947
4. Van Eeden PJ, Joubert JR, van de Wal BW, et al, A nosocomial outbreak of Crimean Congo hemorrhagic fever at Tygerbert hospital. I Clinical features. S Afr Med J. 1985;68:711-717
5. Papa A, Bino S, Llgami A, et al. Crimean Congo hemorrhagic fever in Albania. 2001. Eur Clin Microbiol Infect Dis. 1986;154:39
6. Mardani M, Jahromi MK, Naieni KH et al. The efficacy of oral Ribavirin in the treatment of Crimean Congo Haemorrhagic Fever in Iran. Clin Infect Dis. 2003;36:1613-1618