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CONTENTS

Volume 14 Number 1 Mar 2019

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Procedure for online submission of manuscript

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EDITORIAL

First Trimester Miscarriages: Medical or Surgical Outpatient Management

Saadia Sultana

The World Health Organization reported 93,000 maternal deaths due to miscarriages and abortions yearly in developing countries. This represents more than 13% of all pregnancy-related deaths, and is especially prevalent in settings where access to safe health-care services is difficult. Miscarriages and abortions contribute excessively to maternal morbidity and mortality in under-developed and developing countries.

Complications from unsafe, induced and spontaneous abortions/miscarriages are recognized worldwide as a main public health fear and are one of the foremost reason women seek critical care. A lot of women survive with pelvic inflammatory disease, chronic pain, risk of ectopic pregnancies and infertility. Most mortalities and morbidities are actually preventable through in-time access to safe health-care and contraception services. Post-abortion care, refers to specific services for those females who are experiencing problems from all types of spontaneous miscarriages or induced abortions. This term is commonly used by the 'international community for reproductive health'. Availability of services for post-abortion care must be improved to reduce maternal mortality and morbidity. Midwives, nurses and junior/senior doctors can safely provide post-abortion care even in outpatient settings; only if they receive appropriate support and training. Access to health-care services and contraception facilities decreases the need for it and averts complications. It is much less expensive to prevent unsafe abortion/miscarriage rather to treat resulting complications.

Missed miscarriage is in-utero death of the embryo/foetus before the 20th week of pregnancy. It is not very uncommon, occurring in up to 10-20% of recognized pregnancies. Incomplete miscarriages are incomplete expulsion of products of conception before 20th week of pregnancy. These miscarriages can be managed expectantly, medically and surgically.

Medical treatment of miscarriage is the one that is carried out by taking medications. It is gaining ground as a feasible and low-cost method of uterine evacuation. Multiple drugs are used for this purpose e.g. mifepristone, misoprostol and methotrexate. Vaginal and oral misoprostol are safe, effective and acceptable methods of treating miscarriage with a reported effectiveness of 85–95%. Increasing evidence is coming that tablet misoprostol is a safe, effective, and acceptable method to achieve uterine evacuation for women needing care after miscarriages.

Misoprostol must be readily available especially for women who do not otherwise have access to proper healthcare facilities. According to multiple studies in the recent past, medical management of miscarriages by oral misoprostol was lesser effective than MVA (Manual Vacuum Aspiration), but it was more acceptable to the patients. Medical termination is well-suited for usage in low-resource situations and it must be encouraged to be used as an option for the management of incomplete/missed/ spontaneous miscarriages. Latest researches have questioned the necessity for routine surgical evacuation of uterus, suggesting expectant /medical management to be more appropriate.

Surgical management of miscarriages has been the standard treatment across the globe for many years. Its effectiveness and safety are very well proven. Surgical treatment of spontaneous, incomplete, missed miscarriage, or of induced abortions, includes evacuation of the uterus with sharp curettage or MVA. Manual vacuum aspiration is increasingly being used to treat first trimester miscarriages. It is believed to be safe and cost

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effective in experienced hands. MVA is quite a new (2013) addition to the management of incomplete abortion in our hospital. Multiple studies reveal that MVA is more efficient than medical termination of pregnancy in the treatment of first trimester miscarriages.

If uterine size is lesser than or equivalent to gestational age 13 weeks, either misoprostol or treatment with vacuum aspirator is recommended. MVA is reported to be very useful and effective procedure in low-resource settings for patients with 'incomplete/missed/spontaneous miscarriages' with a uterine size of less than 12-13 weeks.

Complications which are reported only in the misoprostol group and not in the MVA group are: abdominal cramping (usually starting within the first few hours but it may begin as early as 10 minutes after misoprostol administration), fever, vomiting and chills. These are common side effects but are transient. Fever does not necessarily indicate infection. Antipyretic can be used for its relief. Nausea and vomiting usually resolve within 2 to 6 hours. Anti-emetic can be used if required. MVA is generally considered safe, but complications such as infection, bleeding, uterine perforation and decreased fertility can occur in up to 10 % of women. Relatively more complications are reported for the treatment with misoprostol than with MVA. Hospital stay was shorter in the MVA group than the misoprostol group in almost all the studies of the last five years.

After this brief review, we can **conclude**: in low resource settings, misoprostol can be used where MVA is not available or when complete uterine clearance cannot be achieved even after misoprostol (in recommended doses). Health care providers must be optimally educated/trained/drilled (in medial termination as well as in MVA) beforehand, about complete protocols, procedures, complications and timely referrals. In those areas where MVA or operation theatre facilities are not available, health care providers can prescribe misoprostol and they should inform females about the side effects e.g. chills, fever nausea, cramping etc. and guide them about the simple management of these minor complications. Women should also learn to get help when they notice/feel the bleeding as 'too much'. Timely medical/surgical/both management of first trimester miscarriages/ abortions will profoundly help not only to decrease the maternal morbidity and mortality but it will also minimize the social and economic burden on the society and country.

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ORIGINAL ARTICLE

Misoprostol Versus Outpatient Manual Vacuum Aspiration (MVA) for Termination of Pregnancy: A Quasi Experimental Study

Safia Khalil, Nighat Shaheen

ABSTRACT

Objective: To compare the safety and efficacy of Misoprostol versus Outpatient manual vacuum aspiration (MVA) for termination of pregnancy.

Study Design: Quasi experimental study

Place and Duration of Study: Conducted in Cantonment General Hospital from 1/1/2015 -31/1/2015

Materials and Methods: Women eligible for both MVA and Misoprostol were included in this study. Women were allocated to either group according to the option chosen by them. Misoprostol was administered at a dose of 400 microgram sublingual, 4 doses four hours apart. Manual vacuum aspiration was performed according to practice guidelines provided by IPAS Certified Professionals.[1] Primary outcome measure was achievement of complete evacuation within 24 hours.

Results: Out of 101 women, 68 (62.3%) were in MVA group and 33 (32.6%) in Misoprostol group. Complete evacuation was achieved in 28 (82.1%) women in the Misoprostol group and 68 (100 %) in the MVA group within 24 hours (P value 0.003). Five women had failed medical termination. None of the women required hospital admission. There was no statistical difference between the two groups (p value 0.079) in terms of total number of visits or requirement of analgesia.

Conclusion: Both Manual Vacuum Aspiration (MVA) and Misoprostol are safe and effective alternatives for termination of pregnancy but the former is more likely to achieve complete evacuation within 24 hours.

Key Words: *Efficacy, Manual Vacuum Aspiration (MVA), Misoprostol, Outpatient , Safety, Suction Curettag.*

Introduction

About 15 % of all pregnancies result in miscarriage. The world wide abortion rate is 28 per 1000 women aged 15-44 years.[2] The bulk of these miscarriages occur in underdeveloped countries. In low resource settings, there is a high incidence of unwanted pregnancies. Most of these pregnancies succumb due to poor antenatal care, malnutrition or induced miscarriages. Lack of post abortion care is one of the leading causes of maternal mortality, accounting for about 7.9% of maternal deaths. Part of maternal mortality due to sepsis is also contributed by it.[3] In Pakistan, abortions (both spontaneous and induced) are responsible for 5.6 % - 11% of maternal mortality.[4] This is mainly due to overburdened public health sector, high total fertility rate (3.8%) , low contraceptive prevalence (35%) , lack of resources , inability to access to safe abortion care and poor understanding of legislation by health care professionals and patients.[5] In the last 30 years, there has been effort at the international as well as regional level to improve service provision for miscarriages and to evaluate its effectiveness by measuring quantifiable objectives. This included legislation, introduction of misoprostol, evolution and provision of manual vacuum aspiration and monitoring of these efforts.[4] Most of the studies done in this context are based on In patient category. Both MVA and Misoprostol have been shown to be effective methods of termination of pregnancy in admitted patients. Outpatient or community use of MVA has not been studied so far. Few good quality studies from South Asia and Africa (Pakistan, India, Nepal & Bangladesh) have shown that Misoprostol use is safe in community.[6] Outpatient provision of termination facilities has several advantages including cost and convenience. It is extremely useful in far flung areas where access to established medical facilities is not available.

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Our study is designed to bridge this gap. Our study intends to address the provision of safe and effective methods of termination of pregnancy in an Outpatient setting which is likely to have a larger effect in low resource settings. We compared Manual Vacuum Aspiration with medical termination (Misprostol) in cases where both options were suitable for the woman. Apart from assessing the safety & efficacy, it will also give us an insight into the patient preference in our culture. The Objective of our study was to compare Misoprostol Versus Outpatient Manual Vacuum Aspiration (MVA) for Termination of Pregnancy.

Materials and Methods

This Quasi experimental study was conducted in Cantonment General Hospital Rawalpindi from 1/1/2015 -31/1/2015. Approval from institutional ethical committee was obtained. 107 women consented to participate in the study. Nonprobability convenience sampling technique was used to collect sample. All women with an indication for termination of pregnancy with

- A crown rump length of 7-40 mm without cardiac activity (missed miscarriage)or
- Mean gestational sac diameter between 25 and 45 mm without fetal pole (anembryonic pregnancy) or
- Passage of products of conception with the residual anterior-posterior endometrial lining ≥30 mm [retained products of conception (RPOCs)] or
- Uterine size <13 weeks Were included in the study .

Hemodynamically unstable women, septic abortion, suspected ectopic pregnancy, known bleeding disorders, uterine anomalies and those taking anticoagulant therapy were excluded from the study. Women were educated about various options for termination of pregnancy. Those not willing /suitable for expectant management were allocated to either group according to the option chosen by the woman. Group one received misoprostol while group two underwent manual vacuum aspiration in the Outpatient department. Misoprostol was administered at a dose of 400 microgram sublingual, 4 doses four hours apart (total 8 tablets of 200 micrograms). Manual vacuum aspiration was performed according to practice guidelines provided by IPAS by Certified Professionals. A pelvic ultrasound was performed after 24 hours to confirm completion of evacuation. Estimation of blood loss was subjective. Women who had incomplete evacuation were administered 600 microgram misoprostol in line with FIGO recommendation and required follow up visits at 01 week post procedure. Those who had persistant RPOCs despite following this protocol were declared failed medical treatment. These women had to undergo MVA to remove RPOCs. Contraceptive advice was provided afterwards and the woman was advised follow-up after one week. All data was entered on a preformed Performa. Systematic review done by Linet T et al was used as a guideline for protocol development of medical & surgical methods of termination of pregnancy.[7] Primary outcome measure was achievement of complete evacuation within 24 hours. Other outcome measures included duration of hospital stay, no. of hospital visits, procedure related blood loss, requirement of post procedure analgesia, need for repeat / alternate procedure, adverse effects and complications.

Data was analyzed on SPSS 22. Descriptive statistics included means & frequencies. Means were calculated and compared for age, gravidity, parity, gestational age, duration of hospital stay and number of hospital visits. T test was applied to calculate the difference. Frequencies and percentages were calculated for type of selected procedure, indication of termination, achievement of complete evacuation within 24 hours. Chi square test was applied to calculate the p values. Other variables include procedure related blood loss (mild ,moderate , severe) , requirement of analgesia (yes, no) , need for repeat procedure (yes , no) , adverse effects (fever shivering, gastrointestinal side effects) and complications (hemorrhage ,infection , perforation). Frequencies and percentages were calculated for these as well and chi square was applied.

Results

Total 107 women were enrolled in the study, out of which 68 (62.3%) were in MVA group and 33 (32.6%) in Misoprostol group. Six women (5.6%) opted for dilatation and curettage (D & C) and were excluded

from the study.

Mean age of women undergoing MVA was 28.55 ± 5.228 and in Misoprostol group was 27.95 ± 2.681. Mean gestational age in MVA group was 10.59 ± 3.053 and in Misoprostol group was 12.31 ± 3.248. Mean gravidity was 3.35 ± 1.483 in MVA group and 2.78 ± 1.281 in Misoprostol group. Mean parity was 1.84 ± 1.417 versus 1.52 ± 1.051. The two groups were comparable in these respects.

Regarding indications for termination of pregnancy, 31(29%) women had missed miscarriage, 77 (65.4%) had incomplete miscarriage and 6 (5.2%) had termination of pregnancy for other reasons [anencephalic fetus (n=4 ,3.7%), blighted ovum (n=2 , 1.9%)]. One woman (0.9%) had a previous scar of lower segment cesarean section. The two groups did not differ in the indication of termination of pregnancy (p value = 0.07).

Complete evacuation was achieved in 28 (82.1%) women in the Misoprostol group and 68 (100 %) in the MVA group within 24 hours. Five women had failed medical termination. This difference was statistically significant (p value 0.003).

Mean no. of visits required in the Misoprostol group was 2.8 ± 0.837. None of the women had retained products of conception following MVA and the mean no. of required visits in this group was 1. This difference was statistically significant (p value 0.01). Most of the women did not return for follow up visit (n=89, 83.2%). Twelve women (17.6%) in the MVA group returned for follow up and five (17.8%) in the Misoprostol group. There was no statistical difference between the two groups in terms of return to follow up (p value = 0.497). When the total number of visits were considered (procedure related plus follow-up visit), there was no statistical difference between the two groups (p value 0.079).

Only six women (5.6 %) required analgesia and the requirement for analgesia did not differ with the type of procedure undertaken (p value =0.529). None of the women required hospital admission. No complications or adverse effects were reported. There was no report of moderate or severe blood loss during the study in either group. Contraceptive counseling was provided to 79 (73.9%) women.

Discussion

Zaidi S et al has conducted two of the largest studies regarding abortion care in Pakistan and Bangladesh in 2014.[4,5] They explain the prevailing situation of abortion care in detail. It is an alarming situation. There is a large unmet need for abortion care in our country (estimated at around 20% in 2012).[8] It is one of the major target areas that need improvement if we aim to achieve Sustained Development Goals.

Regarding the choice of method NP Khawaja et al report that women are aware of the modern methods which are safe and effective.[9] Similarly in our study most of the women opt for new methods of abortion care. This is because of increased awareness probably because of lesser cultural inhibition and positive role of media. On the other hand complications in underdeveloped countries are common due to widespread illegal abortion. Shahab et al report that a significant percentage of women (5%) have a fear of the pain and complications[10]. These women prefer dilatation and curettage under anesthesia instead of modern methods. In our study a small number of women opt for D& C probably for similar reasons.

At the moment abortion for unwanted pregnancies is restricted in Pakistan to save the life of the mother.[11] Other indications for termination are similar in both groups and were not related to the choice of procedure.

Our study shows that both methods are safe, effective and tolerable to the women with little analgesia requirement. There is no significant blood loss, hospital admission, complication or side effects. Several other studies show similar results. Speedie J et al have developed a one stop evaluation unit for Manual Vacuum Aspiration.[12] They find it safe and acceptable with significant reduction in cost. However they do not compare it with other modalities. Ansari A et al has confirmed its safety in high risk cardiac patients.[13] In our study, only those women who have a failed medical termination are not satisfied with the chosen method. They have two options. One is to take higher doses of Misoprostol with or without Mifepristone as reported by Meena SR et al and Li YT et al respectively.[14,15] The other option is to undergo MVA as suggested by Heller et al.[16] All of the women in our study chose the second option. In our study, all procedures are done by certified doctors. However, in a systematic review Ngo TD et al has shown that Manual Vacuum aspiration is safe in the hands of mid level care providers as well.[17]

Speedie J et al has found that Manual Vacuum Aspiration is a good opportunity to offer long acting reversible contraception.[10] Eighty percent women in

their study opt for long acting reversible contraceptive methods. Korjamo R and Laursen L also find that women are more receptive to contraceptive advice during this period.[18,19] This is in contrast to our study. Although contraceptive counseling is provided to a significant proportion of women, majority (66%) opt for barrier methods or no method at all. This indicates a need for research into the local problems associated with contraception. Counseling methods need to be improved and designed to address the local concerns. Current situation of contraceptive use in Pakistan is unlikely to change until and unless there is a mechanism of continuous positive feedback by women who are successfully using reliable contraceptive methods. Also, backstreet abortion is cheap and readily available. Most women feel it an easy way out instead of regularly using contraception.

Increasing role of community workers for provision of abortion care in the outreach is being explored. Early reports by Constant D et al and Gupta P et al are encouraging.[20,21] They have shown that misoprostol administration by community workers at home is safe and efficacious.

One of the limitations of our study is small sample size. This is mainly because majority of women as well as care providers feel more comfortable with in patient management. The reasons for such preference need to be explored. Another limitation is poor follow-up.This is mainly due to a lack of integrated services. Most of the women who report for follow up are those who are concerned about retained products of conception. Apart from hospital visit, other means for follow-up can be explored e.g. telephonic contact etc. Aiken ARA et al has evaluated newer techniques like telemedicine to facilitate followup.[22] Another limitation is that the reasons for preference of a particular method were not interviewed further.

Termination of pregnancy is a wide area of research. Barriers to implementation of safe and effective abortion care need future research.[6]

Conclusion

Both Manual Vacuum Aspiration (MVA) and Misoprostol are safe and effective alternatives for termination but the former is more likely to achieve complete evacuation within 24 hours, larger prospective studies are needed to confirm these findings.

Table I: Demographic Features of Women in Treatment Groups

	Misoprostol (group 1)	Manual Vacuum Aspiration (group 2)	Failed medical treatment	D& C
Age	27.95 + 2.681	28.55 + 5.228	33.00 + 6.831	23.31 + 4.732
Gestational Age	12.31 + 3.284	10.59 + 3.053	11.00 + 2.646	11.33 + 1.633
Gravidity	2.78 + 1.281	3.35 + 1.483	4.06 + 4.336	2.33 + 1.506
Parity	1.52 + 1.051	1.84 + 1.417	2.40 + 2.302	0.67 + 0.816

Table II: Indication for Termination in Misoprostol & MVA Groups

	Misoprostol (group 1)	Manual Vaccum Aspiration (group 2)
Missed miscarriage	13(12.8%)	11(10.8%)
Incomplete miscarriage	51(50.4%)	15(14.8%)
Therapeutic (anencephalic, blighted)	4(3.7%)	2(1.9%)

Table III: Outcome Measures in Misoprostol vs. MVA

	Misoprostol (group 1)	Manual Vaccum Aspiration (group 2)	P value
Complete evacuation within 24 Hours	23 (82.1%)	68 (100 %)	0.003
Moderate /severe blood loss	0	0	0
Need for hospital admission	0	0	0
Mean procedure no. of related Visits	2.8 + 0.837	1	0.001
No. of follow up visits	5 (17.8%)	12 (17.6%)	0.497
Analgesia requirement	3 (10.7%)	3 (4.4%)	0.529
Complications/ side effects	0	0	0

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ORIGINAL ARTICLE

Comparison of Umbilical Cord Care: Chlorhexidine 4% Versus Dry Cord Care

Noshina Riaz, Rida Tahir, Sidra Tul Muntaha, Shahid Aziz, Rubina Zulfqar

ABSTRACT

Objective: To determine the efficacy of chlorhexidine 4 % versus dry cord in neonatal cord care to prevent infections and reducing healing time in a hospital setting.

Study Design: Randomized Controlled Trial.

Place and Duration of Study: At Department of Paediatrics Cantonment general hospital, Rawalpindi from 1 December 2016 to 30 November 2017.

Materials and Methods: Total 200 newborns were divided into two groups by randomization using lottery method; Group A comprising of 100 newborns were applied with chlorhexidine 4% on umbilical cord, once a day for a week and group B 100 newborns were advised conventional dry cord care. They were called for follow up visit after 1 week and later contacted telephonically till the separation of umbilical cord. Newborns with delayed healing or any signs of infection were called again for follow up examination. The day of separation of umbilical cord and signs of infection were recorded for each neonate.

Results: The mean duration of separation and healing of umbilical cord in group A (chlorhexidine 4%) and group B (dry cord care) was 8.35±3.73 and 6.98±2.59 days respectively. The difference in healing and duration of separation of umbilical cord in two groups was statistically significant (p= 0.003). Signs of infection observed in group A and group B were 5% and 4% respectively. Hence statistically insignificant (p=0.73).

Conclusion: The umbilical cord care with 4% chlorhexidine or conventional dry method has similar results in terms of frequency of infection in a hospital setting. However healing time in terms of mean duration of separation of umbilical cord is prolonged with the application of 4% chlorhexidine to the cord stump.

Key Words: *Chlorhexidine 4 %, Dry cord care, Newborn, Umbilical Cord Care.*

Introduction

Among deaths noted in children younger than 5 years, neonatal deaths account for more than 40%.[1] In high mortality settings, about half of neonatal deaths are caused by infections involving sepsis, pneumonia, meningitis and tetanus neonatorum.[2] In Pakistan 30% of neonatal deaths are due to sepsis.[3] Such a high rate of mortality needs to be addressed by certain cost effective interventions like use of topical antiseptics that limit the growth of bacteria around the umbilical stump.[4]

One of the important causes of neonatal mortality is omphalitis.[5] Any delay in separation of umbilical cord increases the chances of infection as it is ideal for bacterial colonization.[6] Application of harmful substances to umbilical cord like ash, oil, lead based compounds (surma) and rarely cow dung are being practiced in rural areas of Pakistan.[3] Even in urban areas like Karachi, 74 % mothers were applying various substances to the cord stump like mustard oil, coconut oil, butter and turmeric in a study conducted by Gul S et al.[7] Approaches that reduce chances of neonatal infections include hand washing, skin cleansing with antiseptics such as chlorhexidine, use of clean birth kits and early breastfeeding.[3] According to a new recommendation for umbilical cord care issued by WHO in January 2014, for newborns who are born at home in high mortality settings, chlorhexidine 4% should be applied to the cord stump daily for 1 week. For newborns who are born in hospital or at home in low neonatal mortality setting, clean dry cord care is recommended.[8] Dry cord care was the recommendation of WHO in 1998 but in 2016 researchers working on chlorhexidine found that mothers were fond of applying one or the other substance on the cord stump.[9] These traditional practices led to the development of WHO

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recommendation of using chlorhexidine gel instead of applying harmful substances to the cord stump.[8] Regarding safety profile of chlorhexidine , it is generally safe and is extensively used in medical settings since 1950. Only few side effects like contact sensitivity, dermatitis, urticaria and photosensitivity have been reported. Percutaneous absorption of chlorhexidine when used as a body wash or for cord care has no reported side effects .However this absorption is more likely to occur when used on premature and underweight neonates.[10] A high quality evidence suggests that chlorhexidine cord care in the community setting reduces 50% chances of omphalitis.[11] A metanalysis revealing the efficacy of Chlorhexidine 4% in reducing the risk of omphalitis, concludes a reduction of 27 to 56% compared to dry cord care. However the protection is most fruitful in the 1st week of life.[12] WHO recommends dry cord care as a suitable method, though several studies support this recommendation. Still this method is controversial.[6] Two African trials conducted at Tanzania and Zambia failed to show superiority of Chlorhexidine over dry cord care.[8] There is much controversy over the umbilical cord separation time as some studies favor chlorhexidine while others show dry cord care to be more effective in reducing umbilical cord extraction time.[13,14,15,16] Other than one community based study , none of such study is found in a hospital setting in Pakistan. This study was planned to determine the effectiveness of chlorhexidine 4 % in comparison to dry cord care in hospital setting. Also to compare the extraction time of umbilical cord in both groups and risk of possible omphalitis.

Materials and Methods

This Randomized Controlled Trial was conducted at Department of Paediatrics Cantonment general hospital, Rawalpindi from 1 December 2016 to 30 November 2017 with Non-probability consecutive sampling technique. All babies delivered in cantonment general hospital were included in study. Any sick newborn that required admission to hospital was not included in the study. Informed written consent was taken from parents. Gestational age, gender, weight and mode of delivery were noted. All newborn were randomized based on lottery method into two groups. Group A comprising of 100 newborns were applied with chlorhexidine 4% on umbilical cord , once a day for one week and group B 100 newborns were advised conventional dry cord care. They were called for follow up visit after 1 week and later contacted telephonically till the separation of umbilical cord. Newborns with delayed healing or any signs of infection were called again for follow up examination. The duration of separation of umbilical cord and signs of infection were recorded for each neonate. Enrolled subjects completed this study. Signs of infection were further categorized into mild (redness, swelling or pus restricted to the cord stump), moderate (redness, swelling or pus extending to the skin at the base of the cord stump less than 2 cm) and severe (inflammation extending more than 2 cm from the cord stump, with or without pus).[3]

Data were analyzed using SPSS version 23. Frequency and percentage were calculated for categorical variables like gender whereas mean and standard deviation were calculated for numerical variables. Categorical comparisons were made using the chi square test. Numerical comparisons like mean duration of separation of umbilical cord was made using independent samples t-test. A p value < 0.05 was considered statistically significant.

Results

The study population included 106 (53%) male and 94 (47%) female babies. Group A included 27 (41.54%) male and 38(58.4%) female babies. Group B included 34 (52.3%) male and 31(47.7%) female newborns. Gestational age, weight and mode of delivery in both groups are shown in table I. More late preterm were enrolled in group A than group B i.e. 66.7% versus 33.3%.Low birth weight neonates were 66.7% in group B while 33.3% in group A. More neonates delivered by SVD i.e. 80.4% in group B while 61.8% by Caesarean section in group A. These differences had no statistically significant effect on frequency of infection and umbilical cord extraction time.

The mean duration of separation and healing of umbilical cord in group A and group B was 8.35±3.73 and 6.98±2.59 days respectively. The difference in healing and duration of separation of umbilical cord in two groups was statistically significant (p= 0.003) as shown in Table II. Infection observed in group A and group B was 5% and 4% respectively. Hence statistically insignificant (p=0.73) as shown in Table

III. Mild infections were seen in 4% babies in both groups . Moderate infection was found in only 1 baby in group A. None of the neonate developed severe infection in both groups.

Table I: Gestational age, Weight and Mode of Delivery in Group A and B (n=200)

Characteristics		Cord care	
		Chlorhexidine 4% n (%)	Dry cord care n (%)
Gestational age	Late preterm	6(66.7)	3(33.3)
	Term	94(49.2)	97(50.8)
Weight	<2.5kg	2(33.3)	4(66.7)
	>2.5kg	98(50.5)	96(49.5)
Mode of delivery	SVD	11(19.6)	45(80.4)
	Caesarean delivery	89(61.8)	55(38.2)

Table II: Days of Separation of Umbilical Cord in Group A and Group B (n=200)

Statistics	Cord care		P-Value
	Chlorhexidine 4%	Dry cord care	
Mean ± SD	8.35±3.73	6.98±2.59	0.003

Table III: Frequency of Infection in Group A and Group B (n=200)

Signs of infection		Cord care		P-Value
		Chlorhexidine 4% n (%)	Dry cord care n (%)	
Yes	Total	5(5%)	4(4%)	0.733
	Mild	4(4%)	4(4%)	
	Moderate	1(1%)	0	
	Severe	0	0	
No		95(95%)	96(96%)	

Discussion

The present study shows that there was no significant difference between chlorhexidine 4 % and dry cord care group regarding signs of infection in a hospital setting. However duration of separation of umbilical cord was prolonged by application of chlorhexidine to the cord stump. This did not have an effect on the frequency of infection in both groups. In a community based study conducted in a rural district of Pakistan, topical application of chlorhexidine 4% reduced the incidence of omphalitis by 42% in comparison with the dry cord care. This finding is contrary to our results as no difference was observed between the two groups. The mean cord separation time was 6.2±1.3 days in Chlorhexidine group and 5.9 ±1.5 days in dry cord care group that is statistically insignificant but contrary to our results as it was prolonged in chlorhexidine group.[3] A community based study was conducted in Bangladesh to assess the effect of cord cleansing with Chlorhexidine 4% on neonatal mortality. It was found to be lower in neonates who received single application of 4% Chlorhexidine in comparison with dry cord care. There was no effect of application of chlorhexidine for a week to reduce neonatal mortality. However there was a significant difference of serious cord infection in this group versus dry cord care .[1] This finding is again contrary to our results. Moreover mortality risk was not assessed in our study. A Cochrane review published in July 2004 included 21 hospital studies. The conclusion of the review stated that topical application of various agents to the cord was not superior to dry cord care in terms of systemic infections as found in our study.[17] A study conducted in India compared the effectiveness of chlorhexidine with dry cord care and results showed the superiority of chlorhexidine over dry cord care. The mean time to cord separation was 8.92±2.77 days (shorter) in former group and 10.31±3.23 days in latter. This is in contrast to our findings as it was vice versa. However no difference was noted in terms of umbilical sepsis as in our study.[18] A meta-analysis published by Bhutta et al showed the effect of umbilical cord cleansing with chlorhexidine versus dry cord care on omphalitis and neonatal mortality in community settings in developing countries. Three trials were included that were done in Nepal, Pakistan and Bangladesh. In Pakistan trial there was no difference in the time of separation of cord. However, Nepal and Bangladesh trial showed longer separation time in chlorhexidine group (1.08 and 2.41 days respectively) compared to dry cord care group, consistent with our results.[19] Khairuzzaman et al in Bangladesh found that chlorhexidine reduces the risk of mild omphalitis with single application of chlorhexidine and moderate omphalitis both in single and multiple chlorhexidine cleansing group as compared to dry cord care group. None of the neonates developed severe infection in their study. This is in contrast to our study as both groups had similar frequency of mild infection and only 1% developed moderate infection in chlorhexidine

group. However severe category of infection was not found in both groups similar to the above study.[20] A study conducted in Gadaba and Konda Dora tribes for umbilical cord care practices showed that 96% of Gadaba and 95.3% of Konda Dora newborns were applied with oils or ash of vegetative origin and also different powders.[19]Our study also revealed the fact that despite of proper counselling regarding cord care, mothers used to apply different substances like oil, spirit, pyodine, onion and powder to the cord stump.

Current study supports the WHO recommendation of preference of dry cord care in low neonatal mortality settings. Dry cord care is simple, cheap and cost effective. Limitation of our study is that actual practice of hand washing cannot be monitored in both groups. Further studies are required to prove the efficacy of chlorhexidine 4 % in hospital settings.

Conclusion

The umbilical cord care with 4% chlorhexidine or conventional dry method has similar results in terms of frequency of infection in a hospital setting. However healing time in terms of mean duration of separation of umbilical cord is prolonged with the application of 4% chlorhexidine to the cord stump.

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ORIGINAL ARTICLE

The Association of Pro-inflammatory Markers with Suicidality in Patients Suffering from Major Depressive Episode

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ABSTRACT

Objective: To determine the association of pro-inflammatory markers with suicidality in patients suffering from major depressive episode.

Study Design: Descriptive cross sectional study.

Place and Duration of Study: The study was conducted at department of pathology in collaboration with the department of psychiatry, Pakistan Railway Hospital, Islamic International Medical College, Rawalpindi from 18th April, 2017 to 17th March, 2018.

Materials and Methods: Seventy five subjects recruited through convenient non-probability sampling technique were divided into three groups i.e. Group 1 (patients of major depressive episode with suicidality), Group 2 (patients of major depressive episode without suicidality) and Group 3 included healthy controls. Patients of major depressive episode and suicidality were diagnosed through Hamilton rating scale for depression and beck scale for suicidal intention successively. Pro-inflammatory markers i.e. Interleukin-1ß, Interleukin-6 and C-reactive protein were measured in the serum of all participants. Data was analyzed through statistical package for social sciences version 21. Simple descriptive statistics (frequencies, percentages) were computed for each categorical variable. Mean and standard deviation were calculated for the numerical data. Independent t test and one way ANOVA was applied to determine the statistical significance. *P* value of < 0.05 was considered significant.

Results: Out of 75 enrolled patients, Pro-inflammatory markers i.e. Interleukin-1ß, Interleukin-6 and C-reactive protein were raised in patients of major depressive episode with suicidality.

Conclusion: Pro-inflammatory markers i.e. CRP, IL-1ß and IL-6 are markedly raised in patients of major depressive episode with suicidality. So, from the results it is concluded that pro-inflammatory markers have an association with suicidality in patients of major depressive episode and treating inflammation in them can improve their symptoms and reduce suicidal rate.

Key Words: *CRP, IL-1ß, IL-6, Inflammation, Major Depressive Episode, Pro-inflammatory Markers and Suicidality*

Introduction

Suicide is the second major cause behind the death of youngsters among the 15–29 age group in the world.[1] According to one estimation, 40,000 people die of suicide per year in the United States and the global death rate was nearly 800000 in the year 2014 calculated by World Health Organization (WHO).[1] It was predicted by WHO in 2011 that one million people would die by the year 2030 due to suicide, that will contribute to 1.4% of all the deaths globally.[1] The real number of deaths may be higher because suicidal deaths are normally not reported due to societal issues and criminalization of suicide in many societies.[1] Suicidal attempts are also insufficiently reported. Almost twenty or more than that suicide attempts occur for every completed suicide, so suicide attempts are more frequent than completed suicide.[1] There is emerging evidence that this behavioral phenotype is independently associated with a pro-inflammatory state in the body, and that it is not linked to the severity of depression per se.[2]

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Many studies have shown that suicide is associated with an increase in inflammatory markers in the body.[2] **"Cytokine-induced sickness behavior"** (i.e., lethargy, fatigue, depression, failure to concentrate, anorexia, sleep problems, reduced sense of personal hygiene and social withdrawal) has been shown to be linked and mediated by pro-inflammatory cytokines i.e. IL-1 and IL-6.[3] Another important mechanism underlying suicidality is the activation of kynurenine pathway for the catabolism of tryptophan. Abnormalities in this pathway lead to certain biological mechanism which associates inflammation with suicidality and depression.[4] Tryptophan catabolism through kynurenine pathway produces certain neuroactive substances especially quinolinic acid (QUIN) and kynurenic acid (KYNA).[5] The inflammatory cytokines especially IL-1ß, IL-6 and IFN-**γ** are potent activators of indole amine 2, 3 dioxygenase (IDO-1) and tryptophan 2.3 dioxygenase (TDO2). These two enzymes regulate the initial steps in kynurenine pathway.[6,7] Tryptophan is the precursor of serotonin neurotransmitter, so catabolism of tryptophan through this pathway down regulates the amount of serotonin in the body.[8] This mechanism can directly lead to lower levels of monoamines in the CSF and serum of suicidal victims.[1] A meta-analysis by Black & Miller published in 2015 has shown that levels of IL-1 β, IL-6 & TNF-α are elevated in the body fluids of those psychiatric patients who attempted suicide or have strong suicidal ideation.[2] Identification of specific inflammatory markers and important co-existing biological factors can inform us regarding the risk of suicidal behavior. Further, treatments targeting the constituents of the inflammatory pathway are nowadays undergoing trials for a variety of psychiatric conditions, and this approach can lead to the development of novel therapeutic tools for the prevention of suicide.[9] Our proposition is that patients who contemplate or attempt suicide have an inflammatory state in their bodies which can be detected by measuring pro-inflammatory markers like interleukin-1 β, interleukin 6 and C-reactive protein (CRP) in the serum.[10,2] In the extant literature review, no single study has documented the association of IL-1 β, IL-6 & C-reactive protein together with the existence of suicidality.[2] Further, no local studies are available to relate the association of pro-inflammatory markers with suicidality in patients of major depressive episode (MDE) patients. The objective of our study is to find the association of pro-inflammatory markers (IL-1β, IL-6 & CRP) with suicidality in patients suffering from major depressive episode (MDE).

Materials and Methods

This observational cross sectional study was conducted at Chemical Pathology department in collaboration with psychiatry department of Pakistan Railway Hospital, Islamic International Medical College, Rawalpindi from 18th April, 2017 to 17th April, 2018 after getting permission from ethical review committee of Riphah International University. Sample size was decided based on the data from similar previous studies. Participants from both genders were enrolled through non-probability convenient sampling after taking informed consent. Seventy-five participants were inducted in the study between the ages of 18 to 65 years and divided into three groups each having twenty-five participants. Group 1 had twenty-five patients of major depressive episode (MDE) with suicidality, Group 2 had twenty-five patients of MDE without suicidality and Group 3 had twenty-five healthy adults. Participants having any acute or chronic infection or inflammation, obesity, allergies, pregnancy and lactating mothers were excluded. Participants of MDE and suicidality were diagnosed based on Hamilton rating scale for depression (HRSD) and Beck Scale for suicidal intent respectively by a qualified Psychiatrist in OPD of psychiatry department. After filling demographic data, patients were directed towards Pathology lab and 5 ml of venous blood was withdrawn in plain vacutainers considering all necessary measures to avoid pre-analytical errors. Samples were transported in crushed ice to CREAM lab of Army Medical College, Rawalpindi for refrigerated centrifuge at 4° C. Serum was extracted and stored at -80° C. CRP levels of all samples were analyzed through visual agglutination semi quantitative method. IL-1ß and IL-6 were analyzed by ELISA method. Data was analyzed through statistical package for social sciences (SPSS) version 21. Simple descriptive statistics (frequencies, percentages) were computed for each categorical variable. Mean and standard deviation were calculated for numerical data. Independent *t* test and one way

ANOVA were applied to determine the statistical significance. *P* value of < 0.05 was considered significant.

Results

Total 75 patients were divided into three equal group i.e. group 1 consisted of patients of MDE with suicidality, group 2 consisted of MDE patients without suicidality and group 3 consisted of healthy adults with no history of somatic or psychiatric illness.

Descriptive statistics of age (years) was calculated in terms of mean and standard deviation. Mean age (years) of participants was overall 33.32±8.93 whereas mean age (years) of MDE patients with suicidality was 28.44±6.70 and of MDE patients without suicidality was 37.8±8.10. The mean age (years) of healthy adults was 33.72±8.93. Distribution of gender was calculated in terms of frequency and percentage of male and female patients. There were 39 (52.0%) male participants, of these 11 (44.0%) were present in group 1 (MDE with suicidality), 13 (52.0%) male patients were included in group 2 (MDE without suicidality) and 15 (60.0%) male participants were present in group 3 (Healthy adults). There were 36 (48.0%) female participants included in the study, of these 14 (56.0%) were present in group 1 (MDE with suicidality), 12 (48.0%) female patients were included in group 2 (MDE without suicidality) and 10 (40.0%) were present in group 3 (Healthy adults). The association of pro-inflammatory markers (IL-1 β, IL-6 and CRP) with suicidality in patients suffering from major depressive episode (MDE) was assessed using independent *t* test. The results came out to be statistically significant for all the three markers i.e. P-value < 0.05 as shown in Table I and Table II.

Pro-inflammatory markers (CRP, IL-1ß and IL-6) were compared among three groups using one way ANOVA. The results showed statistically significant difference for all pro-inflammatory markers (p value<0.001) as shown in Table III.

Table I: Comparison of Pro-inflammatory Markers between Groups 1 and Group 2

Pro-inflammatory Markers	GROUPS		P-value*
	Group 1* n=25	Group 2** n=25	
CRP level (mg/L)	6.48±5.36	3.04±2.38	0.005
IL-1β (pg/ml)	30.71±40.16	0.73±1.29	0.001
IL-6 (pg/ml)	134.76±157.08	9.95±5.75	<0.001

P≤0.05 was taken as level of significant
Group 1: MDE with suicidality
Group 2: MDE without suicidality

Table II: Comparison of Pro-inflammatory Markers between Groups 1 and Group 3

Pro-inflammatory Markers	GROUPS		P-value*
	Group 1* n=25	Group 3** n=25	
CRP level (mg/L)	6.48±5.36	2.0±0.0	<0.001
IL-1β (pg/ml)	30.71±40.16	0.42±0.88	<0.001
IL-6 (pg/ml)	134.76±157.08	0.31±1.02	<0.001

P≤0.05 was taken as level of significance
Group 1: MDE with suicidality
Group 3: Healthy Adults

Table III: Comparison of Pro-inflammatory Markers among Three Groups (n=75)

Pro-inflammatory Markers	GROUPS			P-value *
	Group 1 n=25	Group 2 n=25	Group 3 n=25	
CRP level (mg/L)	6.48±5.36	3.04±2.38	2.0±0.0	<0.001
IL-1β (pg/ml)	30.71±40.16	0.73±1.29	0.42±0.88	<0.001
IL-6 (pg/ml)	134.76±157.08	9.95±5.75	0.31±1.02	<0.001

P≤0.05 was taken as level of significant

Discussion

The results of present study show that in healthy adults, levels of cytokines are undetectable. In 2013, Kleiner et al. also published a study in which it was concluded that in normal healthy people levels of cytokines were undetectable due to the absence of any inflammatory stimulus.[9] Due to the absence of inflammation in healthy adults, CRP levels are also undetectable or within normal range in them.

In patients of major depressive episode without suicidality, the levels of pro-inflammatory markers like interleukin-1ß, interleukin-6 were high as compared to healthy adults. This shows that some degree of inflammation is also present in patients of major depressive episode irrespective of absence of

suicidality in them. However, the levels are not as much elevated as in patients of major depressive episode with suicidality. In 2013, a study conducted by Valkanova V et al. also concluded that levels of CRP and IL-6 were high in depressed patients.[10] In 2007, a study conducted by Kim YK et al. also showed that levels of cytokines were high in depressed patients.[14] Hughes MM et al. also found out during their study that inflammation and inflammatory markers play a vital role in the pathogenesis of depression and therapies targeting this inflammation might prove beneficial.[11] In 2005, Schiepers OJ et al. published their review article regarding cytokines and depression. They concluded after reviewing many studies related to the subject that cytokines play a pivotal role in the central regulation of certain behavioral changes linked to depression.[12]

In this study it is observed that suicidality in MDE patients is not directly linked to the severity of depression. Another important finding is that certain behavioral traits like aggression, loss of hope and hostile behavior are specially highlighted in patients of MDE with suicidality as compared to MDE patients without suicidality. In 2006, Brezo J et al. reviewed some studies and concluded similar finding that certain behavioral traits were linked to suicidality directly.[13] In 2015, DeShong HL et al. presented a "five factors model" which was based on similar personality traits. They concluded that these behavioral traits could predict future risk of suicidality in MDE patients.[14] So, these findings during this study are in accordance to the previous studies.[13, 14] Importantly among these behavioral aspects "**Hopelessness"** which is a very important factor in diagnosing psychiatric patients especially with suicidality was previously identified to be associated with raised interleukin-6 levels and this finding is also observed and concluded in this study.[15]

CRP was markedly raised in patients of suicidality when compared to non-suicidal depressed patients and healthy individuals. This shows that level of inflammation is higher in patients of suicidality as compared to the non-suicidal depressed patients. Courtet at al. also worked on CRP in both patients with suicidality and non-suicidality and found that high CRP levels were associated with suicidality in MDE patients. They also found that irrespective of low or high risk for suicidality, CRP levels were raised in acute cases. [16] In another recent study, Gibbs et al. did research work on indoor psychiatric patients with and without suicidal behavior. They found out that MDE patients with high degree of suicidality had greater inflammation and increased levels of CRP when compared to MDE patients without suicidality and healthy adults.[17] In 2015, Black and Miller in their meta-analysis of many studies based on inflammation and suicidality also concluded that inflammation was strongly associated with suicidality in MDE patients.[2]

Conclusion

It is concluded that pro-inflammatory markers are significantly high in MDE patients with suicidality. This also throws light on the association of pro-inflammatory markers with suicidality and importance of treating patients with anti-inflammatory drugs in medicine resistant depression with suicidality.

Limitations

This study was conducted in a single outdoor patient department. Due to financial constraints and limited time duration availability, a small group of patients was included. There are some other pro-inflammatory markers and anti-inflammatory markers mentioned in literature like TNF-α, IL-2 etc. They can be considered for further studies. A longitudinal study can be conducted in future to observe the effect of anti-inflammatory drugs in these patients.

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ORIGINAL ARTICLE

Role of Nigella Sativa Seeds on Pyrazinamide Induced Hepatotoxicity

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ABSTRACT

Objective: To determine the hepatoprotective effect of *Nigella sativa* seeds in Pyrazinamide induced hepatic damage in albino mice.

Study Design: An experimental study.

Place and Duration of Study: The study was conducted in pharmacology department of Islamic International Medical College, Riphah International University, in collaboration with National Institute of Health, Armed Force Institute of Pathology and Pakistan Institute of Medical Sciences Islamabad, Pakistan.

Materials and Methods: A total of 68 male albino mice were included in the study. They were divided into 4 group's i.e, seventeen mice in each group. Group A (control group), Group B (Pyrazinamide only treated), Group C (Pyrazinamide + Low dose *Nigella sativa* treated) and Group D (Pyrazinamide+ High dose *Nigella sativa* treated). Group B, C and D received Pyrazinamide through gavage needle once daily for six weeks. Group C and D received *Nigella sativa* along with Pyrazinamide through gavage needle once daily for six weeks. Blood sampling (baseline, end of 3 and 6 weeks) was done to analyze serum ALT and AST levels. Data was analyzed by using SPSS version 21.

Results: Pyrazinamide treatment for 6 weeks increased serum ALT and AST levels. *Nigella sativa* administration along with Pyrazinamide for six weeks decreased the elevated levels of ALT and AST in C and D groups.

Conclusion: *Nigella sativa* seeds exerted hepatoprotective effect by decreasing the raised levels of ALT and AST in Pyrazinamide treated albino mice.

Key Words: *ALT, AST, Hepatotoxicity, Nigella Sativa Seeds, Pyrazinamide.*

Introduction

Tuberculosis is an infectious bacterial ailment caused by several species of mycobacterium.[1] One-third of the global population is infected with tuberculosis, out of whom 5%–10% may develop active tuberculosis in rest of their lives. Tuberculosis is a disease that causes 1.4 million deaths each year.[2] All three major drugs for the treatment of tuberculosis i.e., Pyrazinamide(PZA), Isoniazid(INH) and Rifampicin(RIF) have hepatotoxic effects.[3] The occurrence of liver injury due to antituberculosis treatment (ATT) varies from 2.0% to 28.0%.[4] An important first line and sterilizing tuberculosis drug is Pyrazinamide which aids in condensing the duration of present chemotherapy regimens for tuberculosis.[5] Pyrazinamide causes more hepatotoxicity compared to Isoniazid and Rifampin.[6,7] Hepatotoxicity by Pyrazinamide is either dose dependent or idiosyncratic. Free radical species are produced as result of alteration of nicotinamide acetyl dehydrogenase levels by PZA.INH and PZA may have similar mechanism of injury because of similarity in their molecular structure. In Patients with latent tuberculosis infection, more toxic effects came into observation with RIF and PZA. PZA may cause hypersensitivity reactions and liver damage.[8] *Nigella sativa* has emerged as a miraculous herb with its wide range of pharmacological effects.[9] Hassan et al, (2012) has done research on hepatoprotective effect of *Nigella Sativa* seeds on Isoniazid induced hepatotoxicity and that research was conducted on rabbits. We have conducted the research on male albino mice.

Danladi et al, (2013) has conducted his research on

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protective effects of *Nigella Sativa* in CCl_4 induced hepatotoxicity in rats, while in our research PZA was used.

Nigella sativa (Black cumin) was found to be protective against various hepatotoxic chemicals and drugs.[10] The antioxidant, anti-angiogenesis and anti-inflammatory properties of *Nigella sativa* may play a part in its hepatoprotective role.[11] Thymoquinone (TQ) is an active constituent and is proven to bear hepatoprotective qualities.[9] TQ has immunomodulatory effects on the antioxidant defense mechanisms of the body that underwent toxicity.[12] The hepatoprotective activity was revealed by a reduced release of alanine aminotransferase (ALT), aspartate aminotransferase (AST)and reduced uptake of trypan blue.[9] Many studies have been conducted on hepatotoxic effect of ATT and PZA is a component of this therapy. Its hepatotoxic effect is a main factor in compromising the treatment of TB patients. Therefore to search for a drug that can reduce the effects of PZA toxicity is a challenge for pharmacologists.

This research was aimed to determine the hepatoprotective activity of *Nigella sativa* seeds on PZA induced hepatic damage in male albino mice.

Materials and Methods

An experimental study was conducted at Pharmacology Laboratory, Department of Pharmacology and Therapeutics and Multidisciplinary Research Laboratory, Islamic International Medical College, Rawalpindi with access to Animal House for mice at National Institute of Health (NIH). The project was accepted by the Ethics Review Committee of Islamic International Medical College, Riphah International University,Islamabad. The study took 6 weeks (10^{th} November, 2015 to 22^{nd} December, 2015). Sixty eight adult male Albino mice 2 months of age weighing 25-50gm, purchased from the animal house of NIH were chosen for research project and were then divided into 4 groups i.e. Group A as control group and Groups B, C and D were experimental groups each comprising 17 male albino mice.

Standard nutrition consisting eating regimen and clean tap water was started *ad libitum*. A specific room was assigned for experimental mice at NIH. Mice were retained in a well-ventilated place, humidity 50-70 %, room temperature 24±2°C and 12 hours light/dark cycle was maintained. [12, 13] Study was initiated after one week acclimatization of mice under the standard research facility settings. *Nigella Sativa* (kalonji) seeds were obtained from National Agriculture Research Centre, Islamabad. Plant material was certified by National Agriculture Research Centre, Islamabad through proper taxonomical rules. *Nigella Sativa* seeds were grounded into fine powder in electrical grinder. Suspension was made by adding glucose water into the fine powder. 500mg seeds powder was added to 5ml glucose water equivalent to 100mg of seed powder per ml for group C mice (A dose of 500mg/kg was administered to each mouse in group C). One gram seed powder was added to 5ml glucose water equivalent to 200mg of seed powder per ml for group D mice (A dose of 1000mg/kg was administered to each mouse in group D). [14] 500mg (research grade salt of Pyrazinamide) was dissolved in 5ml glucose water equivalent to 100mg of drug per ml (A dose of 500mg/kg was administered to each mouse in group B, C and D). The powder of Pyrazinamide was dissolved completely to obtain a homogenized solution. The dose of Pyrazinamide was calculated for body weight of mice.[14, 15] Glucose water suspension of Pyrazinamide was prepared in an amount of 500 mg/kg body weight (i.e 20 mg of research grade salt of Pyrazinamide was dissolved in 0.5ml glucose water) and was administered once daily to mice in group B for 6 weeks through a gavage tube and syringe. Glucose water suspension of *Nigella sativa* was used in a dosage of 500 mg/kg body weight in the group C mice (20 mg *NS* seeds powder *was* dissolved in 0.5ml glucose water) along with 500mg/kg body weight of Pyrazinamide(i.e 20 mg of research grade salt of Pyrazinamide was dissolved in 0.5ml glucose water) and in dosage of 1000mg/kg body weight in group D(40 mg *NS* seeds powder *was* dissolved in 0.5ml glucose water) along with 500mg/kg body weight of Pyrazinamide(i.e 20 mg of research grade salt of pyrazinamide was dissolved in 0.5ml glucose water) given once daily to mice for 6 weeks by means of a gavage tube and syringe.[14] Blood samples of mice from all groups were taken three times (baseline, at the end of 3 and 6 weeks). Blood samples of 2 mice from each group were collected at day 0 for baseline liver enzymes (ALT, and AST) and of 5 mice from each group at day

21 for evaluation of progress of research. Finally a blood sample of 10 mice from each group was taken at day 42 for final evaluation of liver enzymes.[16] Intracardiac blood sampling technique was utilized under Chloroform anesthesia to acquire the blood and further biochemical assay measurement. After centrifugation at 3000 rev/min for 10min serum was separated from blood. Bench top centrifuge was used for this purpose. Serum was kept at -20°C until biochemical assay measurement took place.[17] Serum ALT and AST were determined by using ALT and AST kit by (Merck) on Chemistry Analyzer, Micro lab 300 (Merck) by IFCC method.

Statistical analysis was done using SPSS version 21 to analyze the data. Mean ± S.D was calculated for ALT and AST. One way ANOVA was used to evaluate mean difference between control and experimental groups. Post Hoc Tuckey test was applied for the comparison of mean difference between groups. $p<0.05$ was considered significant.

Results

The levels of serum ALT and AST in various animal groups and values of their mean, S.D and Post Hoc comparisons between the groups is given in tables I - VI. Pyrazinamide treatment for 6 weeks has significantly increased levels of ALT and AST in group B. Nigella sativa seeds administration at low (500mg/kg body weight) and high dose (1000mg/kg body weight) along with pyrazinamide for 6 weeks was reduced (P<0.05) the elevated levels of ALT and AST in group C and D respectively.

Table I: Description of Mean and Std. Deviation of ALT in all Groups at day 42

Groups	Minimum	Maximum	Mean	Std. Deviation
A	14	38	27.40	7.291
B	56	124	82.80	21.883
C	29	55	38.40	7.891
D	23	51	36.70	8.179

Table II: Comparison of serum ALT amongst different Groups at the end of experiment by one way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	18440.275	3	6146.758	37.187	.000
Within Groups	5950.500	36	165.292		
Total	24390.775	39			

Table III: Post-hoc Comparison of Total ALT between the Groups Tukey HSD Dependent Variable: ALT (U/L)

(I) Experimental Group	(J) Experimental Group	Mean Difference (I-J)	Sig.
1 Group A	2 Group B(Z)	-55.400(*)	.000
	3 Group C	-11.000	.241
	4 Group D(High dose herb+ Z)	-9.300	.382
2 Group B(Z)	1 Group A	55.400(*)	.000
	3 Group C	44.400(*)	.000
	4 Group D(High dose herb+ Z)	46.100(*)	.000
3 Group C	1 Group A	11.000	.241
	2 Group B(Z)	-44.400(*)	.000
	4 Group D(High dose herb+ Z)	1.700	.991
4 Group D(High dose herb+ Z)	1 Group A	9.300	.382
	2 Group B(Z)	-46.100(*)	.000
	3 Group C	-1.700	.991

* The mean difference is significant at the .05 level.

Table IV: Description of mean and std. deviation of AST in all Groups at day 42

Groups	Minimum	Maximum	Mean	Std. Deviation
A	28	70	48.30	15.011
B	74	159	103.00	28.063
C	44	87	67.40	12.721
D	32	71	54.40	14.683

Table V: Comparison of serum AST amongst different Groups at the end of experiment by one way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17981.075	3	5993.692	17.244	.000
Within Groups	12512.900	36	347.581		
Total	30493.975	39			

Discussion

In our study we observed that oral administration of Pyrazinamide for 06 weeks has significantly elevated the levels of serum ALT and AST in male albino mice. Drug induced hepatotoxicity is a leading health problem with far reaching implications and is the

TableVI: Post-Hoc Comparison of Total AST between the Groups Tukey HSD
Dependent Variable: AST (U/L)

(I) Experimental Group	(J) Experimental Group	Mean Difference (I-J)	Sig.
1 Group A	2 Group B(Z)	-54.700(*)	.000
	3 Group C	-19.100	.119
	4 Group D(High dose herb+ Z)	-6.100	.884
2 Group B(Z)	1 Group A	54.700(*)	.000
	3 Group C	35.600(*)	.001
	4 Group D(High dose herb+ Z)	48.600(*)	.000
3 Group C	1 Group A	19.100	.119
	2 Group B(Z)	-35.600(*)	.001
	4 Group D(High dose herb+ Z)	13.000	.414
4 Group D(High dose herb+ Z)	1 Group A	6.100	.884
	2 Group B(Z)	-48.600(*)	.000
	3 Group C	-13.000	.414

***** The mean difference is significant at the .05 level.

most common cause of termination of drug development programs.[18] More than 900 drugs have been implicated with this side effect.[19] Liver is pivotal to all detoxification processes and adversely targeted by administration of Pyrazinamide.[20] Present study was channeled in order to explore the hepatoprotective effect of seeds of *Nigella sativa* in Pyrazinamide induced hepatotoxicity. Our study revealed that glucose solution of Nigella sativa has marked hepatoprotective activity ($p < 0.05$) in a dose dependent manner. High dose of *Nigella sativa* (1000mg/kg) lowered level of ALT and AST in group D to a greater extent as compared to low dose (500mg/kg) given in group C. Combined administration of 1000mg/kg of Nigella sativa with PZA significantly prevented the elevation of ALT and AST as compared to drug treated group B. Current study is in accordance with multiple studies which proved hepatoprotective effect of *Nigella sativa* oil or the *Nigella sativa* seeds aqueous suspension against several hepatotoxins.[21,22,23] Thymoquinone is an active constituent of *Nigella sativa* that is proven for bearing hepatoprotective activity.[9]

The antioxidant, anti-angiogenesis and anti-inflammatory properties of *Nigella sativa* also play a part in its hepatoprotective role.[11] It is proven that harmful effects of ischemia reperfusion injury of liver are relieved by Nigella sativa.[24] Also its administration protects liver tissue from harmful properties of toxic chemicals and metals.[25]

Our results clearly show that Nigella sativa is hepatoprotective which is indicated by changes in hepatic enzymes. This result is greatly supported by data given in literature.[14,26] Our study shows the hepatoprotective effects of Nigella Sativa. However we recommend search for the hepatoprotective effect of different components of this herb to find the most active component which can further provide a lead for the development of a hepatoprotective drug.

Conclusion

Concurrent administration of *Nigella sativa* seeds with pyrazinamide produce hepatoprotective effect thereby preventing hepatotoxicity caused by pyrazinamide.

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ORIGINAL ARTICLE

The Obesity: A Risk to Iron Deficiency

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ABSTRACT

Objective: To determine association between iron deficiency and obesity in twin cities of Pakistan.

Study Design: Observational Cross sectional Study.

Place and Duration of Study: The study was carried out in Islamic International Medical College and Railway General Hospital Rawalpindi. The duration of the study was one year (April 2017 to March 2018).

Materials and Methods: Eighty obese, eighty overweight and eighty normal weight healthy individuals were recruited. 5ml blood was collected. Blood complete picture, serum iron levels, total iron binding capacity, transferrin saturation and serum ferritin levels were performed. Data was collected and analyzed on SPSS version 21.

Results: Sixty seven (84%) obese adults had iron deficiency out of which 38 (48%) had iron deficiency state and 29 (36%) iron deficiency anaemia. 64 (80%) overweight adults had iron deficiency out of which 46 (57%) had iron deficiency state and 18 (23%) iron deficiency anaemia. 50 (62%) normal weight had iron deficiency among those 33 (41%) had iron deficiency state and 17 (21%) had iron deficiency anaemia. Serum Iron and Transferrin Saturation were significantly low in overweight and obese with a p-value of <0.001. Serum Ferritin was significantly in higher diagnostic range among overweight and Obese than the normal weight with a p-value <0.001.

Conclusion: Iron deficiency and iron deficiency anaemia have higher prevalence among obese individuals.

Key Words: *Ferritin, Iron Deficiency Anemia, Iron Deficiency State, Obese, Overweight, Transferrin Saturation.*

Introduction

Obesity is on surge in developing countries. This is due to rapidly changing lifestyle and dietary habits. There is increasing burden of both obesity and under nutrition in these nations.[1] Worldwide obesity has tripled since 1975. WHO states that 13% of the world population is obese which comprises around 0.65 billion people.[2] In Pakistan 5.4% adult population is obese. It indicates that around 7.2 million people are obese. Pakistan ranks 20th in respect to high number of obese population in world.[3] Obesity is associated with a number of morbid conditions which include Diabetes, Hypertension, Cardiovascular disorders, Liver diseases, Psychological disorders and cancers.[4]

On the other hand iron deficiency is a common health problem worldwide.[5] Although iron deficiency is a problem considered linked with under nutrition, it is also considered present in obese individuals. Iron deficiency state is a condition in which there are decreased iron stores in the body but hemoglobin levels are normal for the age and sex of the individual. Iron deficiency anaemia is a condition in which lack of iron stores in the body lead to hemoglobin levels below normal for the age and sex of the individual.[6,7] Iron deficiency results in a number of health problems. These include impairment of cognitive abilities and memory, stunted growth and development in children, increased risk to pregnancy and related complications which include prematurity and fetal growth retardation, a fall in work capacity and increased risk to cardiovascular disorders.[8,9] Prevalence of anaemia in Pakistan is 30%. Half of this anaemia is due to iron deficiency.[5]

Amongst causes of iron deficiency anemia; inadequate diet, malnutrition, increased demand in growing children and females during reproductive

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period of life, increased blood loss in old males and postmenopausal females are well documented.[10,14] The association of obesity with iron deficiency anaemia is well documented in various studies conducted in developed nations.[15] The data available on this subject in developing countries does not highlight this strong association of obesity with iron deficiency.[15] Scanty data is available from the developing countries. To the author's knowledge, no such study has been conducted in Pakistan. This study has been conducted to determine association between obesity and iron deficiency in our set up where both obesity and iron deficiency are common.

Materials and Methods

It was an observational cross sectional study. The study was carried out in Islamic International Medical College and Railway General Hospital Rawalpindi. The duration of the study was one year (April 2017 to March 2018). It included 80 obese (BMI 30 and above), 80 overweight (BMI 25-29.9) and 80 normal BMI individuals (BMI 18.5-24.9). The sampling technique was Non Probability Convenient sampling. The study was approved by the Ethical Review Committee Riphah International University Islamabad. Inclusion Criteria included: Apparently healthy individuals with age between 18-65 years. Exclusion Criteria included: Subjects with BMI below 18.5 kg/m^2. Subjects suffering from any bleeding disorders. Pregnant and lactating women and individuals having any acute or chronic infection or inflammatory disorder.

Healthy adults were recruited which included patient attendants in the wards and OPD, nursing staff, nursing students, lab technicians, technician students, workers and doctors. Informed consent was taken. Demographic Data was collected which included name, age gender and ethnicity. Each individual was allotted a separate number. Height of the individuals was measured in centimeters and converted into meters. The weight was measured in kilograms. BMI was measured using the formula: Weight in kilograms/ height in meter square

Detailed medical history was taken regarding drug intake, blood loss, pregnancy, lactation, thyroid disorder, and to rule out any acute or chronic disease in the individuals.

Subjects were seated comfortably on a chair. 5ml blood was collected from ante cubital vein by applying the tourniquet 3 to 4 inches above the puncture site. After cleaning the area with 70% isopropanol in circular motion from inward to outward. The needle was inserted at the arm surface at 15-30 degree angle. Tourniquet was removed and sample bottles were filled. Needle from the patient arm were removed and gauze was placed on puncture site. 2ml blood was filled in EDTA vial and 3ml in serum vials. CBC was performed immediately. Serum samples, after centrifugation at 4000gx for 10 minutes, were stored at -20^{o}C until tested.

Blood counts and red cell indices were measured on Sysmex XP100 from EDTA anti coagulated blood using StromatolyserWR (500ml) and cell pack (20ml) provided by Sysmex Asia Pacific Pte Ltd. It was stored at 2-8 ^{o}C.

Serum Iron was quantitatively measured by Ferene Method on MERCK micro lab 300, Netherlands using Human kits. TIBC was quantitatively measured by Precipitation Method on MERCK micro lab 300, Netherlands using Randox kits. Serum Ferritin was quantitatively measured by ELISA technique on 96 Plate Reader, Netherlands using Human Kits.

Statistical analysis was done using SPSS version 21. Mean ±SEM were given for quantitative variables. Chi-square was used for analyzing Iron deficiency state and Iron deficiency anaemia in three BMI groups and between genders. One way ANOVA (Analysis of Variance) and independent sample t test were used to analyze Serum Iron, Serum TIBC, Serum Ferritin and Serum Transferrin saturation. The percentage and number of individuals were calculated using cross tabs. A *p* value of less than 0.05 was considered significant.

Results

A total of 240 individuals were studied. 80 individuals were obese, 80 were overweight while 80 were in normal BMI range. There were 60 (75%) females in each group and 20 (25%) males in each BMI group. There were 20 individuals with age range between 18-29 years, 24 in the range of 30-41years, 18 in range of 42-53 years and 18 in range of 54-65 years in each group.

The mean hemoglobin, serum iron, serum TIBC, transferrin saturation and serum ferritin in all groups are shown in the Table I The frequency of iron deficiency, iron deficiency state and iron deficiency anaemia are given in Table I.

Table I: Comparison of the Frequency of IDA and IDS in different BMI Groups

BMIKg/m^2	Iron Deficiency State		Iron Deficiency Anemia		Total
	Number	P Value*	Number	P Value*	
18.5-24.9	33(41%)		17(21%)		80
25.0-29.9	46(57%)	0.04	18(23%)	0.8	80
30 and above	38(48%)	0.426	29(36%)	0.036	80

P-value versus 18.5-24.9

33 out of 80 were in iron deficiency state and 17 were in iron deficiency anaemia in normal weight individuals. Among overweight there was a significantly higher number of individuals in iron deficiency state with a p value of 0.04. In obese individuals the number of subjects with iron deficiency anaemia was significantly higher with a p value of 0.03. (Table)

Hemoglobin level <12 in females and <13 in males represent anaemia. Normal serum iron is 37-145ug/dl in females and 59-148ug/dl in males and TIBC is 259-388ug/dl (46.4-69.5umol/l). Normal Transferrin Saturation is 20% to 50%. Serum ferritin ranges from 30-300ng/ml in normal individual. In setting of inflammatory disease, ferritin levels below 100ng/ml represent absolute iron deficiency.

Mean serum iron was significantly lower in the obese group with a p value of <0.001. Serum transferrin saturation was significantly low in the overweight and obese groups with a p value of <0.001. Serum ferritin levels were significantly on the higher side of diagnostic range in overweight and obese individuals with a p value of 0.002 and <0.001 respectively. Hemoglobin and TIBC were not significantly affected in overweight and obese subjects. (TableII)

Table II: Comparison of Hemoglobin and Serum Iron profile in three BMI Groups

Parameters		BMI: 18.5-24.9	BMI:25-29.9	P value	BMI:30and above	P value
Hemoglobin (g/dl)	Mean		12.98 ±1.71	0.20	13.00 ±1.78	0.56
Serum Iron (ug/dl)	Mean	69.730 ±35.5	54.540 ±34.4	0.17	44.28 ±35.50	<0.001
Serum TIBC (ug/dl)	Mean	299.8 ±99.96	305.7 ±94.63	0.27	312.68 ±90.9	0.068
Transferrin Saturation (%)	Mean	27.80 ±20.29	18.83 ±11.89	<0.001	15.04 ±7.85	<0.001
Serum Ferritin (ng/ml)	Mean	28.03 ±40.90	30.06 ±62.88	0.002	70.71 ±90.79	<0.001

Discussion

In the present study, Iron deficiency state was present in 41% normal individuals, 57% overweight and 48% obese. Iron deficiency state was significantly higher among overweight individuals with a p value of 0.04. A study conducted on 321 children and adolescents showed that iron deficiency was higher among overweight and obese children with a p value of <0.001.[16] Another study conducted on Sudanese women showed that iron deficiency increased with the increasing BMI in pregnant women with a p value of 0.015.[17]

Iron deficiency Anaemia was 21%, 23% and 36% among normal, overweight and obese individuals, respectively in this study. The p value was 0.036 showing significant increase with increasing BMI. A study conducted on 421 adolescent Iranian girls showed that iron deficiency anaemia was significantly higher among overweight and obese girls than normal weight. The frequency was 27%, 28% and 36% among normal, overweight and obese girls respectively.[18] Another study conducted on 118 obese and 57 normal weight children showed that iron deficiency anaemia was significantly higher among obese children with a p value of <0.001.[19]

In our study mean serum iron and transferrin saturation were significantly lower in overweight and obese individuals while serum ferritin levels were significantly on the higher side of diagnostic range. Low serum iron levels in obese were first reported when Wenzel et al found significantly low serum iron levels in obese patients.[20] A study conducted on 234 obese and 172 non obese adolescents in Washington DC showed that high BMI was associated with significantly low serum Iron levels with a p value of 0.002 while serum ferritin was higher obese with a p value of 0.009.[21] Low transferrin saturation is mentioned in a number of studies conducted on obese individuals. A study carried out on 35 obese and 35 non obese children showed that obese children had significantly low transferrin saturation than non-obese children with a p value <0.05.[22] Another study conducted on 50 obese and 50 non obese Egyptian children showed that serum iron and transferrin saturation was significantly low while serum ferritin levels were significantly higher among obese children.[23] Other studies conducted on obese individuals in developed countries have also shown similar results.[24,25]

Higher levels of serum ferritin can be explained by the fact that ferritin is an acute phase reactant and its levels rise in settings of inflammation.[26] Adipose

tissue releases inflammatory cytokines including interleukin-1, IL-6 and TNF.[27] It creates a proinflammatory environment resulting in increased levels of ferritin in circulation.[28]

Iron deficiency in obesity can be explained by a number of factors which include dilutional hypoferremia, increased basal loses of iron, intake of iron poor diets and decreased absorption in obese individuals due to higher hepcidin levels. [24,27,29]

It is evident from the present study that iron deficiency state is highly prevalent among obese and overweight individuals and should be focused and corrected at an earliest to save the patient from undue complications. It is a known fact that complications set in iron deficiency state before progression to the stage of iron deficiency anaemia. These include impairment of cognitive abilities and memory functioning as well as result in stunted growth in children.[6,26]

The importance of obesity is almost parallel to other causes of iron deficiency anaemia as mentioned earlier. To overlook this important cause of iron deficiency will result not only in aggravation of signs and symptoms of iron deficiency anaemia but also will add to failure in achievement of satisfactory management of this common ailment of our society. The diagnostic workup of iron deficiency should include complete iron profile with a view to consider raised diagnostic value of serum ferritin levels in obese as compared to normal individuals. The management should include iron supplementation along with weight reduction.[30,31]

Further studies can be conducted including C-reactive proteins and serum hepcidin levels to better understand the pathophysiology of iron deficiency in obesity.

Conclusion

Iron deficiency is significantly associated with obesity. It should be considered in all age group obese and overweight patients visiting OPD clinics or hospital departments with or without symptoms of anaemia.

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ORIGINAL ARTICLE

Comparative and Combined Synergetic Effects of Black Coffee and Metformin in Treatment of Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To determine the comparative and combined synergetic effect of Black Coffee and Metformin in treatment of type 2 diabetes mellitus in mice model.

Study Design: Experimental, Randomized control study.

Place and Duration of Study: This study was conducted over a period of one year from May 2017 to April 2018 at Pharmacology laboratory and Multidisciplinary laboratory of Islamic International Medical College in collaboration with National Institute of Health, Islamabad.

Materials and Methods: A total of 50 male Balb/c albino mice were taken, group 1 was non-diabetic normal control (n=10) and diabetes was induced in experimental group (n=40) by using low dose streptozotocin (40mg/kg). After confirmation, diabetic mice were further divided into four groups (10 mice/group). Group 2 was diabetic control and remaining 3 groups were treated with black coffee, metformin and combination of both, respectively for 45 days. Blood samples were taken by intracardiac puncture for HbA_{1C}, which shows the long-term control. Statistical analysis was done applying SPSS 21. Comparisons of means of HbA_{1C} between the groups were analyzed using one way ANOVA (post hoc tuckey test). P value of <0.05 was considered significant.

Results: Black coffee treated (Group 3), metformin treated (Group 4) and combination of black coffee and metformin treated (Group 5) had significantly decreased serum HbA_{1C} levels in comparison with those found in diabetic control (Group 2) (P<0.05).

Conclusion: Combination of black coffee and metformin significantly decreases serum HbA_{1C} levels in diabetic mice as compared to metformin or black coffee treated diabetic mice separately.

Key Words: *Diabetes Mellitus, HbA_{1C}, Metformin, Pancreatic Islets Cells*

Introduction

Diabetes mellitus is spreading worldwide pandemic that is a raising wellbeing concern with numerous difficulties and an expanding ubiquity.[1] Despite stunning change over both essential and clinical therapeutic sciences, diabetes mellitus is at present a hopeless life-long sickness, rapidly affecting both genders.[2] Diabetes mellitus may be not a single illness rather an aggregation of metabolic issue connected with secondary harm on various organ framework that incorporate cardiovascular diseases, stroke, nephropathy, retinopathy, neuropathy, gangrene and even amputations.[3,4] Diabetes mellitus affects 382 million people globally.[5,6] The current prevalence of this disease in Pakistan is 11.77% according to a study conducted in 2015.[7] The pandemic of type 2 diabetes mellitus has been met by emerging approach and clinical tactics, including the generally-accepted commendation to institute drug therapy concomitant with lifestyle changes.[8,9] Metformin an older and broadly acknowledged prime agent, has antihyperglycemic properties and also other important functions such as enhancement in endothelial dysfunction, hemostasis and oxidative stress, insulin resistance, lipid profiles, and fat redistribution.[10] Metformin's efficacy, security profile, beneficial cardiovascular and metabolic effects and its capacity to be associated with other antidiabetic agents makes this drug the first glucose lowering agent of choice when treating patients with type 2 diabetes.[11,12] Even the most explored out oral antidiabetic drugs sometimes fail as monotherapy and eventually different drug combinations are to be

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considered.[10,13] Many new trends in management of diabetes are therefore being considered nowadays and the approach for diabetes treatment is moving towards the incorporation of more organic natural products which tend to counter the root causes of the disease.[14,15] Black coffee is the most commonly used energy boosting beverage worldwide. Recent studies have shown many potential health benefits of black coffee in humans.[16] Besides keeping you alarm and wakeful, coffee is the richest and intense source of antioxidants that work as meager warriors battling and securing against free radicals inside human body.[17]

Caffeine in black coffee regulates the hyperglycemic effect in diabetic patients by increasing insulin release from pancreatic beta cells by the sensitization of the ryanodine receptor and activation of 5'-adenosine monophosphate–activated protein kinase (AMPK). Caffeine also up regulates the insulin-like growth factor 1 signaling, which is responsible for enhanced insulin sensitivity as well as insulin secretion. Chlorogenic acid has an eloquent role in glucose metabolism by decreasing glucose output in the liver and promoting the synthesis of the "homeodomain transcription factor IDX-1", which directs beta cells to counter the increased glucose levels in plasma. Important antioxidants in coffee include hydrocinnamic acids and polyphenols, Hydrocinnamic acids are very effective at neutralizing free radicals and preventing oxidative stress. Polyphenols counter the increased insulin resistance and escalates the insulin sensitivity.[18,19,20,21]

The cumulative body of suggestion about lower frequency of diabetes among coffee users is conclusive enough to prove a positive impact of coffee consumption on the development of type 2 diabetes mellitus. Different studies have been conducted to evaluate the preventive role of black coffee on type 2 diabetes.[22]

To the best of our knowledge, no study has been conducted to explore the effect of black coffee as adjunct to metformin in treatment of diabetes patients. If this agent works to improve insulin sensitivity and decreases the insulin resistance, then this cost effective and easy administered agent with overall beneficial effects on health can be used in place of other antidiabetic agents who are usually given with metformin, when metformin monotherapy fails. This experimental study was done to determine the comparative and combined synergistic effect of Black Coffee and Metformin in treatment of type 2 diabetes mellitus in mice model.

Materials and Methods

This randomized control trial was carried out at Pharmacology laboratory and Multidisciplinary research laboratory at Islamic International Medical College with the collaboration of National Institute of Health (NIH) Islamabad Pakistan. Before starting the study, a formal approval by the Ethics Review Committee of Islamic International Medical College, Riphah International University, was taken. The duration of this study was 12 months (May 2017 to April 2018). A total of 50 healthy, 6-8 weeks old male, weighing 30-50 g albino Balb/c mice were included in the study. All the mice were accommodated in standard cages which were made up of plastic and placed on metallic racks, at the Animal house of NIH, Islamabad. Room number 13 was allocated for the research procedure. The mice had free access to tap water through the inverted bottles of 250ml capacity fixed on top of the cages. These bottles were cleaned and filled on daily basis according to the protocol of the animal house. The normal standard diet was prepared at the NIH, which was served with standard food pellets. Animal house atmosphere was maintained at room temperature of 20 ± 2 oC with relative humidity of 50-70% with a light and dark cycle of 12 hours each. After acclimatization for 1 week, the mice were randomly divided into two groups; 10 mice were allocated to Group 1 and remaining 40 mice were allocated to the Experimental Group. Group 1 was labeled as Normal Control and was given normal diet for 5 days whereas the Experimental group was given normal diet plus streptozotocin, (STZ), (40mg/kg/day)[23] intraperitoneally for consecutive 5 days. After 5 days, confirmation of diabetes in experimental group was done by measuring and comparing fasting blood glucose levels (mg/dl) with Group 1. The blood sample was taken from lateral tail vein of all mice with 1 ml syringe and the blood glucose levels were measured by using EASY GLUCO Ultra Plus Auto Coding meter Iso tech Co. Ltd. Experimental group was then further divided into four groups i.e. 2

(Diabetic control), 3 (Black coffee treated), 4 (Metformin treated) and 5(Combination of Black coffee and Metformin treated).

Group 2 mice were given normal standard diet only. Group 3 mice were given normal diet mixed with Black Coffee (5g/kg/day)[24] orally for 45 days. Group 4 mice were given normal diet along with Metformin (200mg/kg/day)[25] orally mixed in drinking water for 45 days. Group 5 mice were given normal diet mixed with Black Coffee (5g/kg/day) orally and Metformin drug (200mg/kg/day) orally mixed in drinking water for 45 days. After 45 days of treatment, final sampling of the experiment was done from group 3, 4 and 5 which included HbA_{1C} (%) by cardiac puncture. Fixed time nephelometry certified by National Glycohemoglobin Standardization Program (NGSP) was employed for HbA_{1C} estimation (%). For this study, PA50 fully auto specific protein analyzer was used.

Statistical analysis was done by applying the Statistical Package for Social Sciences version 21 (SPSS 21). Results were documented as Mean ± Standard Error of Mean (SEM). Comparison of means of HbA_{1C} (%) among the five groups were analyzed by using the One way ANOVA and Post Hoc Tuckey tests. P value of <0.05 was considered significant.

Results: HbA_{1C} (%)

The result of Mean ± SEM of HbA_{IC} (%)in group 2 (7.82 ± 0.11) was significantly higher than group 1 (P value >0.05) as shown in Table I. While on comparison of Mean ± SEM of HbA_{IC} (%) in group 3 (6.02 ± 0.29), group 4 (5.76 ± 0.45) and group 5 (4.90 ± 0.28) were significantly lower than group 2 (P value <0.05). Table I shows the comparison of mean ± SEM of HbA_{IC} (%) of all the groups.

Table I: Comparison of Mean ± SEM of HbA_{IC} (%) in all five Groups

Groups	Group 1 Control	Group 2 Diabetes	Group 3 Black Coffee	Group 4 Metformin	Group 5 Comb:of Black Coffeeand Metformin
Mean ± SEM of HbA_{1C} (%)	4.75 ± 0.13	7.82 ± 0.11	6.02 ± 0.29	5.76 ± 0.45	4.90 ± 0.28
P value	0.000*				

P value <0.05*

Table II: Multiple Comparison of Mean Difference of Hba1c (%) of Control and Experimental Groups

Groups	Mean Difference	P value
1 vs 2	3.07	0.000*
1 vs 3	1.27	0.020
1 vs 4	1.01	0.102
1vs 5	0.15	0.995
2 vs 3	1.79	0.000*
2 vs 4	2.06	0.000*
2 vs 5	2.91	0.000*
3 vs 4	0.26	0.961
3 vs 5	1.12	0.053
4 vs 5	0.85	0.219

The graphical representation of HbA_{1C} (%) results shows a marked difference between the Black coffee, Metformin, Combination of Black coffee and Metformin as compared to the diabetic control mice. Metformin has a better role in lowering HbA_{1C} (%) than black coffee, yet the role of combination therapy is astonishing in aspect to lower HbA_{1C} (%) nearly to the normal control range.

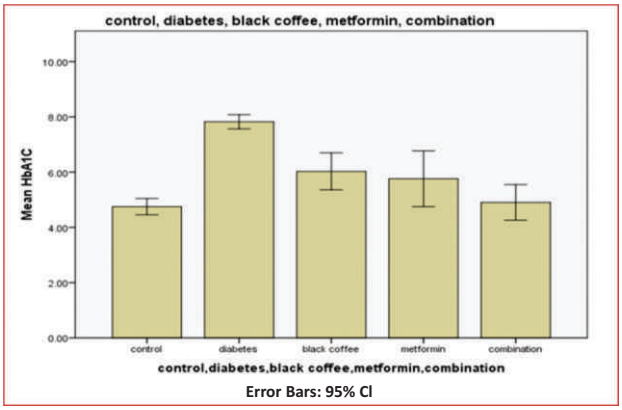

Fig 1: Graphical representation of HbA_{1C} (%) results

Discussion

The results of present study confirm that hyperglycemia induced by streptozotocin, is ameliorated by all the experimental agents to an appreciable extent, yet the result of combination therapy of Black coffee and Metformin is very impressive.

In present study, the antidiabetic effect of Metformin is seen in group 4 and in combination with Black coffee in group 5. Improvement of HbA_{1C} in Group 4 is supported by study of S.H Chung et al., who compared the antidiabetic effect of metformin and compound k in diabetic db/db mice and proposed that normalization of raised plasma glucose levels and improvement in insulin levels in metformin treated group.[26] In present study, improvement in

HbA_{1C} in Groups 3 and 5 along with better improvement in Group 5 which is given combination of black coffee and metformin is observed. Kobayashi M et al., also demonstrated similar results who used black coffee, caffeine extract, decaffeinated coffee against different sets of experiments to analyze the preventive part of black coffee on development of STZ induced diabetes and also the reversal of worsening offered by STZ induced hyperglycemia in male C57 BL/ 6J mice. He demonstrated that continuous Black coffee ingestion prevented the development of STZ induced diabetes mellitus and also revealed that the black coffee can recover the hyperglycemia induced metabolic changes by analyzing the biochemical and histopathological parameters.[27] Mukesh Doble et al., did a study to demonstrate comparative and combined effects of plant phenolic compounds, chlorogenic acid and ferulic acid with metformin and thiazolodinedione on the uptake of 2-deoxyglucose (2DG) by L6 myotubes of rats. He established that a combination of different concentrations of chlorogenic acid and metformin or THZ, has a synergistic effect in the uptake of 2DG with a maximum of 5.0 and 5.3 times respectively, when contrasted to the control. Ferulic acid in combination with metformin or THZ has likewise displayed a synergistic impact and the 2DG uptake increases by 4.98 and 5.11 fold when compared to the control.[28]

Hence when Metformin is given in combination with black coffee, HbA_{1C} which shows the long term control of diabetes is improved demonstrating that black coffee can be used as adjunct to metformin in the treatment of type 2 diabetes mellitus.

Conclusions

Black coffee significantly lowers HbA_{1C} levels in diabetic mice model. Combination of black coffee and metformin significantly decreases serum HbA_{1C} levels in diabetic mice as compared to metformin or black coffee treated diabetic mice separately.

Study limitations

Study should also have involved oral glucose tolerance test, serum insulin level estimation, morphological study of pancreas, and immune histochemistry of histology of pancreas but owing to the cost and availability issue, the above mentioned parameters could not be explored.

Recommendation

- Further explorations need to be directed on active constituents of black coffee and highlight their individual hypoglycemic role.
- The pharmacokinetic properties and interaction of black coffee with other drugs should be studied.
- The comparative and combination therapy of black coffee with modern glucose lowering drugs should be investigated.

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ORIGINAL ARTICLE

Association of Portal Vein Doppler Parameters with Chronic Liver Disease Child Pugh Classes: A Single Center Experience at Rawalpindi, Pakistan

Abdul Ahad Farooq[1], Ahsen Farooq[2], Muhammad Rashid[3], Junaid Haris Farooq[4], Salma Umbreen[5], Rabia Waseem[6]

ABSTRACT

Objective: To determine the association between Doppler Parameters of average peak portal vein velocity and flow direction and Child Pugh classes of patients suffering from the chronic liver disease.

Study Design: It was a descriptive study.

Place and Duration of Study: The study was conducted from December 2013 to January 2015 at the Radiology Department of Holy Family Hospital, Rawalpindi, Pakistan.

Materials and Methods: Selected chronic liver disease (CLD) patients were examined with gray scale and Doppler USG for assessment of portal vein (PV). Average peak portal venous velocity (PVV) and direction of flow in the main portal vein were recorded. Doppler findings were correlated with clinical features and laboratory findings in three classes (A,B & C) of patients using Child Pugh criteria to establish any probable association between them (appendix: I).

Results: Out of total 115 CLD patients studied, 47.8% were in Child Pugh class C. The main portal vein average PVV (cm/sec) in 24.34% patients with Child Pugh class A was 18.75 + 1.88, in 27.82% patients with Child Pugh class B was 14.25 + 0.98 and in 47.82% patients with Child Pugh class C was 8.15 + 1.84. This showed significant fall in portal vein average PVV with advancing Child Pugh class of cirrhosis. Only 10.4% patients showed continuous hepatofugal flow and 4.3% showed bidirectional flow. It was recorded only in Child Pugh class C patients.

Conclusion: Doppler findings of average peak velocity in the main portal vein fall progressively with worsening of the Child Pugh class. In addition, the direction of flow is also reversed in cases of the Child Pugh class C cirrhosis.

Key Words: *Child, Cirrhosis, CLD, Doppler, Direction, Flow, Hypertension, Hepatic, Liver, Portal, Pugh, Transplant, Ultrasound, Vein, Velocity.*

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Introduction

Pakistan has been positioned in intermediate prevalence zone for viral hepatitis B and C that is responsible for >75% of cirrhosis and hepatocellular carcinoma (HCC) in WHO EMRO region.[1] The burden of the disease and its end-stage complications are a huge challenge for the healthcare providers because a majority of patients remain asymptomatic and unaware, leading to a silent epidemic and therefore resulting in significant morbidity and mortality in our country.[2,3] The chronic liver disease leads to many complications such as portal hypertension (ascites, hypersplenism, varices), systemic dysfunction (hypoalbuminemia, coagulopathy), hepatopulmonary and hepatorenal syndromes, encephalopathy, hepatocellular carcinoma, and the notorious cirrhosis which is the common endpoint of a wide variety of CLD processes causing hepatocellular necrosis.[4,5] The World Health Organization (WHO) has defined Cirrhosis as "a

diffuse process characterized by fibrosis and conversion of normal liver architecture into structurally abnormal nodules."[6] The Child Pugh classification system is the most commonly used tool for clinically predicting the prognosis in cirrhosis. Child and Turcotte first presented their criteria in 1964 that was later revised by Pugh in 1973.[7]

Chronic liver disease, especially in asymptomatic patients has been diagnosed mainly by the liver biopsy which helps in its staging and grading.[8] However, the risks and limitations associated with this invasive method have led to the development of safer methods of investigation.

Grayscale USG is now an integral part of the routine liver examination and Doppler studies have been considered as the gold standard tool for evaluating the velocity and direction of blood flow in the portal venous system.[4,9] It is a non-invasive, rapid and cost-effective investigation and well accepted by the patients.[4,10]

In Pakistan, now there are centers offering liver transplant as prospective management of CLD, therefore, use of Child Pugh classification and Doppler studies have become a vital assessment tool. Only a few studies have been conducted evaluating the role of Doppler ultrasound in the assessment of chronic liver disease patients in Pakistan.[2] The aim of this study was to explore the value of Doppler ultrasonography in the assessment of PV hemodynamics in CLD patients and to determine the association between Doppler Parameters of PPV and flow direction and Child Pugh classes of the Pakistani patients suffering from the chronic liver disease.

Materials and Methods

This descriptive cross-sectional study was conducted in the Department of Radiology in collaboration with the Department of Medicine at Holy Family Hospital Rawalpindi. Approval was taken from the hospital ethical committee. Nonprobability convenience consecutive sampling technique was used. Using WHO sample size calculator, the sample size of 115 was calculated by taking a confidence level of 95 % and population mean of 18.33. All the patients of either gender and of age more than 20-year, having clinical, biochemical and radiological evidence of chronic liver disease, presenting to the departments of medicine and radiology from December 2013 onwards was included in the study. Child Pugh classification (A, B and C) was done as per standard criteria by the medical and radiological department.[7] (Appendix 1). Patients having portal vein thrombosis (PVT) were excluded from the study. Informed consent was obtained from all the patients fulfilling the inclusion criteria. Patients were subjected to conventional grayscale and Doppler ultrasound using Toshiba Nemio ultrasound machine equipped with multi-frequency linear and curvilinear transducer probes. The examination was performed or supervised at least by a registrar level radiologist. All the patients were examined preferably in the supine position. During the examination, every patient was required to hold breath in deep inspiration, to avoid motion artifacts. The scanning of the portal vein was carried out longitudinally throughout its entire length from an anterior abdominal subcostal and/or right intercostal approach. It was analyzed for the presence or absence of intra-luminal thrombus, blood flow velocity in cm/s and direction of blood flow within the portal vein. All the findings were reconfirmed by another consultant radiologist.

Data were collected by the principal investigator on the study proforma. Data were entered and analyzed by Statistical Package for the Social Sciences (SPSS) version 22. Descriptive statistics were calculated. The quantitative variable of the study included; average peak venous velocity in the main portal vein compared with different Child Pugh classes A, B & C and expressed as Mean ± SD. The qualitative variable of the study included; the direction of portal vein blood flow compared with different Child Pugh classes A, B & C and expressed as frequency.

Results

A total of 125 CLD patients presented from December 2013 till January 2015. Ten patients were excluded from the study because of either incompletely available medical record or loss to follow up and portal vein thrombosis in 2 cases. Therefore, a total number of 115 CLD patients without PVT were included in this study. The age ranged from 21-77 years with a mean age of 40.6$\pm$11.54 years. Most of them (43, 37.4%) ranged between 31-40 year age group. Males were 70 (60.87%) and females 45 (39.13%) with M:F of 1.6:1 approximately. Majority of patients (47.8%) were in

Child Pugh class C (Table-I). Doppler findings of the average peak venous velocity (PVV) and the direction of blood flow in the main portal vein were evaluated in all the subjects and were compared with Child Pugh classes A, B, and C. The main portal vein average PVV (cm/sec) in 28 (24.34%) patients with Child Pugh A was 18.75 $\pm$ 1.88, in 32 (27.82%) patients with Child Pugh B was 14.25 $\pm$ 0.98 and in 55 (47.82%) patients with Child Pugh C was 8.15 $\pm$ 1.84 (Table-II). Only 17 (14.7%) patients demonstrated non hepatopetal flow (hepatofugal / bidirectional), 12 (10.4%) showed continuous hepatofugal flow and 5 (4.3%) showed bidirectional flow (Table-III). It was recorded only in Child Pugh class C patients.

Table I: Distribution of Child Pugh Classes A, B, and C among the Patients

Child Pugh Class	Frequency	Percent	Valid percent
Child Pugh A	28	24.3	24.3
Child Pugh B	32	27.8	27.8
Child Pugh C	55	47.8	47.8
TOTAL	115	100.0	100.0

Table II: Comparison of Average Peak Venous Velocity in the Main Portal Vein in Child Pugh Classes A, B, and C

Child Pugh Class	Average Pvv	No. Of Patients	Std. Deviation
Child Pugh A	18.75	28	1.878
Child Pugh B	14.25	32	0.984
Child Pugh C	8.15	55	1.840
TOTAL	12.43	115	4.722

Table III: Patterns of Flow in the Main Portal Vein among Child Pugh's Classes A, B, and C.

Child Pugh Class	Hepatopetal	Hepatofugal	Bidirectional	Total
Child Pugh A	28	0	0	28
Child Pugh B	32	0	0	32
Child Pugh C	38	12	5	55
TOTAL	98	12	5	115

Discussion

Accurate evaluation of liver parenchymal damage and altered vascular hemodynamics is crucial for therapeutic decisions, surveillance, and assessment of the prognosis in chronic liver diseases of various aetiologies. Since long, liver biopsy has been considered as the gold standard for diagnosing, staging and grading the liver damage in CLD, especially in asymptomatic patients.[8] This invasive method has several risks and limitations, including morbidity and mortality, sampling error, diagnostic inaccuracy, interobserver and intraobserver variability, and difficulties in follow-up.[11] Therefore, it led to the development of safer methods for evaluation of CLD and its complications. The non-invasive Grayscale and Doppler USG studies have now been considered as an essential part of the investigation battery for assessing the CLD patients.[9,10.] With increasing accessibility of liver transplant facility for the management of end stage CLD world over, the use of Child Pugh classification as a valuable indicator of the prognosis in CLD patients have become vital as well.[9] Combined together imaging, pathological and clinical assessment methods help explore new therapeutic avenues.

The role of ultrasound imaging in the evaluation of CLD / cirrhosis is well known.[4,12,13,14,15] Doppler USG is an important diagnostic modality for the analysis of the hepatic vasculature and blood flow.[9,10,16,17] The standard dynamics of blood flow in the hepatic artery, hepatic veins, and the portal vein, have been well defined in the literature.[4,9,10,15,17] Changes in hepatic blood flow dynamics might be alternate markers for significant parenchymal changes in chronic liver diseases and their complications.[9.10,11]

The Child Pugh classification is mostly used to stage chronic liver disease based on clinical and laboratory parameters that is ascites, encephalopathy, bilirubin and albumin levels, and prothrombin time. There are three stages of the Child Pugh classification system, A, B and C depicting increasing severity of CLD.[7] (Appendix-I)

On Doppler US, the characteristic portal vein hemodynamic features of liver cirrhosis include reducing blood-flow velocity, absent pulsatility, and change in flow direction from hepatopetal to hepatofugal in more advanced cases.[9,10,11,15,16,17] The normal portal vein velocity ranges from 16 to 40 cm/sec with variations caused by respiration.[15,16] Portal venous flow velocities progressively fall with rising portal venous pressure because back pressure limits forward velocity that is characteristic for portal hypertension and also due to collaterals formation,

Appendix I: Child Pugh Classification for Liver Cirrhosis

Parameter	Points assigned		
	1	2	3
Ascites	None	Suppressed with meds	Refractory
Bilirubin	<34 uM	34-50 uM	>50 uM
Albumin	>35 g/L	28-35 g/L	<28 g/L
INR	<1.7	1.7-2.2	>2.2
Encephalopathy	Grade 0	Grade 1-2 or suppressed with meds	Grade 3-4 or refractory
Grade 0: Normal Cognition Grade 1: Euphoria, Fluctuation in level of Consciousness, slurred/disoriented speech Grade 2: Drowsiness, Inappropriate behavior, loss of sphinteric control Grade 3: Marked Confusion, Stupor, incoherent Speech Grade 4: Coma			
Class A	5-6 Points	"Well- Compensated"	
Class B	7-9 Points	"Significant functional impairment"	
Class C	10-15 Points	"decompensated liver function"	

which is caused by cirrhosis in most of the cases.[4,16]

With this background, not many studies have been conducted evaluating the role of Doppler ultrasound in the assessment of chronic liver disease patients in Pakistan.[2] Likewise, few regional studies have examined the association of Doppler parameters of direction and velocity of flow in the main portal vein with the Child Pugh class.[9,18]

Our study has determined that Doppler ultrasonography is a valuable modality for the assessment of portal vein hemodynamics in the Pakistani patients suffering from the chronic liver disease. It has established an association between Doppler findings of average peak velocity and flow direction in the main portal vein and Child Pugh classes of cirrhosis. Doppler findings of average peak velocity in the main portal vein fell progressively with worsening of the Child Pugh class. In addition, the direction of flow was also reversed in cases of the Child Pugh class C cirrhosis.

A study by Mittal, et al published in 2011, have examined the association of Doppler findings of flow velocity and direction in the main portal vein with the Child Pugh class in the Iranian population. This study shows average peak portal venous velocity of 18.33 $\pm$ 2.22,14.59 $\pm$ 3.57 and 10.96 $\pm$ 2.33 cm/s in the Child Pugh class A, B and C respectively and non-hepatopetal flow (hepatofugal or bidirectional) in 12 % of patients which all corresponds to the Child Pugh class C.[9] Afif, et al in 2017, studied the maximum velocities in portal vein, hepatic vein and hepatic artery and hepatic artery resistive index in liver cirrhosis patients of the Singapore population. They found that flattening of the hepatic vein waveforms was related to the degree of liver cirrhosis. The cirrhotic patients showed higher maximum hepatic vein velocity. However, they had lower maximum portal vein velocity and there was a further decrease in average maximum portal vein velocity as the severity of liver cirrhosis worsened.

In our study of 115 Pakistani CLD patients, the main portal vein average PVV was 18.75 $\pm$ 1.88, 14.25 $\pm$ 0.98 and 8.15 $\pm$ 1.84 cm/sec in the Child Pugh classes A, B and C respectively (Table-II). This showed a significant fall in portal vein average PVV with the increasing severity grade of cirrhosis based on the Child Pugh class. The results are consistent with results of Chawla et al, Shi et al and by Vyaset al studies.[19,20,21]

Normal individuals display a hepatopetal portal venous blood flow.[15,16,17] In deteriorating CLD, portal venous flow progressively slows down with rising portal venous resistance and pressure, first nearing the level of stagnation demonstrated by the phenomenon of a to-and-fro (bidirectional) flow.[16,17] Later with advancing cirrhosis, obstruction of the hepatic venules and sinusoids due to fibrosis and architectural distortion, and further by arterioportal and porto-systemic shunting, eventually leading to hepatofugal (reversed) portal venous flow and fall in velocity.[4,16,17]

In our study, only 4.3% patients showed bidirectional flow and 10.4% showed continuous hepatofugal flow (Table-III). It was recorded only in the Child Pugh class C. None of the patient in the Child Pugh class A and B demonstrated hepatofugal flow. Gaiani et al have reported the similar patterns.[22] It implies that with more reversal of the portal venous flow, there is increased deterioration of liver function and hence poorer the Child Pugh score.

Despite the single center's small study and some limitations of the final diagnoses and follow up, the results of our study are by and large supported by the various international studies. This study imparts a deeper insight into the non-invasive and cost-effective mode for the assessment of progression

and subsequent management of the patients suffering from the deadly disease of chronic liver disease in Pakistan.

Conclusion

Doppler ultrasonography is a valuable modality for assessing the complex hemodynamics of hepatic vasculature (portal vein in our study), in patients with chronic liver disease and its progression as well. Doppler findings of average peak velocity in the main portal vein fall progressively with worsening of the Child Pugh class. In addition, the direction of flow is also reversed in cases of the Child Pugh class C cirrhosis. The findings of our study are in line with other published studies.

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ORIGINAL ARTICLE

Caries Susceptibility of Proximal Surfaces in Permanent First Molars: A Cross Sectional Survey

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ABSTRACT

Objective: To report the caries frequency of proximal surfaces of permanent first molars.

Study Design: Descriptive study.

Place and Duration of the Study: Department of Operative Dentistry, Dow Dental College, Dow University of Health Sciences Karachi. One month from 1st October 2015 to 31st October 2015.

Materials and Methods: The study was conducted on retrospective data of 374 patients presenting to the department of Operative Dentistry, Dow University of Health Sciences. Diagnosis of dental caries was made on selected periapical radiographs using criteria provided by Senel. Caries data and other independent variables e.g. age; gender, arch and side were analyzed with SPSS for windows version 17. Chi square test was used to measure association between proximal caries and qualitative variables such as gender, age group and tooth group.

Results: There were more mesial proximal lesions (56.4%) than distal lesions (43.6) in all age groups except for 41-50 years and above 50 years (p=0.109). When maxillary and mandibular teeth were compared with proximal surfaces, mesial surface of maxillary first molar was found to be more carious statistically (p=0.000326)

Conclusion: Mesial surface of maxillary permanent first molar was associated with increased risk for dental caries.

Key Words: *Caries Diagnosis, Dental Caries, Permanent First Molar, Mesial Proximal Surface*

Introduction

Permanent first molar due to its early eruption and posterior location is most caries prone and most treated tooth in dentistry.[1,2] A different caries risk has been associated with its different surfaces. A study from USA reported a higher caries incidence for buccal and occlusal surface as compared to proximal.[1] Hopcraft reported a possible association of age with site of tooth, occlusal caries been more common in young while proximal caries was observed more in older subjects.[2] Similarly, early diagnosis of caries in these teeth is of paramount importance. For occlusal and smooth surfaces, only visual clinical examination might be enough.[3] Proximal surfaces on the other hand, are more likely to be under-diagnosed even with radiographs.[4-6] previous caries experience especially in early childhood might also be used to assign a higher risk of developing proximal caries.[7] The proximal surfaces of first molar therefore, must be thoroughly examined and accurately diagnosed. It has been reported that proximal decay is the most common reason for endodontic therapy in this tooth.[8,9] Site specific studies have been published internationally reporting a different caries incidence for proximal and smooth surfaces of permanent first molar. Lith reported an increased incidence of dental caries in mesial surface of first molar.[10] Distal surface of first molar was found more at risk in a study that reported caries risk in proximal surface according to eruption order.[11] A Swedish longitudinal study on adolescents also found more caries incidence on distal surface.[12] In contrast, proximal surface of first molar was not considered at more risk in another study.[1] Although there is abundant caries data on local population, site specific studies have not been conducted so far.[13,16] Few studies reported caries incidence, however data on specific sites was pooled and tooth

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level site specific data was not provided.[17,18] It was therefore, the objective of this study to report the site specific caries data for proximal surface of permanent first molar. The aim of the study was to evaluate the caries frequency of mesial and distal surfaces of permanent first molar.

Materials and Methods

This descriptive study was undertaken at the Department of Operative Dentistry, Dow Dental College. The study was based on retrospective patient data stored in the records of the Department. Duration of study was one month, during the month of October 2015. Ethical approval for the study was obtained from the institutional review board (IRB-585/DUHS/Approval/2015/110). The sample size for this study was calculated at 95% confidence level and 80% power, a sample size of 362 was calculated(62% occurrence of class 2 lesions).[19] Sample size calculation was made by using Open Epi version 2 (open source calculator SS-proper). We included 374 permanent first molars in our study by consecutive sampling method based on following criteria. We included permanent First molars in either arch, teeth with primary proximal decay and radiographs of acceptable quality. Cases with more than one carious first molar were also included. The exclusion criteria included teeth with secondary carious lesions and a tooth with both the proximal surfaces involved. Selected radiographs were viewed under standard conditions. An illuminator was used to view and score selected radiographs. A calibrated examiner made all the readings. The radiographic diagnosis of dental caries was made according to the criteria modified from Senel, where 0 was scored for no caries and 1 for caries confined to enamel or involving dentin.[20]

A specially designed proforma was used to collect the data. Data analysis was performed with SPSS version 17 for Windows. Mean and standard deviation of qualitative variable like age and percentages of qualitative variables e.g. gender and proximal surface were calculated. Chi square test was used to determine Association between the two proximal surfaces and categorical variables like gender, age group and tooth group was determined with chi square test at 95% of significance.

Results

The current study included 374 permanent first molars based on our inclusion and exclusion criteria. The basic descriptive details of our sample are summarized in table no. I. The mesial surface was found to be carious (n=211, 56.4%) more than the distal surface (n=163, 43.6%). The association of proximal carious surface to the age groups (p=0.109) and gender (p=0.423) was found to be insignificant (Table No. II). The percentage of mesial and distal surfaces was found to be equal for <20 age group, where-as for age group 20-40 mesial surface had an increased occurrence. However, age group 40-50 had both surfaces equally affected and in >50 age group an increased percentage of distal carious lesions was found (Graph no. I). The association of proximal decay on mesial surface of maxillary permanent first molar was found to be significant (p=0.00032) (Table No. III).

Table I: Basic Demographic Data

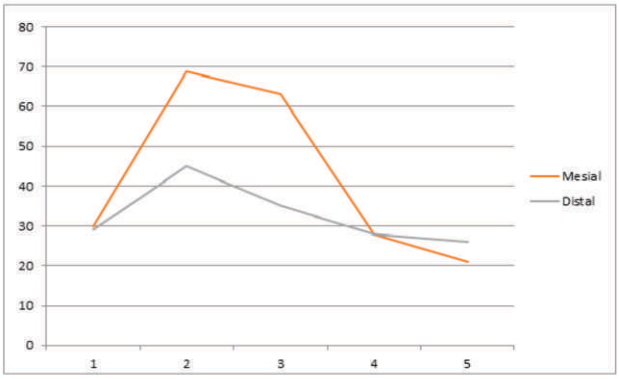
	Frequency	Percentage	Mean ± SD
Age			**34.36±13.39**
Gender Male	174	46.5	
Female	200	53.5	
Age Group	59	15.8	
<20	114	30.5	
21-30	98	26.2	
31-40	56	15.0	
41-50	47	12.6	
>50			
Proximal Surface	211	56.4	
Mesial	163	43.6	
Distal			
Tooth	85	22.7	
Maxillary Right	97	25.9	
Left	100	26.7	
Mandibular Right	92	24.6	
Left			

Table II: Association of Proximal Caries Frequency with age

		Site		Total	P Value
		Mesial	Distal		
Age Groups	1	30	29	59	0.109
	2	69	45	114	
	3	63	35	98	
	4	28	28	56	
	5	21	26	47	
Total		211	163	374	

Table III: Association Of Proximal Caries frequency with Individual Tooth

		Site		Total	P Value
Tooth No		**Mesial**	**Distal**	**Mesial**	0.00032
	Right Maxillary First Molar	55	30	85	
	Left Maxillar First Molar	68	29	97	
	Left Mandibular First Molar	41	51	92	
	Right Mandibular First Molar	47	53	100	
Total		211	163	374	



Graph No. 1 Relationship between Age Groups and Proximal Caries

Graph No.1 Relationship between Age Groups and Proximal Caries

Discussion

We included data of 374 permanent first molars in our cross-sectional study. We found carious lesions more frequently on mesial proximal. Also mesial surface of maxillary first molar was found to be more carious statistically. However a reverse trend was observed for age group of >50 where distal surface was found to be affected more. Moreover, the association of proximal decay with age and gender was non-significant. DMFT score of Pakistan is high. In 12 year olds it has increased from 1.2 in 1998 to 1.6.[14] Therefore, it is important to be aware of local demography before one can draw any conclusions or make comparisons with other studies. Our study was the first of this kind that was done locally. Although local caries burden is known, site specific study on permanent first molar was lacking.[13,14] Our results differ from studies reported from other parts of the world. Regional variation in rate and pattern of dental caries may be responsible for these differences. Our main finding that mesial surface of first molar may be more caries susceptible agrees with Lith.[10] This longitudinal study followed patients from 6 years of age till age 20, and found this condition remained at an elevated level throughout the period of study. They also found a high frequency of carious and restored distal surface of second premolar, a finding which may partly explain a high caries rate on mesial surface of first molar. This was one of a limitation of our study that we did not consider the caries status of adjacent teeth. Bachelor on the other hand reported buccal pits and occlusal fissures in first molars to be most caries susceptible, while proximal surfaces were found to be less prone.[1] Better access to health care facilities, a regular checkup and fluoride intake may be responsible factors for a different disease pattern in this American study. A longitudinal Swedish with a cohort study design found distal surface of first molar to be more caries affected as compared to mesial surface.[12] Since the methodology of this study differed considerably from our study due to its cross sectional study, this fact could help explain the differences in results. A difference in the proximal surface morphology and enamel maturation has been proposed previously as a possible reason for the conflicting result of our and other studies.[21] the mesial surface of maxillary first molar presents a flatter surface with a more buccally placed contact area with a concavity cervical to this contact area. Distal surface on the other hand, has a more convex profile and a wider contact area and a minimal sub contact concavity. This anatomical difference may be responsible for increased plaque retention and caries experience. In contrast, the mandibular first molar lack a concavity and it's both mesial and distal contact areas are broad and much flatter.[22] These observations support our results of significantly greater involvement of the maxillary first molar when compared to the mandibular first molar. Edward and Burchell have proposed another interesting association, that the proximal surfaces of a tooth that erupts earlier and thus exposed for longer periods of time are more susceptible.[11,23] This explanation however don't support the results that were derived in our study. The distal surface of the first molars have a longer exposure time to the oral environment as second molar is one of the last teeth to erupt. Whereas the mesial surface forms a contact with deciduous second molar immediately after

eruption and is therefore less exposed. In a study by Norblad and Larmas no such association was found.[24] In fact the early contact formation could be used to explain the greater risk of mesial surface. Also in case of a carious deciduous second molar, it can inoculate its permanent neighbor with infection. Studies have shown this relationship.[25] This fact combined with a high prevalence of dental caries in local pediatric population supports the results of our study.[14]

Conclusions

We found mesial surface of maxillary first molars with greater caries frequency.

Limitations

We report following limitations of our study

- We did not take the caries status of adjacent tooth into account. This can alter the caries susceptibility and help explain the results better.
- We did not consider the overall oral hygiene status of the patients and this can potentially act as a confounder.
- Our study was a snap shot; a better design may be a longitudinal one which is also a limitation of our study.
- Single calibrated examiner made all the readings and inter-examiner reliability could not be calculated.
- Our results conflict with published data.

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Disclaimer

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ORIGINAL ARTICLE

Establishing Validity of SLICE –As an Assessment Instrument of Long Case

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ABSTRACT

Objective: To establish the content and construct validity of Structured Long Interview and Clinical Examination (SLICE) as an instrument for the assessment of long case.

Study Design: This was a quantitative analytical study.

Place and Duration of Study: The study was conducted at Pakistan Railway General Hospital during the period between March 01, 2016 to August 31, 2016.

Materials and Methods: SLICE is a tool of Long Case assessment, however, it's content and construct validity are not established. Examiners, who had used SLICE for the long case assessment, were requested to fill the questionnaire. The questionnaire contained questions about the relevance and clarity of SLICE. Each examiner individually rated the relevance and clarity of all the items on the SLICE using a five point Likert Scale. Content validity index of SLICE was established for individual items and overall scale. The construct validity of SLICE was determined by factor analysis using principal component analysis method.

Results: Content Validity Index of SLICE (S-CVI), for relevance and clarity was 0.92 and 0.90 respectively. KMO value of SLICE was 0 .655. Bartlett test value of SLICE was 0.00.

Conclusion: The content validity index for overall scale (S-CVI) and construct validity indicates that SLICE is a valid instrument for the long case assessment.

Key Words: *Assessment, Long Case, SLICE, Validity.*

Introduction

The long case examination assess the clinical competency of the medical students with real patients, in real clinical environment.[1] In undergraduate setting, the conventional method for the assessment of long case has many drawbacks. During undergraduate long case examination by conventional method, the student's performance in history taking and clinical examination is un - observed by the examiner.[2] The assessment of the long case examination is also un-structured. The examiners are devoid of the structured checklist and the marking scheme, so the examiners are free to award marks, depending on their personal inclinations, personal will and perceptions about the examinee.[3,4] Another problem is that difficulty of long case is not marked in the assessment. The result of long case assessment depends considerably on difficulty of the clinical problems of the patient allocated to the examinee. If the examinee gets a difficult case with multiple clinical problems, he may fail or get poor grades as compared to the clinically less competent fellow who gets a patient with a single problem.[5,6,7,8]

In Pakistan each class of MBBS, consists of 100-300 students. It is impractical to observe the history taking and clinical examination of every student by the examiner, because this would require a lot more resources in terms of time, faculty involvement and the patients' commitment. The only practical way to hammer the drawbacks, confronted during the long case assessment is to assess the long case by using the structured assessment tool, so that the results are valid, reliable and free of bias. For the structured assessment of long case, many instruments had been designed as OSLER (Observed Structured Long Examination Record), PBAC (Practice Based Assessment of Clerks in internal medicine), and SCCP (Structured Clinical Case Presentation).[9] Similarly SLICE was established for the structured assessment

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of long case, of final year medical students at Islamic International Medical College. The SLICE (ANNEXURE. 1) has 13 items for the long case examination record. The examiners assess the examinee on these 13 items over 15 minutes. The examiner after giving marks for each individual item on the SLICE sheet, calculate the overall marks and takes pass/ fail decisions. Rehan et al established the reliability and face validity of SLICE, however, the content validity and construct validity of SLICE were not established.[10]

The purpose of this study was to determine the content and construct validity of SLICE sheet, which is a new instrument for the structured assessment of the long case.

Materials and Methods

This quantitative analytical study was planned to establish the content validity and construct validity of SLICE. The study was conducted in Pakistan Railway General Hospital, which is a teaching hospital affiliated with Islamic International Medical College. The study was conducted between March 01, 2016 to August 31, 2016. SLICE is already in use for the assessment of long case examination of final year MBBS students at Islamic International Medical College. Examiners who had used SLICE, as an assessment instrument of long case at Islamic International Medical College were included in the study. These examiners were from the Departments of Medicine, Pediatrics, Gynaecology/Obstetrics and Surgery of Pakistan Railways General Hospital. Examiners, who came from other teaching hospitals for the summative assessment of final year MBBS and used SLICE as assessment instrument, were also included in the study. Examiners and faculty, who had never used SLICE for long case assessment, were excluded from the study.

The questionnaire was designed and approved from the ethical committee of Islamic International Medical College. The questionnaire with the covering letter explaining the purpose of the study was distributed among participants by the researcher. The questionnaire comprised of two parts. The sheet 1 of questionnaire comprised of questions about the relevance of SLICE items, on a five-point Likert scale. The five responses were, not relevant, some -what relevant, un- decided, relevant, very relevant. The sheet 2 of questionnaire comprised of questions about clarity of items, of SLICE, on a five-point Likert scale. The five responses were, not clear, some -what clear, undecided, clear, and very clear. Purposely chosen 16 experts, who used SLICE sheet for assessment of long case, were asked to review the 13-items SLICE. Each reviewer independently rated the relevance of each item on the SLICE using a 5-point Likert scale.

Content validity index for the relevance and clarity of individual items and overall scale was determined. The I-CVI was calculated by number of expert giving 4 or 5 rate to the individual items on the scale (4 or 5 rate show relevance of the individual items in the scale under study) and then dividing it by the total number of experts. I-CVI = Number of experts giving a rating of either 4 or 5 to individual item in scale / Total number of experts. The S-CVI was calculated by the following method, S-CVI/Ave as (.90+.90+.90+ .90+.90+.90)/6= 0.90

SPSS version 21 was used to analyze the data. The data was non parametric. The construct validity of SLICE was determined by factor analysis using principal component analysis method.

Results

Relevance

History taking domain of SLICE consists of 03 items. The CVI – I of all three items of history taking domain turned out to be +1 (Table I). Examination domain of SLICE consists of 02 items. The CVI–I of the two items of examination domain was 0.94 and 0.88 (Table I). Defending diagnosis domain of SLICE consists of 02 items. The CVI –I of two items turned out to be 0.94 and +1 (Table I). Investigations domain of SLICE consists of 02 items. The CVI - I of the two items was 0.88 and +1 (Table I). Management domain of SLICE consists of 04 items. The CVI –I of 02 items of management domain was 0.88, the other two items had CVI-I 0.94 and 0.75 (Table I).

Clarity

History taking domain of SLICE consists of 03 items. The CVI –I of one item had value of +1 and the other two had values 0.93 and 0.94(Table I). Examination domain of SLICE consists of 02 items. The CVI –I of both the items was 0.88 (Table I). Defending diagnosis domain of SLICE consists of 02 items. The CVI –I of both the items was 0.94 and+1(Table I). Investigations domain of SLICE consists of 02 items. The CVI –I of both the items had value of +1(Table I).

Management domain of SLICE consists of 04 items. The CVI – I of these four items was 0.81, 0.69, 0.88 and 0.75 respectively (Table I).

S-CVI of SLICE regarding the relevance of SLICE is 0.92 (Table 1). The S-CVI regarding the clarity of SLICE is 0.90 (Table I).

Table I: Content Validity Index of SLICE

ITEMS OF SLICE	*CVI-I Relevance of SLICE SHEET*	CVI-I Clarity of SLICE sheet
HISTORY TAKING		
Presenting complaints in chronological order with Relevant comprehensive, logical history of presenting complaints in orderly manner	1	0.93
Systemic review, Past history , Family history, Socioeconomic history, Allergic, Drug and transfusion history	1	1
Presentation skills	0.94	0.94
Examination		
General Physical Examination	0.88	0.88
Relevant Regional Examination	0.94	0.88
Defending Provisional Diagnosis		
Making a provisional diagnosis and providing relevant points to defend it	0.94	0.94
Providing a list of relevant D/D and excluding them logically	1	1
Defending Investigations		
Suggest and justify relevant routine investigations	0.88	1
Suggest and Justify relevant specific investigations	1	1
Defending Management		
Suggest and justify the appropriate treatment scheme	0.88	0.81
Describe the complications of treatment	0.94	0.69
Describe the follow up plan for the patient	0.88	0.88
Describe Recent advances	0.75	0.75
S-CVI of SLICE	**0.92**	**0.90**

Factor Analysis

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO), measures the sampling advocacy and determines if the responses given are supportive or not. KMO should be close to 0.5 for a satisfactory factor analysis. Kaiser recommended values of 0.9 as superb, values between 0.7 to 0.8 as acceptable and 0.5 as minimum. Table II shows that KMO value of SLICE was 0.655, which is close to acceptable and above the minimum requirement. Barlett test also indicates the strength of the relationship among different variables. The significant is less than 0.05. Table II shows Bartlett test value of SLICE was 0.00, which also shows a strong relationship among the different variables.The Scree plot (Fig 1) is the graphic representation of the Eigen values against all the factors. The graph helps in determination of how many factors to be retained. The point of importance is where the curve begins to flatten. So it can be recognized that the curve starts to flatten between factor 5 and 6. It can be noted that factor 6 onwards have Eigen value of less than 1, so only 5 factors have been retained.

Table II : Kaiser-Meyer-Olkin and Bartlett's Test of SLICE

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.655
Bartlett's Test of Sphericity	Approx. Chi-Square	194.433
	df	78
	Sig.	.000

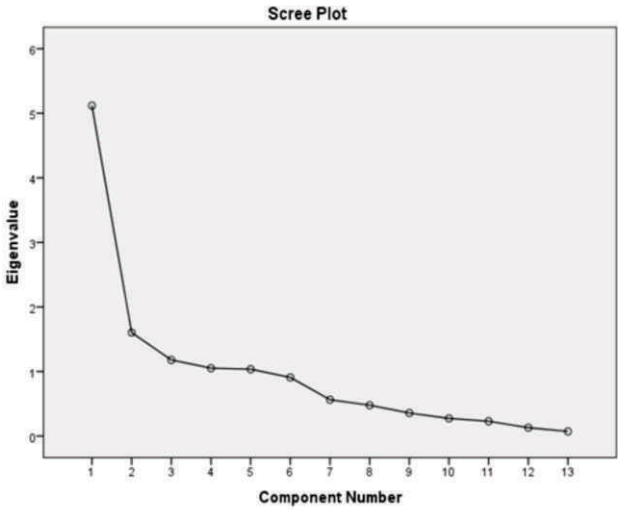

Fig 1: Scree Plot for SLICE

Discussion

SLICE, including 13 items, was designed and used for the assessment of long cases at Islamic International Medical College. It proved to be useful and an appropriate effort to increase the reliability and validity of long case examination. Examiner can generalize the results after objective assessment of fixed number of items of SLICE. SLICE can also be considered examiner friendly as it reminds the examiner to check the same domains for all students.

SLICE is also feasible because it assesses the long case over 15 minutes while many other tools assess the examinee over 20 – 30 minutes.

The CVI – I of individual items regarding their relevance were found to be 88% and more except for one item, which is describe the recent advances. However, CVI – I of this domain is still in the acceptable range i.e.75%. The content validity index of overall scale for relevance was above 92% (Table I). These results strongly determine the relevance of items of SLICE thus endorsing its content validity. The CVI –I of individual items for clarity was 81% and more for eleven, out of thirteen items. These two items were, to describe the complications of treatment and the recent advances. The CVI – I of these two items were also in acceptable range i.e. 69% and 75%. The content validity index of overall scale for clarity was above 90% (Table I). These results strongly prove the clarity of items of SLICE thus endorsing its content validity.

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO), measures the sampling advocacy and determines if the responses given are supportive or not. Table II shows KMO value of SLICE was 0.655, which is close to acceptable and above the minimum requirement. Barlett test also indicates the strength of the relationship among different variables. The significant value is less than 0.05. Table II shows Bartlett test value of SLICE 0.00, which also shows a strong relationship among the different variables. The Scree plot (Fig 1) is the graphic representation of the Eigen values against all the factors. The graph helps in determination of how many factors to be retained. The point of importance is where the curve begins to flatten. So it can be recognized that the curve starts to flatten between factor 5 and 6. It can be noted that factor 6 onwards have Eigen value of less than 1, so only 5 factors had been retained. The results of factor analysis show that different items in SLICE had strong relationship among themselves and significant Eigen values. The results of factor analysis shows that the SLICE had good construct validity.

Rehan et al established the reliability and face validity of SLICE, however, the content validity and construct validity of SLICE were not established. SLICE had good face validity and the reliability of the SLICE had been found to be 0.87.[10] The results of present study show that SLICE had good content and construct validity. Considering the present results and those contributed by Rehan et al study,[10] SLICE turned out to be valid instrument for the long case assessment.

Some other instruments for the structured assessment of long case includes Objective Structured Long Examination Record (OSLER), Structured Long Interview and Clinical Examination (SLICE), Structured Clinical Case Presentation (SCCP), Practice Based Assessment of Clerks in Internal Medicine (PBAC), Long Case Assessment (LCA), Observed Long Case in Clinical Assessment (OLC), Partially Observed Long Case Exam (POLE), Direct Observation Clinical Encounter Examination (DOCEE), Integrated Direct Observation Clinical Encounter Examination (IDOCEE). These different instruments which were designed for structured assessment of long case vary in the observation of history taking and clinical examination by the student, number of examiners assessing the student at one time and time required by the examiner for assessment of the students. The Objective Structured Long Examination Record (OSLER) was introduced by Gleeson as a method to introduce better standardization to the long case. The student conducts an hour long observed history and examination with a patient followed by 20-30 minutes of structured questioning by the examiner using a 10 item analytical record. As a part of the effort to reduce “the luck of the draw” aspect, examiners are asked to formally document the difficulty of the case. Unfortunately there is no evidence as to reliability and validity of the OSLER.[11]

In short, long case assessment with itemized list would lead to enhancement in validity of long case assessment, satisfaction of the students, better learning of the students and motivation of the students.

Conclusions

The content validity index for overall scale (S-CVI) of SLICE and construct validity indicates that SLICE is a valid instrument for the long case assessment.

Limitations

SLICE is used at Islamic International Medical College only; the questionnaire was filled by the examiners of final year MBBS examination. Better evaluation of SLICE could be done if the number of experts is increased and experts from different medical colleges are contacted for the responses.

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CASE REPORT

Skin Pigmentation Effects of Psoralea Corylifolia: A Case Study of Vitiligo

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ABSTRACT

Vitiligo is an abnormal skin condition characterized by depigmentation of normal skin colour. It negatively affects emotions, psychology, and self-esteem of a patient, especially conspicuous in the females who are more conscious about their aesthetic appearance. It prevails equally in society affecting both sexes. Vitiligo has no permanent cure. Psoralea corylifolia (PC) has been reported to yield significant positive outcomes in vitiligo management. In current study, after approval from the ethical review board, a male patient (17 years old) having vitiligo patches distributed on his skin symmetrically and having history of using solution of honey and milk for facial partial pigmentation, was instructed to take half teaspoonful (equivalent to 05 grams) of powdered seed of PC orally and to apply topically a cream containing hydroalcoholic extract (5% w/w) of the PC seed regularly. The subject was naturally exposed to sunrays during his daily life activities. Liver and renal functions were regularly (before treatment and 30 days thereafter) monitored to avoid any untoward effects of oral therapy. Gradual pigmentation was recorded through photographs taken on regular basis. The self-controlled study design was followed. Local (topical cream) and systemic (oral powder from PC) therapy was found to have an additive effect in complete restoration of the pigmentation of skin of the volunteer as compared to self-controlled study design. Full facial pigmentation was obtained in six-month therapy. Liver and renal functions remained undisturbed throughout the course of treatment. The powder form of P.C and its extract incorporated in cream were found safe and efficacious for pigmentation of the vitiliginous skin.

Key Words: *Depigmentation, Pigmentation, Psoralea Corylifolia, Vitiligo.*

Introduction

Vitiligo is a depigmenting and an acquired idiopathic disorder of the skin that causes the loss of melanocytes (pigment cells). Vitiligo patches are well defined having a milky-white appearance of skin. It can be stigmatic cosmetically, especially in the population with a dark color of skin. Vitiligo is prevalent (1 to 2%) in all races, more common in Indian population.[1] Loss of pigments occurs more commonly in teen ages. Although Vitiligo equally affects males and females, however, its psychological complications are comparatively more conspicuous in females because it hinders their natural beauty and appearance. There is no definitive cause of Vitiligo. Studies have pointed out it to be an autoimmune disorder, genetic stress, harm to skin, a stressful incident, skin burn, hereditary (familial link), exposure to chemicals and viral etc.[2] Vitiligo disease is not contagious. It is classified into non-segmental Vitiligo (symmetrical) and segmental Vitiligo (asymmetrical). Non-segmental vitiligo is sub-classified intoacrofacial, focal, generalized, mucosal,and universal Vitiligo. There are many remedies that can help to decrease the appearance of Vitiligo on the affected skin e.g phototherapy with UV light, skin camouflage, topical corticosteroids, calcipotriene, calcineurin inhibitors. psoralen, skin grafts, tattooing, and depigmentation. A successful treatment is not yet available for Vitiligo. There is a need for randomized controlled trials, using PC for this disease.[3]

Case Report

A 17 years old male with the history of generalized Vitiligo since the age of 06 years. He had used solution of milk and honey that had resulted partial pigmentation on his face. He had no family history of the disease. The patient was otherwise healthy without symptoms of any other illness. Prior consent of the patient and his guardian treatment protocol was developed considering the drug profile. Approval was obtained for human studies by the ethical review committee of University. Based on the reported anti-vitiligo potential of the PC, oral, as well

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as topical regimens were selected to be used concomitantly after successful patch testing of a cream containing hydroalcoholic extract of *Psoralea corylifolia*. The subject was instructed to take daily a half teaspoonful (equivalent to 5 grams) of the powdered seeds of PC orally and to topically apply the cream containing PC extract (5% w/w) once a day in the morning. The cream was used only on face rendering the remaining body parts as self-control for this topical therapy. The subject was naturally exposed to sunrays during his daily life activities (around 2 to 3 hours in average) resulting UV mediated activation of psoralens for their therapeutic action. Liver and kidney functions were monitored regularly (before treatment and 30 days thereafter) to rule out side effects of psoralens used orally. Complete blood count (CBC) of the patient was undertaken twice (before the start and after completion of therapy). Pigmentation effect of the therapy was recorded by photographs of the patient taken at regular intervals to measure the clinical outcome. After 15 days of starting this medication, the Process of repigmentation was observed. Development of visible dots on other parts of the body and rehabilitated pigment on facial skin was noted. The treatment was continued uninterrupted for up to 24 weeks for obtaining full facial pigmentation (Figure 1). Liver and renal function parameters remained normal during this period with a minor elevation of serum bilirubin up to an upper normal limit. CBC remained normal at the completion of treatment.

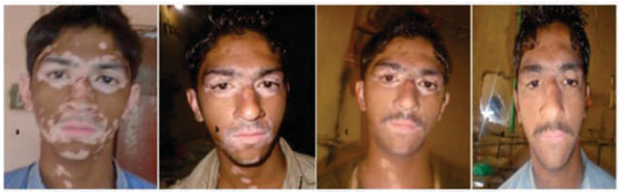

Fig 1: (a-d). Evolution of Facial Pigmentation

a. Before start of Therapy
b. After 60 days of Therapy
c. After 120 days of Therapy
d. After 180 days of Therapy

Discussion

A skin-friendly emulsion containing hydroalcoholic extract of PC was found compatible for the case along with oral powder form of PC. Interestingly, the combination of oral and topical remedy remained safe as well as efficacious in complete restoration of the pigmentation of the vitiliginous facial skin. A half teaspoonful (equivalent to 5 grams) of the powder form of PC was adjusted as an oral dose that was well tolerated by the volunteer. Full facial pigmentation was observed comparative to self-control. Psoralens have been found effective for pigmenting the leucoderma of the vitiligo with possible safety concerns related to hepatic injuries.[4] Cholestatic Jaundice have been reported in a case study, with the use of powdered seeds of *Psoralea corylifolia* at higher doses (10 times the usual dose). Liver biopsy revealed degenerating cells, zone three necrosis, infiltrations and cholestasis.[5] Topical and oral psoralens have been used with variable results in the mitigation of vitiligo.[6] Topical therapy has been employed in the management of vitiligo by incorporating psoralens in the topical formulations. Although dermaceuticals have an important role in vitiligo treatment, poor efficacy and side effects influence their usage and patient compliance. Strategies to design different formulations can have a pivotal role in improving the topical drug delivery systems.[7] Ointment containing powder form of *Psoralea corylifolia* has been reported as an effective remedy for small circular vitiliginous lesions.[8] The oral therapy of *Psoralea corylifolia*was observed safe in current case study although acute drug induced hepatotoxic reactions have been reported with the use of *Psoralea corylifolia* in two separate case studies; one on a 48 years old and other on a 52 years old female patient, respectively. The use of powdered drug had resulted elevation of ALT, AST and bilirubin along with the symptoms of decreased appetite, weakness, dark urine, jaundice, pruritus, vomiting and abdominal pain.[9,10]

Conclusion

Combination of topical and oral PC can be safe and effective for the pigmentation of viltiliginous skin.

Disclosure Author declares no conflict of interest for this submission.

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