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Aims & Scope

The Journal aims to publish research in all fields of clinical, diagnostic, experimental & preventive areas related to medical sciences to disseminate scholastic work among clinicians and scientists around the globe.

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Editorial

CANCER IMMUNOTHERAPY: ADVANCES AND CHALLENGES

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ABSTRACT

Cancer immunotherapy has emerged as a transformative approach in cancer therapeutics, offering new hope to patients by harnessing the power of the immune system to target and eliminate cancer cells. It falls under the category of targeted therapy where an immune target i.e. molecule is identified and then immunotherapy i.e. antibodies are given to target it. Recent advances have brought a number of novel therapeutic options but they are also tagged with various challenges. These challenges include but not limited to the identification of appropriate target, later resistance to the therapy and most importantly the cost of the treatment.

Key Words: Cancer Immunotherapy, Targeted therapy of cancer, Cancer antibodies

INTRODUCTION

Cancer immunotherapy has emerged as a transformative approach in cancer therapeutics, offering new hope to patients by harnessing the power of the immune system to target and eliminate cancer cells. It falls under the category of targeted therapy where an immune target i.e. molecule is identified and then immunotherapy i.e. antibodies are given to target it. Over the past few decades, significant advancements in immunotherapy have revolutionized cancer treatment, leading to remarkable improvements in patient outcomes and survival rates in many cancer types including those which were considered having no treatment. However, as with any therapeutic option there are challenges related to side effects, cost, selectivity and many others, that necessitate ongoing research, innovation, and collaboration to explore the full potential of immunotherapy in oncology.

ADVANCES IN CANCER IMMUNOTHERAPY:

Immunotherapy encompasses a diverse range of strategies designed to enhance the body's natural immune response against cancer molecules mainly proteins expressed in cancer cells. The first monoclonal antibody introduced in the clinical practice was trastuzumab i.e. Herceptin for breast cancer where human epitheial growth factor receptor -2 (HER2) was positive. The HER2 positive breast cancer used to be considered as poor prognostic type before the use of transtuzumab. Following the success of immunotherapy many other targets have been identified and immunotherapy is introduced in many cancer types. Such as the advent of cancer immunotherapy, particularly immune checkpoint inhibitors and chimeric antigen receptor (CAR) T-cell therapy, has transformed the landscape of cancer treatment. Immune checkpoint inhibitors, incldluing PD-1 and CTLA-4 inhibitors, have demonstrated unprecedented clinical responses in patients with advanced melanoma, lung cancer, and other malignancies. These therapies unleash the immune system's

ability to recognize and attack cancer cells, leading to durable responses and prolonged survival. Key advances in cancer immunotherapy include:

Immune Checkpoint Inhibitors: Drugs targeting immune checkpoint molecules such as PD-1, PD-L1, and CTLA-4 have demonstrated unprecedented efficacy in various cancers, including melanoma, lung cancer, and renal cell carcinoma. These agents enhance the immune system's ability to recognize and attack tumor cells, leading to durable responses and long-term survival in some patients.

CAR-T Cell Therapy: Chimeric antigen receptor (CAR) T cell therapy involves genetically modifying a patient's T cells to recognize and kill cancer cells expressing specific surface antigens. CAR-T cell therapies have shown remarkable efficacy in hematological malignancies, leading to complete remissions in patients with refractory or relapsed disease including refractory acute lymphoblastic leukemia and non-Hodgkin lymphoma, offering a lifeline to patients with limited treatment options.

Cancer Vaccines: Therapeutic cancer vaccines stimulate the immune system to recognize and target tumor-specific antigens, triggering an immune response against cancer cells. Advances in vaccine technology and personalized neoantigen vaccines hold promise for improving treatment outcomes and overcoming tumor heterogeneity.

Adoptive Cell Transfer: Adoptive cell transfer (ACT) involves isolating and expanding tumor-infiltrating lymphocytes (TILs) or genetically engineered T cells ex vivo before reinfusion into patients. ACT has shown promising results in melanoma and other solid tumors, offering a personalized and targeted approach to cancer treatment.

Furthermore, ongoing research in cancer immunotherapy continues to uncover novel targets, biomarkers, and combination therapies that hold promise for improving treatment outcomes and expanding the reach of immunotherapy to a broader spectrum of cancer types and patient populations.

CHALLENGES IN CANCER IMMUNOTHERAPY:

Despite the remarkable progress in cancer immunotherapy, significant challenges remain on the path to widespread adoption and optimization of these therapies. One of the foremost challenges is identifying predictive biomarkers to stratify patients who are most likely to benefit from immunotherapy. While PD-L1 expression and tumor mutational burden have shown utility as biomarkers, they are not universally predictive, highlighting the need for more robust predictive markers to guide treatment decisions. Understanding the factors influencing treatment response and resistance is essential for optimizing patient selection and treatment strategies. The definition of the targets is also essential aspect to be explored. Related to that, these cancer molecules are also expressed on normal non cancer cells, thus there is higher risk of immune-related adverse events (irAEs) affecting various organs, including skin, gastrointestinal tract, liver, and endocrine glands. Thus it is fundamental to define the target to the level where it is only expressed in cancer cells. While immune checkpoint inhibitors have revolutionized cancer treatment, they can also unleash the immune system's attack on healthy tissues, leading to a spectrum of autoimmune-like toxicities. Effective management of irAEs requires close monitoring, timely intervention, and multidisciplinary collaboration to mitigate treatment-related complications. Tumors employ various mechanisms to evade immune surveillance, including downregulation of antigen presentation, induction of immune checkpoint expression, and recruitment of immunosuppressive cells. Overcoming tumor

immune evasion mechanisms is critical for enhancing the efficacy of immunotherapy and overcoming treatment resistance.

Another important issue is the cost of the treatment. Immunotherapy drugs are often expensive, limiting access for many patients, particularly in low- and middle-income countries. Addressing cost barriers and ensuring equitable access to immunotherapy remains a significant challenge in cancer care. Additionally, resistance to immunotherapy remains a challenging barrier to achieving durable responses in patients. Tumor immune evasion mechanisms, including upregulation of alternative immune checkpoints, tumor heterogeneity, and immune-suppressive tumor microenvironments, can limit the effectiveness of immunotherapy and lead to treatment resistance. Overcoming resistance mechanisms requires innovative strategies, such as combination therapies targeting multiple checkpoints or enhancing T-cell functionality within the tumor microenvironment.

CONCLUSION:

Cancer immunotherapy represents a paradigm shift in cancer treatment, offering unprecedented opportunities to transform patient care and outcomes. Despite the remarkable progress achieved, significant challenges remain that require collective efforts from researchers, clinicians, policymakers, and industry stakeholders to overcome. By addressing the challenges of response heterogeneity, immune-related adverse events, tumor immune evasion, and access barriers, we can unlock the full potential of immunotherapy and comprehend its promise as a cornerstone of modern cancer treatment. Continued investment in research, innovation, and collaboration is essential to advance the field of cancer immunotherapy and improve outcomes for patients worldwide.

Research article

EVALUATION OF OUTCOME OF SYSTEMIC TRANEXAMIC ACID INJECTION IN DRAIN OUTPUT IN VENTRAL HERNIA REPAIR

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ABSTRACT

The primary objective of the study was to determine the mean drain output after systemic tranexamic acid injection in patients undergoing ventral wall hernia repair. This was a descriptive cross-sectional study conducted in the Department of General Surgery, Hamdard University Hospital Karachi, Pakistan for a period of six months from Oct 2020 to April 2021. The patients undergoing ventral hernia repair were included. Injection tranexamic acid 1gm intravenous twice a day for 1st post-operative day and then capsule Tranexamic acid 500mg per oral three times a day for two consecutive days was given and drain output was measured for 3 days. A total of 63 patients with ventral hernia were included, mean age of patients was 41.476 (±SD= 8.693). There were 41 male patients (65.1%) and 22(34.9%) females. The mean drain output on first day, second and third day was 115.047+14.678 ml/day, 91.919+9.947 ml/day, 66.914+7.070 ml/day and 273.254+29.165 ml/day respectively. The results showed overall reduction in postoperative drain output after oral and intravenous tranexamic acid for 72 hours. However further large scale randomized controlled trials are recommended for confirmation of these findings.

Key Words: Tranexamic Acid Injection, Drain output, Ventral wall hernia repair

INTRODUCTION

Ventral hernias contribute to about 10% and are the commonest with challenging risk of recurrence. They are defined as non-hiatal non inguinal fascial defects in abdominal wall. They can develop anywhere in the abdominal wall (1). Mostly incisional hernia develop at the post-surgical incisions. These ventral hernias have been categorized into congenital an acquired depending on the cause with latter being more common. The common acquired causes of ventral hernias are postsurgical at the site of incision, repetitive stress at weakened abdominal wall, trauma or causes with raised intra-abdominal pressures like coughing, child birth, chronic constipation (3). After surgery abdominal wall restores only 80% tensile strength and operating at incisional site restores around 64% of tensile strength in a healthy individual (2-5). Clinically the presentation of ventral hernia varies among individuals some may remain asymptomatic for many weeks to months while some patients present with strangulation or in-carceration with the need to be operated in emergency (6, 7). Elective repairs have good outcomes as compared to emergency repairs. Mortality after strangulated hernial repairs have been reported in 5% of cases (6-8).

Studies have shown reduction in complications in ventral hernia with use of tranexamic acid. Recently (in 2023) a cross-sectional study by Tarar et al has shown reduced risk of seroma formation in patients with ventral hernia, where in 81% of the patients seroma formation was improved within 5 days (9). Also, reduced number of complications have been reported with topical use of tranexamic acid in different surgeries (10). Khan et al has also reported reduction in seroma formation in 82.7% of patients with reduced drain output (11). Tranexamic acid has anti-fibrinolytic action and limits conversion of plasminogen to plasmin and therefore reducing hemostatic fibrin from getting dissolved and stabilize fibrin, therefore reduces seroma formation. It is

also observed that in wound healing phase excessive bleeding is also reduced. However, there are still limited use of tranexamic acid in clinical practice, therefore, this study was designed to evaluate the effects of tranexamic acid in our population with drain output monitoring regularly as required at our Hamdard University Hospital which is a tertiary care hospital, in order to represent local statistics related to ventral hernia.

METHODS

This was a cross-sectional descriptive study, conducted in the Department of General Surgery, Hamdard University Hospital Karachi, Pakistan. The study duration was six months from 28th Oct 2020 to 28th April 2021. A total of 63 patients were included in this study. The sampling technique was non-probability consecutive sampling. The study included all patients presenting with ventral hernia and undergoing repair, with age range between 18 to 70 years, both genders. All the patients underwent open mesh repair and the redivac drain was placed. The study excluded patients not given informed consent, patients with history of carcinoma, patients with intestinal obstruction (i.e. assessed by history, clinically and X-ray abdomen suggestive of intestinal obstruction). Systemic tranexamic acid is defined as starting injection tranexamic acid 1gm intravenous twice a day for 1st post-operative day and then capsule Tranexamic acid 500mg per oral three times for 2 days and drain output was measured. The Redivac drain was placed over the mesh. Output in drain was measured daily with labels, if there is more than 25ml per day of output was considered positive, however, less than 25ml per day of output was labelled negative. After the ethical approval study was conducted.

Patients meeting the inclusion criteria admitted in general surgery department with ventral hernia diagnosis undergoing open mesh repair in whom redivac drain was placed over the mesh were enrolled in the study. Prior to inclusion patients were explained about benefits of the study and written informed consent was taken. Brief history regarding duration of sign and symptoms was taken & clinical examination was done. In all these patients injection tranexamic acid 1gm intravenously given twice a day for 1st post-operative day and then capsule Tranexamic acid 500mg per oral three times a day for 2 days was given and drain output was measured on daily basis for 3 days, drain output was labeled as positive if it was more than 25ml/day, while was labeled as negative if it was less than 25ml/day. The drain was removed when the output was negative (i.e. less than 25ml/day).

Statistical analysis

Data was entered and analyzed by using Statistical Package for Social Sciences (SPSS) version 22.0. The variables like age, height, weight, BMI, duration of sign and symptom of ventral hernia and drain output mean and standard deviation were calculated. Frequency and percentages were calculated for gender, comorbid conditions (DM/hypertension), type of ventral hernia, drain removed on day, drain output (positive/negative). Effect modifiers was controlled through stratification of age, gender, BMI, duration of sign and symptoms of ventral hernia, comorbidities, type of ventral hernia.

RESULTS

A total of 63 patients with ventral hernia repair undergoing open mesh repair were selected to conduct this study. Mean age was 41.476+8.693 years. In Table 1 the descriptive statistics for age are shown. The mean height was 1.548+0.213 m & weight was 53.859+11.910 kg. In our study 41 patients (65.1%) were males & 22 patients (34.9%) were females. Mean BMI was 21.216+3.250 kg/m2. Diabetes mellitus was seen in 13 (20.6%) patients, Hypertension was noted in 16 (25.4%) patients, the type of ventral hernia was paraumbilical hernia in 25(39.7%) & incisional hernia in 38(60.3%) patients. The mean drain output on day 1 was 115.047+14.678 ml/day. The mean drain output on day 2 was 91.919+9.947 ml/day. The mean drain output on day 3 was 66.914+7.070 ml/day. The mean total drain output was 273.254+29.165 ml/day. The descriptive statistics of drain output on day 1, drain output on day 2, drain output on day 3, total drain output are presented in Table-2.

Table 1. Demographic characteristics of patients undergoing ventral hernia repair

Descriptive	Frequency
Age in years (Mean ±SD)	41.476 ±8.693
Diabetes mellitus	13 (20.6%)
Hypertension	16 (25.4%)
Type of ventral hernia Para-umblical hernia Incisional hernia	 39.7% 60.3%

Table 2. Summary of the drain output per day after ventral hernia repair

Day	Drain output (ml/d)
Day 1	115.047 (±14.678)
Day 2	91.619 (±9.947)
Day 3	66.904 (±7.070)
Total	273.254 (±29.165)

The frequencies age groups, gender, BMI, duration of ventral hernia, diabetes mellitus, hypertension and type of ventral hernia according to mean drain output on day 1, mean drain output on day 2, mean drain output on day 3 and mean total drain output. In our study there was no significant difference of mean drain output on day 1, mean drain output on day 2, mean drain output on day 3 and mean total drain output was noted in age, gender, BMI, diabetes mellitus, hypertension and type of ventral hernia.

Table 3. Drain output in patients undergoing ventral hernia repair

Variables	Mean drain output on day 1	Mean drain output on day 2 (ml/day)	Mean drain output on day 3 (ml/day)	Mean total drain output (ml/day)
Age in years **25-43** **44-60**	 112.473±15.60 118.960±12.43	 89.000 ±10.33 95.000 ±7.97	 66.105 ±7.34 68.120 ±6.48	 267.31 ±31.01 282.28 ±23.94
Gender **Male** **Female**	 116.146 ±14.57 113.000 ±14.98	 92.00 ±10.049 92.00 ±10.049	 67.29 ±7.103 66.18 ±7.115	 274.95 ±28.89 270.09 ±30.07
BMI Kg/m2 **17-25** **26-35**	 115.17 ±14.471 114.00 ±17.473	 92.00 ±9.842 88.57 ±11.058	 67.41 ±7.026 62.85 ±6.517	 274.23 ±28.728 265.42 ±33.400
Diabetes Mellitus	114.61±18.40	91.23 ±13.479	64.15±10.24	270.00±40.78
Hypertension	116.75 ±15.22	90.87 ±9.769	66.25±7.224	273.87 ±29.61
Types of Ventral hernia • **Paraumbilical hernia** • **Incisional hernia**	 115.60 ±15.567 114.68 ±14.266	 92.64 ±10.403 90.94 ±9.717	 67.88 ±6.489 66.26 ±7.442	 275.72 ±30.66 271.63± 28.436

DISCUSSION

One of the most common problems are the Abdominal wall hernias with predominant cause being the increased intra-abdominal pressure (7-10). In 1962, tranexamic acid was discovered, essential medicines used to treat major traumatic injuries, excessive bleeding injuries, post-partum bleeding, surgeries, bleeding from nose, removal of tooth, and heavy menstrual bleeding (8-11). Tranexamic acid is an easily available drug and its cost-effective with no dose adjustment required and safe in hepatic impairment (11-15). Our study therefore designed to explore use of tranexamic acid in drain output.

Studies have shown postoperative reduction in complications when tranexamic acid was used in patients of ventral hernia with reduced seroma formation, serous discharge and postoperative wound leakage as reported previously (16-20). A Study by Ahmed et al has found 81% patients with seroma formation but reduced in 5 days postoperatively and reduced drain output with use of tranexamic acid 1gm twice daily with short hospital stay (20). Another study by Khan et al has found that mostly females were presented with ventral hernia i.e. 67.3%, whereas 82.7% have seroma formation, reduced within 1 week, 41 patients had drain output <150ml, while 55 patients had 150-300ml and 14 patients had >300ml drain output (21). Tarar et al has also found reduced risk of seroma formation in 81% of patients with tranexamic acid given postoperatively. Seromas are commonly seen in patients with hernia repair but their cause is largely not defined. Seroma has increased risk of developing infection if not treated and therefore most studies have shown that tranexamic acid effectively reduces seroma formation. Another cross-sectional study by Lashari et al has found statistically significant correlation among patients treated with tranexamic acid and those in which it was not given with reduced risk of complications and shortened hospital stay with early removal of drain output. Established role of tranexamic acid has been seen in orthopedic surgeries as well. Some studies have suggested topical use of tranexamic acid in orthopedics. Even in patients with mastectomy tranexamic acid has well established role (23, 24). Albatonanny has also found reduced risk among patients with single use of tranexamic acid in patients with ventral hernia (12). Use of Tranexamic acid in our study reduced post-operative drain output in patients with ventral hernia repair. The limitation of our study was single center study, smaller sample size. Further studies with larger sample size and randomized controlled trial design are required.

CONCLUSION

The use of tranexamic acid in patients postoperatively for 72 hours reduced risk of drain output and also seroma formation in patients operated for ventral hernia.

Conflict of interest:

Authors declare no conflict of interest

Funding source:

The study did not receive any external funding

Ethical Approval:

The study was approved by local research ethics committee.

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Research Article

NEGATIVE AIR IONS INDUCED AMELIORATION OF BIOCHEMICAL PARAMETERS IN CEREBRAL PALSY PATIENTS

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ABSTRACT

Cerebral palsy (CP) is an umbrella term for a group of neurological abnormalities that impair a person's ability to stand, balance, walk, and maintain posture. It is caused by injury or anomalies in the developing brain, which tend to happen before or soon after birth or in early childhood. The study aimed to explore the influence of negative air ions (NAIs) on biochemical parameters in CP-inflicted patients and was conducted from February 1st to March 30th, 2021. Thirty-one structured sessions of exposure to NAIs were conducted for six weeks after randomly allocating CP-inflicted participants into control (n=12; Median age: 14±5 years) and intervention groups (n=16; M age: Median age=14.5±7.1 years). Biochemical parameters included blood urea nitrogen, calcium, creatinine, electrolytes (bicarbonate, chloride, potassium, and sodium), liver function tests (alkaline phosphatase, alanine aminotransferase, aspartate transaminase, bilirubin total, direct bilirubin, and γ-glutamyl transpeptidase), protein tests (albumin, globulin, A/G ratio, and total proteins), and random blood sugar. For all tests, a trained phlebotomist collected blood samples from the premises of the rehabilitation center at baseline and at sixth week. The biochemical parameters were normal in both groups at baseline and sustained normality in assessment at the sixth week. The NAI intervention showed no negative effects, indicating that it can be a progressive, non-pharmacological, cost-effective, and a potential method to improve the quality of life of CP-inflicted patients.

Key Words: Cerebral Palsy, Biochemical parameters, Intervention, Negative Air Ions

INTRODUCTION

Cerebral palsy (CP) is an umbrella term for a group of neurological abnormalities that impair a person's ability to stand, balance, walk, and maintain posture. It is caused by injury or anomalies in the developing brain, which tend to happen before or soon after birth or in early childhood. CP may alter normal blood parameters, cognitive ability, hearing, metabolism, muscle coordination, motor movement, oral health, speech, vision, and many other functions (1,2). Some of the key etiological factors include birth asphyxia, consanguinity, genetic abnormalities, head injuries, home labor, hypoxic-ischemic encephalopathy, intrauterine growth restriction, infections, low gestational weight, microcephaly, multiple pregnancies, smaller gestational age, and many more (1,3). The majority of identified risk factors for CP are associated with the male gender and the prenatal developmental period (3,4). Nearly 17 million people are affected by CP all over the world (1) but the epidemiology and the etiology of CP are yet to be determined in Pakistan (5,6).

Certain abnormal biomarkers including amino acids, ammonia, calcium, cholesterol ethers, creatine phosphokinase, electrolytes, iron, phosphate, protein, urea, uric acid, and growth status have been reported in CP-inflicted patients (7-11).

Interventions are efforts done to enhance cognitive, motor, sensory, or social components of one's surroundings when there are clear cognitive or motor deficiencies. Although CP is a lifelong impairment, several interventions can assist in modifying its impact on the body and activities of daily life. Healthcare providers have employed a variety of interventions to deal with behavioral and emotional issues, bowel problems,

controlling pain, drooling, dysphagia, epilepsy, hearing and vision problems, insomnia, intellectual disability, movement issues, problems with communication, and spinal and hip abnormalities in CP-inflicted patients (12). Enriched environment techniques can modulate behavior, spasticity, and cognitive outcomes in CP-inflicted patients (13).

The negative air ionizer/generator generates negative air ions (NAIs) by charging air molecules in the surrounding environment with high voltage. NAIs' biological function is reliant on superoxide and activated oxygen. Ion deficiency is the primary cause of many ailments, and inhaling NAIs is a successful treatment. Studies have demonstrated NAIs induced improvement in aging, allergies, attention, asthma, depression, cancer, cognition, depression, memory, mental energy, metabolism, mood, performance, reaction time, respiratory issues, sleep, spasticity, stress, and numerous physiological functions (14-18). Exposure to negative ionizers can be used as part of environmental enrichment strategies to benefit individuals inflicted with CP. Some studies suggest potential positive effects on humans and animals, while others find no significant impact or even report negative outcomes. Despite several findings, no studies have demonstrated the use of NAIs in the rehabilitation of individuals with CP. As a result, health practitioners remain dubious about employing negative air ionizers to treat CP. It was also an attempt to replenish the scarcity of data on NAI intervention-induced alterations in biochemical parameters of CP-inflicted patients. Thus this study was designed including CP-inflicted patients.

METHODS

The study was conducted from February 1st to March 30th, 2021, after ethical permission from the Institutional Bioethics Committee (Ref: IBC-2017) and the Al-Umeed Rehabilitation Association (AURA), where the intervention study was carried out. The pediatric psychiatrists at AURA identified patients with CP and these patient's participated in this study. Parental consent was taken for the inclusion of their children in the study. A total of 30 parents gave consent for the inclusion of their children in the study. Two participants were later excluded, and the remaining twenty-eight were divided into seven classes, each class with four participants. Each classroom measured roughly forty square feet. The administration of AURA employed a concealed randomization method to allocate all inducted participants to one of two groups: (a) Control (n=12; median age: 14±5 years) or (b) intervention groups (n=16; median age=14.5±7.1 years).

Due to a limited number of consents, participants were chosen regardless of age, gender, muscle tone, mode of transition, topographic presentation, or other additional impairments. Participants with any medical condition, a history of surgery or medical procedure, or blood transfusions in the previous six months were excluded. Participants who were denied permission by the institute were also barred from participating.

The control group was chosen to ensure that the comparisons and results were valid, acceptable, and generalizable. The intervention group participated in 31 scheduled sessions of negative ionizer exposure for 5 days/week for six weeks. Participants in the intervention group attended all of the intervention sessions regularly. 'Negative ionizer JHQ- 801' and 'KT-401 mini air ion counter' were purchased from the Daraz online shop for intervention administration and ion concentration monitoring, respectively. It was ensured that a concentration of 10000 NAIs/cm3 was present in whole session. Throughout the intervention, all study participants in each group continued to receive regular speech, occupational, physical, and medical care. All participants were already receiving several interventions at the center and the negative ionizer was added to the interventions offered to the intervention group.

Biochemical parameters included blood urea nitrogen, calcium, creatinine, electrolytes (bicarbonate, chloride, potassium, and sodium), liver function tests (alkaline phosphatase, alanine aminotransferase, aspartate transaminase, bilirubin total, direct bilirubin, and γ-glutamyl transpeptidase), protein A/G ratio (albumin, globulin, A/G ratio, and total proteins), and random blood sugar. For all blood tests, a trained phlebotomist collected blood samples from the premises of the rehabilitation center at baseline and sixth week. The samples were transported carefully to perform tests at 'The Laboratory' situated in Karachi, Pakistan.

Serum was collected from whole blood using the 'Labofuge 400R Centrifuge'. The 'Roche/Hitachi Cobas 6000 c 501' was used to measure blood urea nitrogen, calcium, creatinine, liver function tests, protein, and random blood sugar levels in serum using a photometric method. The 'Nova biomedical 4+ electrolyte analyzer' was used to do electrolyte tests using the direct ion-selective electrode technique.

Statistical analysis

Statistical Package for Social Sciences (SPSS version 28.0) was used to analyze the collected data. To examine the demographical characteristics of the inducted CP-inflicted patients, descriptive statistics was used for calculating the frequencies and percentages. The demographic characteristics data encompassed gender, gross motor functional classification system (GMFCS) levels, muscle tone, topographical presentation, mode of transition, and additional impairments. For the biochemical parameters, the mean, standard deviation, standard error, and 95% confidence were determined. The data was parametric so, paired t-test was applied to measure the within-group mean differences for each parameter at baseline and the sixth week.

RESULTS

Demographic characteristics

Out of 28 participants, 9 (32.2%) were males and 19 (67.8%) were females. Table 1 shows the characteristics of inducted participants. In the intervention group, dominant cases presented GMFCS level IV in 43.7%, spastic muscle tone in 62.5%, wheelchair-bound mobility in 81.2%, and diplegic presentation in 56.2%, whereas, in control group, dominant cases presented GMFCS level IV in 50%, spastic muscle tone in 58.9%, wheelchair-bound mobility in 58.3%, and triplegic presentation was seen in 58.3%.

Biochemical parameters

In the control group, paired t-tests showed significantly increased blood urea nitrogen ($p<0.01$), chloride ($p<0.001$), creatinine ($p<0.01$), direct bilirubin ($p<0.01$), and sodium ($p<0.001$) (Tables 2, 3 and 4). In the intervention group, paired t-tests showed significantly increased chloride ($p<0.01$), creatinine ($p<0.01$), globulin ($p<0.05$), sodium ($p<0.001$), and total protein ($p<0.05$) (Tables 2, 3 and 4) along with a significant decrease in the A/G ratio test ($p<0.05$) (Table 2).

Table 1: Characteristics of the study participants (n=28)

Characteristics	Control group n(%)	Intervention group n(%)
Gender		
Male	1(8.3)	8(50)
Female	11(91.6)	8(50)
Gross Motor Functional Classification System (Levels)		
I	3(25)	2(12.5)
II	1(8.33)	2(12.5)
III	2(16.6)	4(25)
IV	6(50)	7(43.7)
V	0	1(6.25)
Muscle tone		
Spastic	7(58.3)	10(62.5)
Hypotonic	5(41.6)	6(37.5)
Topographical presentation		
Monoplegic	0	1(6.25)
Hemiplegic	1(8.33)	2(12.5)
Diplegic	4(33.3)	9(56.2)

Triplegic	7(58.3)	4(25)
Quadriplegic	0	0
Mode of transition		
Wheelchair	7(58.3)	13(81.2)
Independent	3(25)	1(6.25)
Walker	2(16.6)	2(12.5)
Additional impairments		
Epilepsy	2(16.6)	1(6.25)
Poor attention	4(33.3)	2(12.5)
Speech impairment	3(25)	2(12.5)

Table 2: Summary of blood parameters: Intervention versus control group

Group	Evaluation	Mean±SD	Std. Error	95% Confidence Interval for Mean		*p*-value
				Lower Bound	Upper Bound	
Total proteins (Normal: 6.6-8.7 G%)						
Control	Baseline	7.6±0.3	0.11	7.35	7.84	0.09
	Sixth week	7.8±0.4	0.12	7.53	8.08	
Intervention	Baseline	7.5±0.4	0.10	7.32	7.78	0.01
	Sixth week	7.8*±0.3	0.09	7.63	8.06	
Albumin (Normal: 3.2-4.5 G%)						
Control	Baseline	4.6±0.3	0.09	4.48	4.89	0.07
	Sixth week	4.8±0.3	0.10	4.58	5.02	
Intervention	Baseline	4.5±0.2	0.07	4.41	4.71	0.19
	Sixth week	4.6±0.2	0.06	4.51	4.81	
Globulin (Normal: 1.9-2.8 G%)						
Control	Baseline	2.9±0.2	0.07	2.74	3.06	0.30
	Sixth week	3.0±0.2	0.05	2.87	3.12	
Intervention	Baseline	2.9±0.4	0.11	2.73	3.23	0.01
	Sixth week	3.1*±0.4	0.10	2.96	3.40	
Albumin/Globulin ratio (Normal: 1.1-2.2)						
Control	Baseline	1.6±0.2	0.05	1.49	1.75	0.75
	Sixth week	1.6±0.1	0.04	1.51	1.70	
Intervention	Baseline	1.5±0.2	0.07	1.41	1.72	0.04
	Sixth week	1.4*±0.2	0.05	1.37	1.59	
Random sugar (Normal: Upto 180 mg%)						
Control	Baseline	83.9±11.9	3.44	76.3	91.4	0.46
	Sixth week	87.5±15.4	4.47	77.6	97.3	
Intervention	Baseline	90.0±18.2	4.56	80.3	99.7	0.25
	Sixth week	94.1±18.5	4.63	84.3	104.0	
Blood urea nitrogen (Normal: 7-21 mg%)						
Control	Baseline	10.4±3.23	0.93	8.36	12.4	0.004
	Sixth week	11.9**±3.72	1.07	9.54	14.2	
Intervention	Baseline	11.1±3.05	0.76	9.55	12.8	0.88
	Sixth week	11.0±2.11	0.52	9.93	12.1	

Creatinine (Normal: 0.6-1.3 mg%)						
Control	Baseline	0.55±0.17	0.04	0.44	0.66	0.003
	Sixth week	0.65**±0.17	0.04	0.54	0.76	
Intervention	Baseline	0.48±0.14	0.03	0.40	0.55	0.006
	Sixth week	0.53**±0.16	0.04	0.44	0.61	
Calcium (Normal: 8.1-10.4 mg%)						
Control	Baseline	9.83±0.30	0.08	9.63	10.0	0.79
	Sixth week	9.85±0.35	0.10	9.63	10.0	
Intervention	Baseline	9.70±0.41	0.10	9.48	9.92	0.10
	Sixth week	9.82±0.39	0.09	9.61	10.0	

Values are significant at $^{*}p$ <0.05 and $^{**}p$<0.01.

Table 3: Summary of Liver Function Test: Intervntion versus control groups

Group	Evaluation	Mean±SD	Std. Error	95% Confidence Interval for Mean		*p*-value
				Lower Bound	Upper Bound	
Bilirubin total (Normal: 0.1-1.2 mg%)						
Control	Baseline	0.38±0.19	0.05	0.25	0.50	0.20
	Sixth week	0.45±0.18	0.05	0.33	0.57	
Intervention	Baseline	0.37±0.31	0.07	0.20	0.53	0.98
	Sixth week	0.37±0.36	0.09	0.17	0.57	
Direct bilirubin (Normal: 0.1-0.4 mg%)						
Control	Baseline	0.14±0.05	0.01	0.10	0.17	0.001
	Sixth week	0.19**±0.05	0.01	0.15	0.22	
Intervention	Baseline	0.13±0.09	0.02	0.08	0.18	0.12
	Sixth week	0.15±0.09	0.02	0.09	0.20	
Aspartate transaminase (Normal: Upto 46 U/L)						
Control	Baseline	25.9±11.1	3.22	18.8	33.0	0.43
	Sixth week	23.5±5.85	1.68	19.7	27.2	
Intervention	Baseline	23.5±7.41	1.85	19.5	27.4	0.68
	Sixth week	23.2±6.77	1.69	19.6	26.8	
Alanine aminotransferase (Normal: Upto 49 U/L)						
Control	Baseline	22.9±18.3	5.30	11.2	34.5	0.27
	Sixth week	17.0±6.06	1.74	13.1	20.8	
Intervention	Baseline	17.3± 6.8	1.71	13.7	21.0	0.05
	Sixth week	14.8± 5.01	1.25	12.2	17.5	
Gamma-glutamyl transferase (Normal: 7-32 U/L)						
Control	Baseline	12.4±4.64	1.33	9.46	15.3	0.15
	Sixth week	13.1±4.70	1.35	10.1	16.1	
Intervention	Baseline	12.1±4.19	1.04	9.95	14.4	0.56
	Sixth week	11.8±3.03	0.75	10.2	13.4	
Alkaline phosphatase (Normal: 35-105 U/L)						
Control	Baseline	178.4±90.5	26.1	120.9	235.9	0.85
	Sixth week	176.1±78.1	22.5	126.5	225.8	
Intervention	Baseline	167.1±75.5	18.8	126.8	207.3	0.33

	Sixth week	179.5±100.5	25.1	125.9	233.0	

Values significant at $^{**}p<0.01$.

Table 4: Summary of serum Electrolytes: Intervention versus control groups

Group	Evaluation	Mean±SD	Std. Error	95% Confidence Interval for Mean		*p-value*
				Lower Bound	Upper Bound	
Sodium (Normal: 136-149 M Eq/L)						
Control	Baseline	139.1±0.90	0.26	138.5	139.6	<0.001
	Sixth week	144.5***±2.24	0.64	143.1	145.9	
Intervention	Baseline	139.5±2.09	0.52	138.4	140.6	<0.001
	Sixth week	144.0***±2.07	0.51	142.9	145.1	
Potassium (Normal: 3.8-5.2 M Eq/L)						
Control	Baseline	4.59±0.39	0.11	4.34	4.84	0.19
	Sixth week	4.39±0.33	0.09	4.18	4.60	
Intervention	Baseline	4.47±0.38	0.09	4.26	4.67	0.25
	Sixth week	4.35±0.42	0.10	4.12	4.57	
Chloride (Normal: 98-107 M Eq/L)						
Control	Baseline	102.9±1.00	0.29	102.3	103.6	0.0002
	Sixth week	105.0***±1.55	0.44	104.0	106.0	
Intervention	Baseline	102.6±2.04	0.51	101.5	103.7	0.006
	Sixth week	104.6**±2.64	0.66	103.2	106.0	
Bicarbonate (Normal: 25-29 M Eq/L)						
Control	Baseline	28.4±1.08	0.31	27.7	29.1	1
	Sixth week	28.4±1.67	0.48	27.3	29.4	
Intervention	Baseline	29.3±1.62	0.40	28.4	30.1	0.06
	Sixth week	28.4±1.45	0.36	27.6	29.2	

Values significant at $^{**}p<0.01$, and $^{***}p<0.001$.

DISCUSSION

In the present study, the participants in the intervention group were exposed to 10000 ions/cm3 for 40 minutes which significantly improved chloride, creatinine, globulin, total proteins, and sodium levels within the normal range.

Electrolytes play a significant role in maintaining blood clotting, blood pressure, chemical reactions, muscle contraction, and water equilibrium for the normal functioning of the human body. The intervention group participants of this study presented an increase in the concentration of electrolytes (i.e. chloride and sodium) and total proteins within the normal range. These changes are consistent with previous study (10) that suggested these changes could be associated with the hypohydration status (19).

NAIs can increase the affinity of hemoglobin for oxygen, raising the partial pressure of oxygen and decreasing the partial pressure of carbon dioxide in the blood. In this way, it reduces the rate of respiration, which improves the metabolism of water-soluble vitamin C and B-complex. Vitamin B12 is required for metabolism, red blood cell formation, and the maintenance of proper central nervous system function. Vitamin B7 is necessary for carbohydrate, lipid, and protein metabolism, and a lack of it can cause anemia, melancholy, cardiac problems, and muscular pains. Vitamin C is an antioxidant that helps with iron absorption, tissue health, bone and collagen formation, wound healing, immunity, and blood vessel strength. Vitamin C deficiency can result in scurvy, poor tissue growth, tooth loss, and impaired wound healing (20). Deficiency in water-soluble vitamins has significant clinical consequences or even death. NAIs activate

various biological systems. As a result, NAI-induced improvements in water-soluble vitamins may have contributed to considerably higher levels of globulin and total proteins in the intervention group within the normal range.

The participants in the present study were exposed to a concentration of 10000 ions/cm3 and a concentration over 1000 ions/cm3 has been found to increase immune functioning (21). For a healthy immune system, there must be an equilibrium between the negative and positive ions in the body. NAIs can increase the cell membrane permeability to enable nutrient absorption and waste removal from the cells by re-activating the Na-K pump. NAIs enhance oxygen (O2) absorption across RBCs by increasing the oxygen-carrying capacity of the cells (14). An insufficient immune system has been reported to trigger several diseases including autoimmune diseases, cancers, and inflammatory diseases. NAIs can boost the immune system by strengthening the immune cells and making them healthier and more energetic to boost their ability to fight foreign antigens (13). Therefore, it is anticipated that the exposure to NAIs may have contributed to the strengthening of the immune system.

Serotonin (5-HT) is a neurotransmitter that controls several endocrine, metabolic, and neurovascular functions including addiction, aggression, appetite, anxiety, autism, bladder, brain homeostasis, breathing, circadian rhythms, depression, fear, gut motility, immunity, intestinal absorption, memory, mood, neurogenesis, pain, sleep, thermoregulation, and vascular tone (22). Though serotonin levels were not monitored in the participants, it is anticipated that NAIs may have normalized the 5-HT levels in the intervention group.

Hence, it is anticipated that there may be possible involvement of multiple factors including the enhancement of water-soluble vitamin metabolism, immune system strengthening, restoration of acid-base disturbances in the brain and other systems, and serotonin reduction that contributed to normalizing the blood parameters. NAIs may have improved the digestive, cardiovascular, circulatory, endocrine, immunological, integumentary, lymphatic, neurological, respiratory, and urinary systems in the participants. It is also possible that the provided NAI intervention may have ameliorated undiagnosed biochemical issues that contributed to the improvement of chloride, creatinine, globulin, total proteins, and sodium within the normal range.

The present study has a small sample size due to the limited number of consents obtained from the parents of CP patients at the rehabilitation center. The recruited participants had normal blood parameters at the time of induction, which might be attributed to the therapies provided at the rehabilitation center. Several biochemical parameters were improved in the present study, it would be interesting to follow the intervention-induced changes in naive CP patients. This study is an endeavor to pool the existing and insufficient data on the blood parameters of CP-inflicted patients.

CONCLUSION

The NAI intervention showed no negative effects, indicating that it can be a progressive, non-pharmacological, cost-effective, and a potential method to improve the quality of life of CP-inflicted patients.

Conflict of interest:

Authors declare no conflict of interest

Funding source:

The study did not receive any external funding

Ethical Approval:

The study was approved by the ethics review committee of the University of Karachi. Verbal and written consent was taken from the parents of all inducted CP-inflicted participants.

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Research Article

ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT DATE PALM (PHOENIX DACTYLIFERA L.) SEED VARIETIES: AN IN VITRO ASSESSMENT

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ABSTRACT

The date palm (Phoenix dactylifera L.) fruit contains variety of bioactive constituents including phenolic compounds. This research aimed to analyze the phenolic constituents and antioxidant potential of crude methanol extract samples from seeds of different date varieties, Aseel (As), Ambar (Am), Ajwa Saudi (AJS), Ajwa Khairpur (AJK), Khipro (KP) and Karblyan (KB). Date seeds are regarded as waste product. However, it contains secondary chemicals of biomedical significance. Colorimetric methods were used for the evaluation of phytochemical and antioxidant capacity. The Folin-Ciocalteu method was used for the assessment of phenolic content. The antioxidant potential was evaluated through phosphomolybdenum test, potassium ferricyanide test, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate scavenging test methods. All samples differ significantly in terms of antioxidant activities and quantity of secondary metabolites (phenolic and flavonoid contents). When all samples were compared, As was shown to have the highest amount of phenolic and flavonoids, while AJ(S) had the lowest content. The KP sample had the highest overall antioxidant and reducing potential, while the AJ(K) and Am samples showed the lowest, respectively. Significant DPPH scavenging capacity has been demonstrated in all samples. In order to extend the shelf life of food products, date seeds may consequently provide ideal material for the bio-functional food industry.

Key Words: Date palm; Phytochemicals; Phenolic content; Antioxidant; Flavonoids

INTRODUCTION

Date palm is the primary plant that characterizes, represents, and defines dry and semiarid-areas of North Africa and the Middle East. Due to its adaptation to tropical or subtropical temperatures, this crop is the world's oldest perennial fruit tree (> 4000 B.C). With around 1 million metric tons of date seeds, the world produced up to 8.5 million metric tons of dates in 2016. Among the nations that produce dates, Pakistan comes on the sixth number (1). Its taxonomical classification is given in Figure 1. Dates are rich source of nutritional and bioactive constituents. The seeds make about 10-15% of overall weight of the fruit and are enclosed in a fleshy pericarp. In addition, date seeds continue to be a concern for the date processing business. Sometimes seeds are utilized as feed for livestock in Middle Eastern and South Asian nations (2). In comparison to controls, broilers fed diets enriched with date seeds gained more weight (3). Additional research suggests that seeds contain nutrients that are beneficial for animal diets and reports a positive supplementary effect on animal diets (4). When comparing date seeds with the wheat bran and oats, they are a richer source of fiber (5). Consuming date seeds lowers the risk of dyslipidemia, diabetes, obesity, hypertension, and other metabolic disorders (6). Protein, fiber, vitamins, and bioactive substances are some examples of components could be associated with these effects (7). Phenolic compounds are among the bioactive substances that have been found to have antioxidant, anti-carcinogenic, anti-microbial, anti-mutagenic, and anti-inflammatory properties.

Date seeds may contain nutritious and bioactive components with high added value that may be extracted and used to bio-functional foods. Despite the 300 different date variants growing in district Khairpur, there is little information available on the phytochemical analysis and antioxidant activity of the seeds produced from these trees as byproducts. So, the primary objective of the current study was to evaluate and compare the existing variations in phytochemical content and antioxidant activities of seeds from six distinct varieties of date (P. dactylifera L.) seeds.

Kingdom:	Plantae
Sub-kingdom:	Tracheobionta
Division:	Magnoliophyta
Class	Monocotyledon
Order:	Arecales
Family:	Arecaceae
Genus:	Phoenix
Specie:	Dactylifera
Botanical name:	*P. dactylifera*

Figure 1. Taxonomy of date palm (P. dactylifera L.)

METHODS

COLLECTION AND PROCESSING OF THE SAMPLE

Various verities of dates were collected from Shah Abdul Latif University or purchased from Saudi Arabia. The seeds were taken from all date varieties, washed, and shade dried. Coarse powder was prepared using Grinder. After that, extract was prepared by soaking the sample powder in methanol (1:4 w/v) for three days at room temperature and frequent shaking. The procedure was repeated, filtered through Whatman No.1 filter paper and concentrated using rotary evaporator (R-200 Buchi, Switzerland). Finally, the samples were completely dried in an oven (Vacucell, Einrichtungen GmbH). The six samples were labeled, including: Aseel (As), Ambar (Am), Ajwa Saudi (AJS), Ajwa Khairpur (AJK), Khipro (KP); Karblyan (KB) and kept in air-tight containers at 4°C until further analysis.

ASSESSMENT OF PHYTOCHEMICALS

Quantification of Total Phenolic Content

The reaction mixture was prepared by mixing the sample, Folin-Ciocalteu reagent, and sodium carbonate (1:9:9, V/V) for the assessment of the phenolic content. The mixture was incubated at 30°C for one hour. The optical density was recorded at 725 nm. The presence of phenolic compounds was evaluated from the regression curve of the Galic acid (y= 0.0617x-0.0525, R2=0.9834), used as standard phenolic compound. While, phenolic compounds were quantified as Gallic acid equivalents (GAE).

Quantification of Total Flavonoid Content

Reaction mixture was prepared by mixing sample, potassium acetate and aluminium chloride (2:1:9, V/V) for the assessment of the flavonoid content. The mixture was incubated at ambient temperature for half an hour. The optical density was recorded at 415 nm. The presence of flavonoid compounds was evaluated from the regression curve of the Quercetin (y= 0.0429x-0.1092, R2= 0.9909), used as a standard flavonoid compound. While, flavonoid compounds were quantified as Quercetin equivalents (QE).

ANTIOXIDANT ACTIVITIES

Assessment of free radical scavenging potential

As a stable radical with strong absorbance at 517 nm, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was utilized to examine the capacity of samples to scavenge this free radical. Assay was performed following the previously described method with minor modification (8). The reaction mixture consisting of sample and DPPH solution was incubated at room temperature for one hour in a dark environment. Then, absorbance was measured at 517 nm. Vitamin C was used as the standard scavenging chemical. The following formula was used to determine the percentage of scavenging activity:

$$\%SA = (1 - ODS/ODNC) \times 100$$

SA: Scavenging activity; ODS: optical density of sample; ODNC: optical density of negative control.

Assessment of total antioxidant potential

The total antioxidant potential of the extracts was estimated using a phosphomolybdenum-based colorimetric assay by following previously described method (8). Total antioxidant reagent comprised of ammonium molybdate, sulfuric acid and sodium phosphate at the concentrations of 4 Mm, 0.6 M and 28 mM, respectively. The sample and total antioxidant reagent (1:9 v/v) was poured in Eppendorf tubes. Then, reaction mixture was incubated in boiling water for one and half hour. Followed by cooling the mixture at room temperature and measuring the optical density at 630 nm. Standard curve of Vitamin C was prepared and vitamin C equivalents were used to express the overall antioxidant activity of the samples. The assay was performed in triplicate and repeated three times.

Assessment of total reducing potential

Total reducing potential of the samples was investigated by following previously described method (9). Reaction mixture for assessment of total reducing potential comprised of extract sample, phosphate buffer (pH 6.6), potassium ferric cyanide at the concentrations of 400 µg, 0.2 M, 0.6 M and 1%, respectively and mixture was subjected to incubated for half an hour in water bath at 50°C. Subsequently, trichloroacetic acid was added to stop the reaction. At the end optical density was measured at 630 nm. Standard curve of Vitamin C was prepared and vitamin C equivalents were used to express the overall total reducing potential of the extract samples. The assay was performed in triplicate and repeated three times.

Statistical analysis

All experiments were carried out in triplicate, repeated thrice and presented as Standard Deviation (± SD). GraphPad Prism (version 5.01 for Windows, California, USA) was used to analyze the data and determine statistical significance at the level of p-value < 0.05.

RESULTS

PHYTOCHEMICAL ANALYSIS

The class of phytochemical and their quantities in extracts depends on the nature of the solvents. The biological potential of plants is generally thought to depend on phytochemical profile such as polyphenolic constituents and flavonoids. Because of this, the quantification of phenolic and flavonoid constituents in each date palm cultivar was measured through in vitro assays using the calibration curve of the corresponding standards. Total six cultivars of date palm, As, Am, AJS, AJK, KP, and KB, were examined for their phytochemical quantification.

Among all tested sample Aseel showed the highest concentration of the phenolic content. While, AJ(S) were observed to have the lowest level of the flavonoid content. The date seed samples used in our investigation revealed phenolic compounds as gallic acid equivalents in various kinds, with As > KP > AJ(K) > KB > Am > AJ(S), respectively. The results of the phenolic compounds are shown in figure 2.

Flavonoid are the important secondary metabolite phytochemical and date seed sample were explored for presence of these compounds. The experiment displayed the quantity of flavonoid compounds in order of As > AJ(K) > KP > Am > KB > AJ(S), respectively, as equivalents of quercetin in all samples (Figure 3).

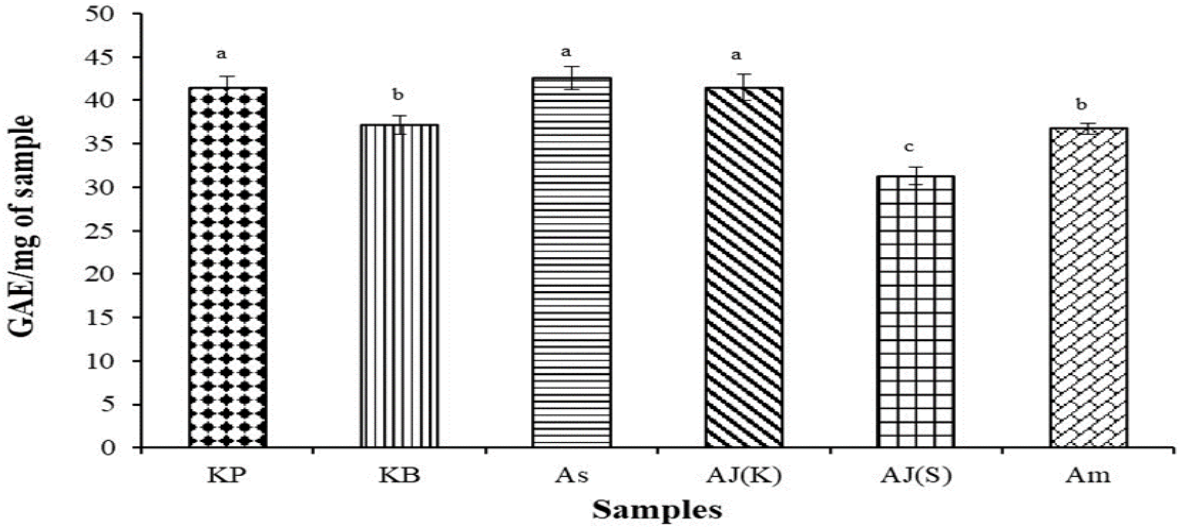

Figure 2. Quantification of total phenolic content in crude methanol extract of date seed samples. The results are shown as mean ± SD of three experiment performed in triplicate and phenolic quantity was expressed as gallic acid equivalent (GAE)/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am, respectively.

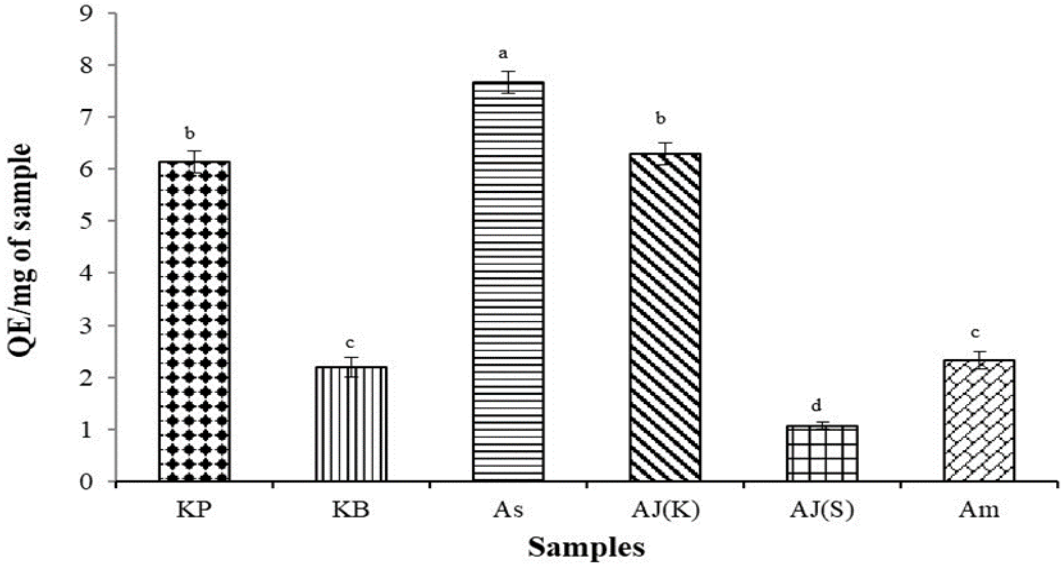

Figure 3. Quantification of total flavonoid content in crude methanol extract of date seed samples. The results are shown as mean ± SD of three experiment performed in triplicate and phenolic quantity was expressed as quercetin equivalent (QE)/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am, respectively.

ANTIOXIDANT ACTIVITIES

In the present study multimode antioxidant activity was performed using different *in vitro* assays such as total antioxidant assay through ammonium molybdate reduction potential, total reducing potential through potassium ferricyanide colorimetric method and free radical (DPPH) scavenging assay for six different cultivars of date palm seeds including As, Am, AJS, AJK, KP, and KB.

Crude methanolic extract of all samples showed the total antioxidant capacity in order of KP > Am > As > AJ(S) > As > KB > AJ(K), respectively as quantified as ascorbic acid equivalents. The results are shown in Figure 4. Among all tested sample KP was observed to highest Total antioxidant activity and AJ(K) has lowest activity.

The total reducing power seed samples in the increasing order of KP > AJ(K) > AJ(S) > As > KB > Am, respectively. The results are presented in Figure 5. Among all tested sample KP was observed to highest total reducing potential, while Am was observed to have lowest.

In addition to these, all samples exhibited the > 90% DPPH scavenging potential at the concentration of 200 µg/ml. Therefore, again evaluated at lower concentration of 7.4, 22.2 and 66.6 µg/ml and significant activity was observed by the samples as shown in Figure 6.

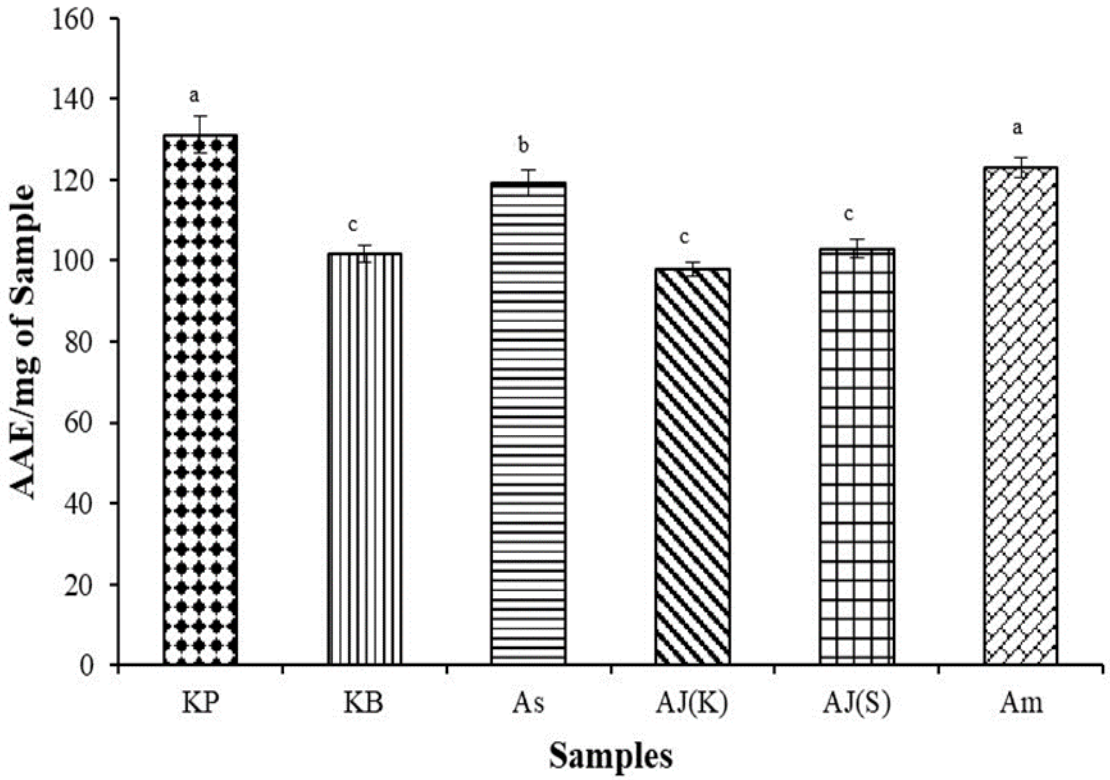

Figure 4. Assessment of Total antioxidant potential of crude methanol extract of date seed samples. The results are shown as mean ± SD of three experiment performed in triplicate and expressed as µg AAE/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

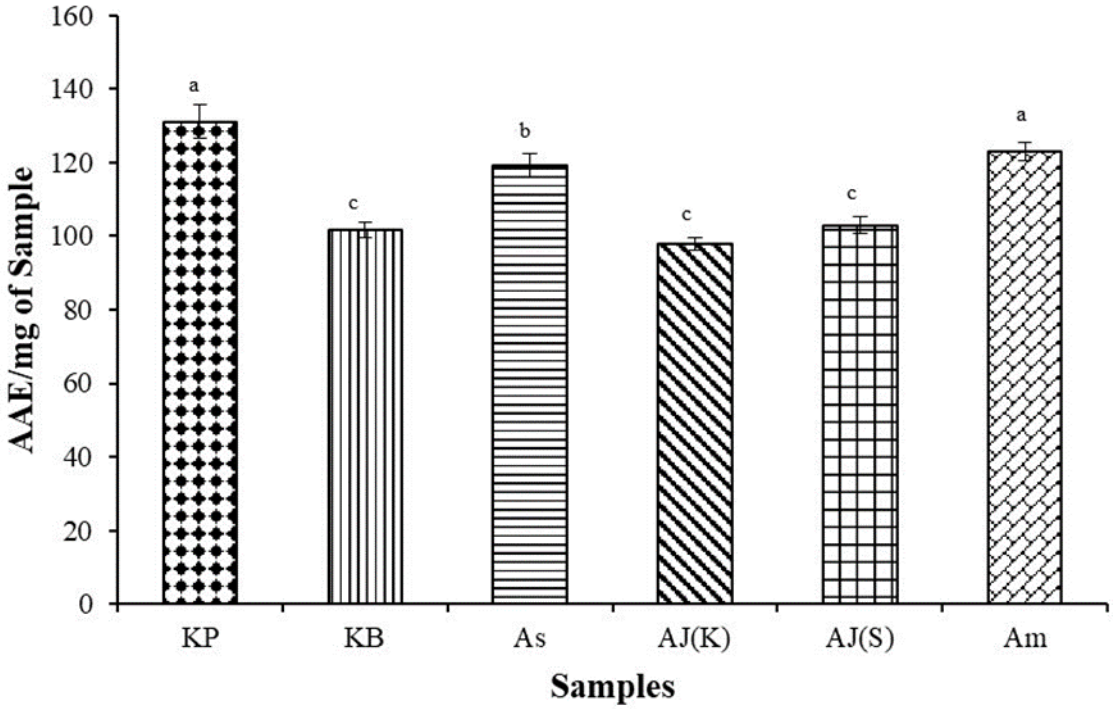

Figure 5. Assessment of total reducing potential of crude methanol extract of date seed samples. The results are shown as mean ± SD of three experiment performed in triplicate and expressed as µg AAE/ mg of extract. Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

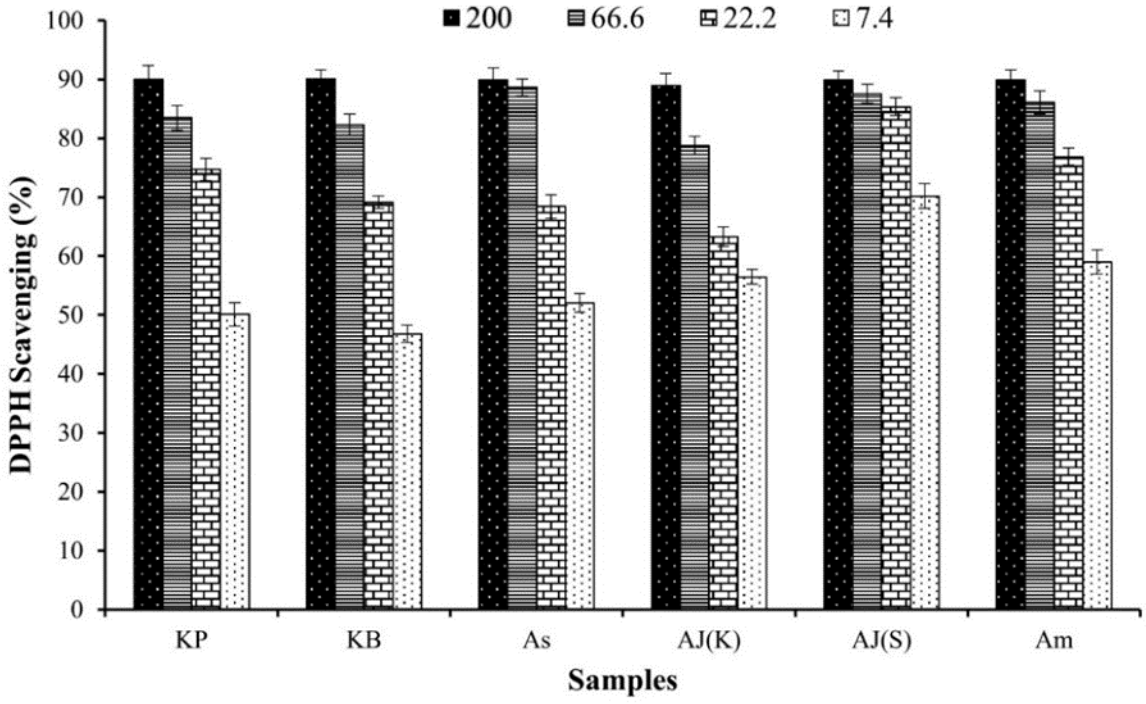

Figure 6. Free radical (DPPH) scavenging potential of the crude methanolic extract of date seeds. The results are shown as mean ± SD at indicated concentrations (µg/ml). Whereas Khipro, Karblyan, Aseel, Ajwa Khairpur, Ajwa Saudi and Ambar are abbreviated as KP, KB, As, AJ(K), AJ(S), and Am respectively.

DISCUSSION

Plants have long been used for the treatment and management a wide range of diseases. The fruit of date palm is a rich source of nutrients and high content of bioactive substances like phenolic compounds. It also possesses variety of therapeutic potential such as antioxidant, antimicrobial, anti-inflammatory and others (10). Date palm is supposed as a complete diet and medicinal plant as phytochemical studies have explained that the date palm contains anthocyanins, phenolics, sterols, carotenoids, and flavonoids. Due to the existence of distinct bioactive constituents, several parts of the tree, including the edible portion of fruits, seeds, leaves, and the spathe, have been linked to a variety of health benefits and therapeutic potential (11, 12).

Secondary plant metabolites with potential benefits for farm animals include polyphenols and flavonoids. These phytoconstituents are related to the taste, and astringency of plant-based products. They are divided into various groups, with the main bioactive components being flavonoids, lignans, phenolic acids, and stilbenes (13). Plant flavonoids have been considered as functional secondary metabolites with potential advantages, such as antioxidant and radical-scavenging properties or protection against various chronic, cardiovascular, or carcinogenic illnesses (14). Recently, a large number of *in vitro* and *in vivo* studies, as well as the identification and quantification of several classes of phytochemicals, have been conducted worldwide in response to the many health advantages of dates (15). Dates fluctuate in form, size, and weight according on the area. Furthermore, they differ in terms of their physical, chemical, and organoleptic properties (16).

In the current study, six variants of date palm, As, Am, AJS, AJK, KP, and KB, were examined for their phytochemical quantification, including their phenolic and flavonoid content and antioxidant activity, using several in vitro tests. Date seeds are continuously distinguished by a high concentration in phenolic compounds despite the fact that many standards have been utilized for phenolic measurement. More phenolic constituents are found in date seeds than other by-products of date (17). A previous study supports our findings, where phytochemicals of three date kinds (Shahal, Um-sellah, and Mabseeli) have been reported (7). In addition to this, the chemicals found in other date seeds and cultivars, obtained from other nations and under various conditions, are positively correlate with present study (18).

Phenolic and flavonoids are one of the important groups of secondary metabolites with significant bioactivities and therapeutic potential. The date seed samples used in our investigation revealed the presence of significant quantity of phenolic and flavonoid compounds (Figure 2). Variations of phytochemicals and biological activities in different studies can be attributed towards variety of factors such as environmental variables, cultivars, fruit maturity, and extraction conditions (19). Other variables like variety, geographic origin, growth circumstances, fertilizer, soil type, season, maturity, sunshine during ripening, or storage conditions might partially account for the observed variation among studies. Date seeds often contain more flavonoids than date fruits (20). Furthermore, the types of solvents and their polarity have an impact on the solubility of polyphenols (14, 21), and the date-seeds contain a variety of polyphenols, each of which may have a distinct polarity. In comparison to a solvent that is just aqueous, acetone in aqueous solution dissolves hydrophilic and high molecular weight molecules (10). One of study reported that phenolics and flavonoids had comparable extraction patterns, with 50% aqueous acetone producing the best results comparative to pure acetone (7). However, in our case methanol was used as solvent for extractions. Date seeds can be utilized to make extracts high in phenolics and flavonoids that could be used as useful substances for both people and animals, according to their chemical makeup.

Since antioxidants scavenge free radicals associated to a number of illnesses, including cancer, arthritis, heart diseases, diabetes, and Parkinson's disease, they have drawn a lot of interest. Catalase, superoxide dismutase, and glutathione peroxidase are examples of the antioxidants that the body naturally synthesizes to defend against free radicals. Altered production in these antioxidant results oxidative stress which is associated with diseases (16). Furthermore, antioxidants can be taken in nutrition to cope with these oxidative stress related conditions. Due the presence of the bioactive chemicals like phenolic constituents, date seeds have the potential to be used as function foods (16).

Possible natural antioxidant action of plant extract, phenolic and flavonoid compounds is well recognized (21). In this investigation, the widely used multimode antioxidant approach was utilized to measure the antioxidant capacity of plant products. Date seeds' high antioxidant capacity may thus promote their usage as natural antioxidants for therapeutic, nutraceutical, or pharmacological applications (22). Through the phosphomolybdenum technique, total antioxidant capacity was assessed and substantial activity was observed in all samples. Date seed samples were shown to have overall significant reducing power as measured through potassium ferricyanide technique. Total antioxidant potential and total reducing power activity was expressed as Ascorbic acid equivalents. All sample exhibited significant free radical (DPPH) scavenging activity. DPPH has frequently been used to evaluate the antioxidant capability of substances, such as plant extracts, and may be used to precisely titrate the oxidisable groups of biomolecules (23). In the present study all sample were found active in scavenging the DPPH free radical. Seeds samples of all six cultivars have shown considerable antioxidant activity. For thousands of years, dates have been a staple meal throughout the Middle East, and different parts of the world. There are many different cultivars of dates around the globe. Each kind of date has demonstrated therapeutic benefit in the prevention of different diseases (24).

The presence of flavonoids, phenolics, and other antioxidant substances (Vitamin-C, -E, carotenoids and other phytochemicals) in these by-products is responsible for their antioxidant action (25, 26). A prior study demonstrated that the phenolic content of date fruit was substantially linked with its ability to scavenge DPPH radicals. Compared to other fleshy fruits like figs, prunes, or raisins, dates and their pits would have more antioxidant activity (7, 27). For this reason, exploiting natural resources like plants, algae, and their products is a better, safer choice when searching for bio-functional or therapeutic compounds

CONCLUSION

Date palm (P. dactylifera L.) seeds are regarded as a problematic waste product, contain secondary chemicals that have biological activities, and initiated the research direction for promising applications in various fields. The present investigation highlights the antioxidant value and phytochemical assessment of different varieties of date seeds. All samples differ significantly in terms of antioxidant activities and quantity of secondary metabolites (phenolic and flavonoid contents). When comparing all samples, As was shown to have the highest amount of phenolic and flavonoids, while AJ(S) had the lowest levels. The KP samples had the highest overall antioxidant and reducing potential, whereas the AJ(K) and Am samples showed the lowest, respectively. The more research is recommended to characterize, isolate the phytochemicals and investigate other properties for possible use in food and biomedical industries.

Conflict of interest:

Authors declare no conflict of interest

Funding source:

The study did not receive any external funding

Ethical Approval:

The study was approved by local research ethics committee.

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Research Article

TITANIUM CLIPS FOR VASCULAR CONTROL OF RENAL VESSELS DURING LAPAROSCOPIC NEPHRECTOMY: A SAFE AND COST EFFECTIVE TERTIARY CARE CENTER EXPERIENCE

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ABSTRACT

This study aimed to evaluate the safety and cost-effectiveness of practice of titanium clip application on renal vessels in laparoscopic nephrectomy (LN) in a tertiary care hospital. A total of 143 patients were included in our study undergoing laparoscopic nephrectomy from January 2018 to April 2023. In our study, 3 titanium clips were applied on each renal artery and vein 1-2 mm apart from each other in order to achieve vascular control of the renal pedicle. This data was obtained retrospectively through medical records. We reported 31 (22%) patients in our study with malignant conditions and 112 (88%) patients with benign diseases. Of these, 4 (3 %) patients required blood transfusion. There were four cases where laparoscopic nephrectomy was converted to open procedure. No incidence of slippage of titanium clips used for vascular ligation was reported in our study neither did we encounter any clip dislodgement during or after the surgery. No significant post-operative complications pertaining to clip application were seen. Cost analysis revealed that most cases required a total of 6 titanium clips costing around 8 USD (4 USD per cartridge of 6 titanium clips) in total for vascular control. Titanium clip application for securing renal vessels during laparoscopic nephrectomy is a safe and cost-effective approach.

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Key Words: Titanium Clips, Renal Vessel, Laparoscopic Nephrectomy

INTRODUCTION

Laparoscopic nephrectomy was recommended by Ralph Clayman in 1991 for the first time ever as a safe and effective surgical approach for both benign and malignant kidney tumors (1). Ever since the proposal, this approach has been adopted and endorsed over open nephrectomy by urologists worldwide (2, 3). Laparoscopic nephrectomy has shown benefits over open technique due to its ability to provide a magnified field for surgery; and it results in shorter hospital stay and better cosmetic outcomes over the latter. The previously encountered reservations of longer duration of surgery have also been tackled with increasing expertise of surgeons and with better techniques to bring this surgery to fruition (4).

The two most pivotal steps in open and laparoscopic nephrectomy are hilar dissection and vascular control of the renal pedicle. Any mishandling encountered in attaining vascular control can result in catastrophic outcomes even if the morbidity and mortality reported due to any such event is around 0.03% to 0.07% (5). The US Food and Drug Association (FDA) has recommended usage of Gastrointestinal Anastomosis (GIA) stapling technique as the safest way to obtain vascular security; however, instances of its malfunctioning may result in dire complications (3, 6). There have been many other techniques that are being used by surgeons worldwide. These techniques include suture ligation, Hem-o-ok application and usage of titanium clip (7).

It has been presumed that using metallic titanium clips may be unsafe and risky pertaining to the chance of its slippage based off on the limited available data, but in a resource limited country, these are being widely used

in laparoscopic surgeries. Other than that, there is lack of sufficient data of its usage on diseased kidneys undergoing laparoscopic nephrectomy. Keeping this in mind, we conducted a retrospective study in our tertiary care hospital to assess the safety and reliability of using titanium clips to obtain vascular control in laparoscopic nephrectomy.

METHODS

We conducted a retrospective observational study in the Urology department of Dow University Hospital Karachi, Pakistan from January 2018 till April 2023 after seeking approval from Ethics Review Committee (IRB-3108/DUHS/EXEMPTION/2023/283). Using non probability convenient sampling, 143 patients were enrolled in our study that underwent laparoscopic nephrectomy for benign and malignant kidney diseases. Patients from all age groups were included in our study and patients undergoing laparoscopic donor nephrectomies were excluded from the study. The primary outcome studied included intraoperative blood transfusions, conversion to open and if additional surgery required to control bleeding. The secondary outcome studied include the length of hospital stay, post-operative complications and change in hemoglobin levels post operatively. We collected this data from medical records and clinical registers.

Material involved in this study included Ligaclips of sizes 400 and 300 used for renal vein and renal artery ligation respectively using a clip applicator. A total of 3 titanium clips was applied on each renal vessel 1 to 2mm apart. Once the clips were placed adequately, the renal vessels were transacted with the help of Ligasure. Each titanium clip cartridge that consists of 6 clips each costs 4 USD. We use 3 clips from each cartridge of sizes 300 and 400 but those cartridges are not used thereafter so we used the cost of 2 cartridges instead of 6 clips in our study analysis. Titanium clip application and renal vessel stump is shown in figures 1 and 2.

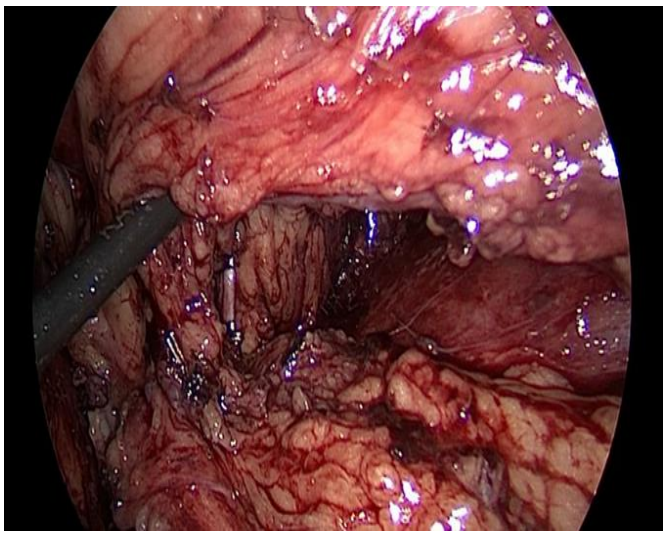

Figure 1. Placement of titanium clip on a renal vessel

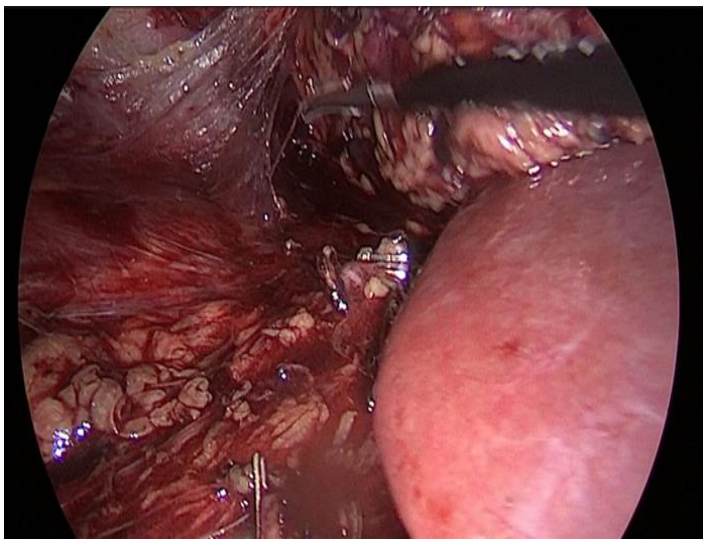

Figure 2. Residual renal vascular stump after ligation of vessel

Statistical analysis

Data analysis was done using SPSS version 21. Categorical variables were represented using metrics of frequencies and percentages and continues variables were represented as means and standard deviation. T-test and chi-square test were applied where needed and p-value of < 0.05 was considered significant.

RESULTS

To achieve renal vascular control, titanium clips were used on 143 consecutive patients with benign and malignant conditions of kidney undergoing laparoscopic nephrectomy. The mean age of the patients included in the study was 46.33±1.25 years and their mean Body Mass Index (BMI) was

30.2±1.34 kg/m2. Of this cohort, 74 (52%) patients were males and 69 (48%) were females. The demographics of the patients included the study are presented in Table 1.

Demographic variables		No. (percentages)
Gender	Male	74 (52%)
	Female	69 (48%)
Mean age in years		46.33±1.25
Mean BMI in Kg/m2		30.2±1.34
Comorbid	Asthma	4 (2.8%)
	DM	21 (15%)
	HTN	13 (9%)
	DM + HTN	2 (1.4%)
	IHD	3 (2%)

Table 1. Demographic characteristics of the patients who underwent laparoscopic nephrectomy

Amongst our study group, 31 (22%) patients had malignant conditions for which they underwent laparoscopic nephrectomy and 112 (78%) patients had benign diseases which included 38 cases with Pelvi-Ureteric Junction Obstruction (PUJO) with nonfunctioning kidney and 74 cases with renal and ureteric stone and nonfunctioning kidney. A total of 74 (52%) left sided and 69 (48%) right sided laparoscopic nephrectomies were performed. The mean operative wheel in to wheel out time was 125±23.4 minutes and estimated blood loss was 70±10.5ml.

123 (86%) of our patients undergoing this surgery had a single renal vein and 128 (90%) of them had a single renal artery. A total of 4 patients required blood transfusion, 2 of which were done intra-operatively and 2 were done post operatively, 4 cases were converted to open owing to dense adhesions encountered during kidney dissection and 12 patients developed post-operative complications that included intra-abdominal collection in 4 patients, post-operative ileus in 7 patients and surgical site infection in 5, all of which were managed conservatively. The operative parameters of our study population are given in table 2.

Titanium clips were sufficient in all cases to achieve renal hilar control. It is noteworthy that there were no incidences of slippage of clips applied on renal vessels and no intraoperative or post-operative instance of difficulty to control hemorrhage due to the clips' dislodgement.

Each titanium clip costs 8 USD. The cost of titanium clips (6 clips) used per nephrectomy was 48 USD and 15 patients with two renal arteries and 20 patients with two renal veins were charged 72 USD in total. The price of titanium clips with those of endovascular GIA Staplers and Hem-o-lok are compared in the table below. We have included price of two cartridges of titanium clips; one of size 300 another of size 400, instead of individual clips' cost as those cartridges are used in only a single case to ensure sterilized instrumentation.

DISCUSSION

Securing renal vessels is a vital step in minimally invasive nephrectomy and is the main determinant of its success. There has been an ongoing debate on the safety and quality of various methods available in the market to achieve this control. Using FDA database, various studies have been done. One such study indicated malfunctioning of titanium clips like scissoring and malformation and one manufacturer of titanium clips suggested against its use on renal arteries (8, 9). Despite such reported issues, serious complications with titanium clips have rarely been reported.

The literature has conflicting outcomes of usage of titanium clips on renal vessels. Kerbl et al occluded renal artery with 9mm titanium clips and observed that they were as safe as securing renal vessels with 0 and 2-0 silk ligature. In the same study, it was also postulated that 2.5mm staples

placed on renal arteries did not yield equally safe results. Based on these observations, they suggested usage of 3 titanium clips on each vessel stump during the renal pedicle dissection step in the surgery (10).

Table 2. Intraoperative and Postoperative parameters of patients who underwent Laparoscopic nephrectomy

Operative parameters		**Statistical metrics**
Mean operative time (Wheel in - wheel out time in minutes)		125±23.4
Operative site	Right	74(52%)
	Left	69 (48%)
Number of Renal Artery	1	128 (90%)
	2	15 (10%)
Number of Renal Vein	1	123 (86%)
	2	20 (14%)
Number of ports	3	128 (90%)
	4	15 (10%)
Estimated Blood Loss (ml)		70±10.5
Blood transfusions		4(3%)
Conversion to open		4(3%)
Length of hospital stay (pre-operative day until the day of discharge)		3.35±0.94
Post-operative complications	**Ileus**	7 (5%)
	Port site infection	5 (3.5%)
	Intra-abdominal collection	4 (3%)
Hemoglobin levels	**Pre-operative**	13.4±1.09
	Post-operative	11.8±0.8

Table 3. Comparison of devices' cost for renal hilar control.

Material for vascular control	**Maximum quantity of material required**	**Price incurred per case (in USD)**
Titanium clips	6-8	8 (4 USD Per cartridge)
Hem-o-lok clips	6-8	64
Endovascular GIA staplers	2	250

It has been suggested that the failure of clips to control renal vasculature could be attributed to surgeons' technique and expertise more than the physical limitations of the clips itself (5). Leaving a vascular cuff of 1 mm from the point of titanium clips applied on renal vessels has proven to be safe at both physiologic pressures and those higher than that (11). Other studies have demonstrated that multiple clips application adds to this safety (12, 13). One of the largest scale study conducted in Canada concluded that in the 489 cases of laparoscopic nephrectomy where titanium clips were used to secure renal artery, only 5 intraoperative events were noted. These events included crossed clips

and dislodgement of clips but there was no major event that warranted conversion to open nephrectomy (14).

Papaioannou et al was able to perform a test on silicone tubing which showed there was no leakage of blood when titanium clips were applied to it (15). Joseph et al tested various methods for renal vessel ligations all of which were able to tolerate pressures of more than 800mmHg (16). Sean et al also concluded that titanium clips safely attain vascular control at physiological pressures (17). No instance of migration of titanium clips was seen on a 4 year follow up by Chibber et al (18).

Liu et al concluded that residual vascular length was greater when clips were applied making it ideal for donor nephrectomies as well. The same meta-analysis showed that using titanium clips is also cost effective when compared with GIA Staplers and Hem-o-lok clips (19). The cost saving benefit has been shown by other researchers as well (18).

Our study has also shown that titanium clips when used in laparoscopic nephrectomies yielded good outcomes in terms of vascular ligation and were a safe and less expensive approach in a resource limited country when performed by experienced surgeons. These clips have made laparoscopic nephrectomies a financially feasible surgery in developing countries such as our own and the theories of clip dislodgement was not validated in our study.

The limitation of our study was that it was conducted in a single center and only one modality to secure renal pedicle was studied. However, in order to offer comparative study, it is important that surgeons should have expertise in performing all methods of renal hilar control. Therefore, we recommend a multicenter study to evaluate these techniques. Our experience clearly suggests that titanium clips are safe and cost effective means of securing renal vessel in a resource limited country.

CONCLUSION

The safest and most economically feasible method for renal vascular control is still controversial. However, as there was no episode of major bleeding during or after the surgery in our study, it is safe to say that titanium clips when applied by trained surgeons taking necessary precautions and following the guidelines for safety are a safe and cost effective means for achieving renal vascular control in laparoscopic nephrectomy. It is still important that the surgeons using these clips have adequate experience and expertise in terms of surgical technique in order to achieve successful results.

Conflict of interest:

Authors declare no conflict of interest

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Ethical Approval:

The study was approved by local research ethics committee.

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Research Article

ANALYSIS OF PATTERN OF SALIVARY GLAND CANCERS: DESCRIPTIVE ANALYSIS FROM A SINGLE CENTER

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ABSTRACT

Salivary gland carcinomas are the malignant tumours arising from major or minor salivary glands, accounting for 5% of all head and neck cancers. There is scarce data available regarding pattern of presentation and clinical outcome of salivary glands in Pakistan. Thus this retrospective analysis of the patients presenting with salivary gland tumours was designed. A total of 305 patients were diagnosed with salivary gland cancers from 2008 till 2023, including 127 (41.6%) of females and 178 (58.4%) of male patients. There were 191 (62.6%) patients with parotid gland cancer and 114(37.4%) with submandibular and other minor glands. Mucoepidermoid carcinoma was the most prominent histological type followed by Adenoid Cystic carcinoma. Grade 2 (44.1%) was most frequently reported followed by Grade 1 (33.9%) and 3 (22.0%). These cancers did not show any significant association with age or gender. Salivary gland cancers are relatively rare cancer. Further studies to explore biological characteristics and long term clinical outcome are recommended.

Key Words: salivary gland cancers, head and neck cancer, clinical presentation

INTRODUCTION

Salivary gland cancers are relatively rare comprising of 5% of all head and neck cancers(1). Ionizing radiation is well established risk factor for salivary gland cancers, other associated risk factors include previous history of head and neck cancers. History of smoking and industrial exposure of certain toxins has been linked with the cancer, though with weak evidences(2). These cancers are rare and present in a variety of histological types. Till date at least 20 histological types have been reported(2). The histological types include Mucoepidermoid, adenoid cystic, adenocarcinoma, pleomorphic adenocarcinoma, salivary duct carcinoma, carcinosarcoma and other types. The standard treatment offered as per guidelines is surgery as primary therapeutic option if not operable then radiotherapy as general protocol. The data regarding treatment and clinical outcome is also based on small scale studies. The rate of salivary gland malignancies reported from India was 77.5% among all tumour of salivary glands where only 22.5% were benign tumours(3). The benign tumours of salivary gland also occur thus biopsy is essential part of diagnosis. However, fine needle aspiration cytology is suggested as primary line making diagnosis supported by MRI imaging. Contrast CT scan is only advised when MRI is not recommended.

The recent advances in oncology with emergence of novel therapeutic options there is still lack of robust evidence in these cancers due to their low incidence. In general operable tumours are treated by surgery while inoperable cancers are offered radiotherapy(2). These cancers in general show good prognosis with >90% 5-year survival rate(4). There has been limited literature presented on salivary gland cancers in Pakistan. Therefore, this study was designed to report pattern of salivary gland cancers in Pakistan.

METHODS

This was a retrospective study including cancer patients diagnosed and treated at Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro Pakistan and their all clinical data available in Cancer

Research database at Cancer Research Laboratory, Medical Research Centre (LUMHS). The database is prospectively developed and regularly updated. This includes patients from 2008 till date.

For this study patients with confirmed diagnosis of primary salivary gland cancer were included. The histological types and grades were taken from biopsy reports. Salivary gland cancers were defined as the cancers arising from parotid, submandibular and from other minor glands. Where parotid cancers were included as one category while h other salivary glands were merged together as submandibular and other minor glands.

Statistical analysis

Data was entered and analyzed by using Statistical Package for Social Sciences (SPSS) version 22.0. The continues variables were analyzed for central tendency and dispersion and presented with median and Standard deviation. Categorical data was analyzed for frequency. Chi-squared test was used for analysis of categorical variables, whereas a p-value of <0.05 taken as significant.

RESULTS

A total of 39044 patients were presented with cancers, out of which 305 had primary salivary gland cancers including 191 (62.6%) in parotid gland and 114 (37.4%) in submandibular and other minor glands. There were 178 (58.4%) male patients and 127 (41.6%) female patients. Median age of the patients was 48 years (±SD=16.47). Age distribution of males and female patients is presented in Figure 1. There was no significant association of age with gender (Figure 2) or the site of cancer (Figure 3). Mucoepidermoid carcinoma was the most prominent histological type followed by Adenoid Cystic carcinoma.

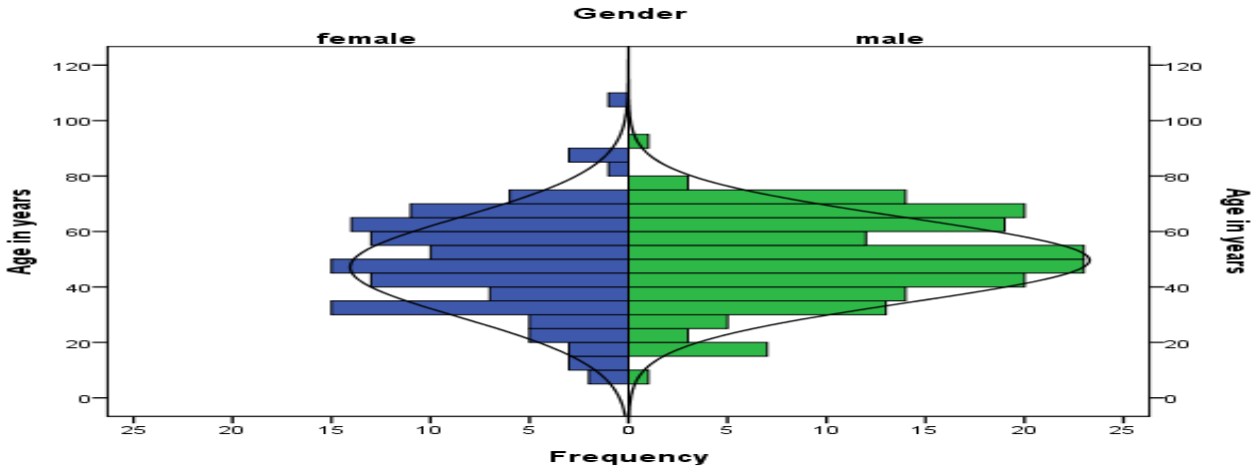

Figure 1. Age distribution of patients presenting with salivary gland cancers: Male versus Female

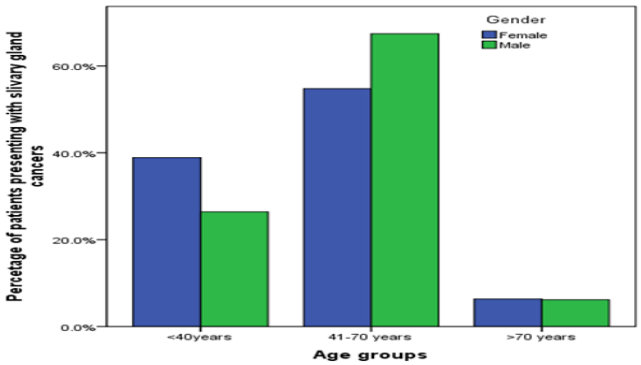

Figure 2. Association of gender with age in patients presenting with salivary gland cancers

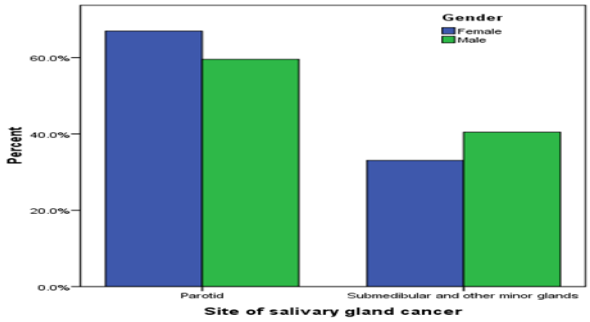

Figure 3. Association of gender with site of salivary gland cancers

The was no significant association of age and the site of the cancer (Figure 4). Grade 2 was dominant with 44.1% of patients followed by grade 1 (33.9%) and grade 3 (22.0%) (Figure 5). There were 43.3% of patients with stage IV disease. A summary of stage and association with the site of tumour is presented in Figure 6 and 7.

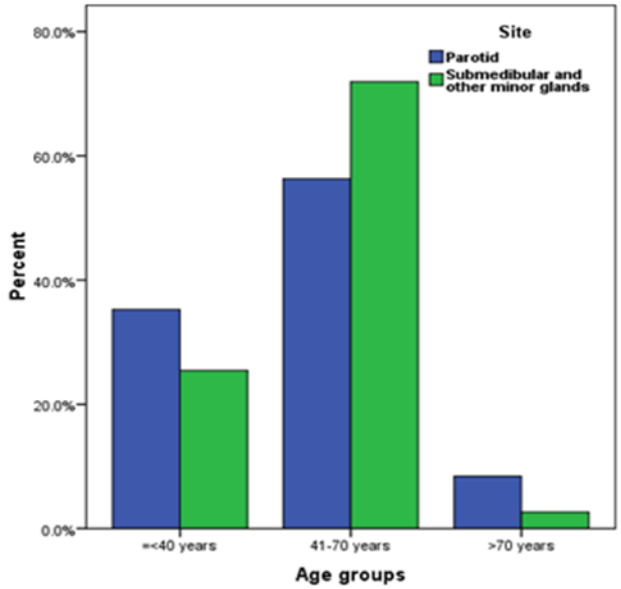

Figure 4. Association of age with site of salivary gland cancers

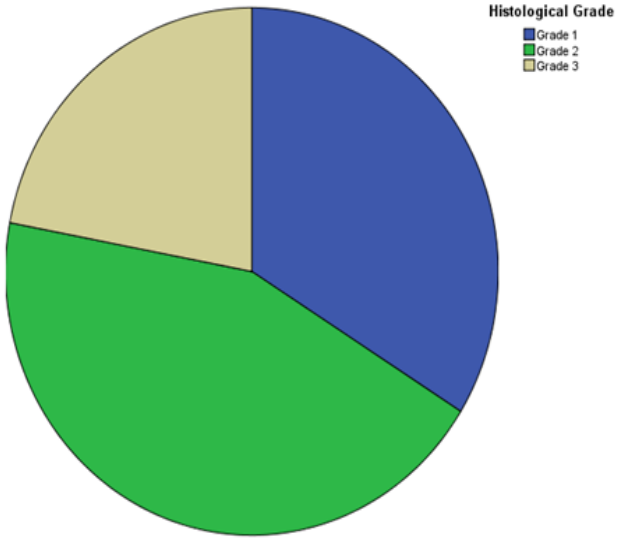

Figure 5. Pattern of histological grade in salivary gland cancer

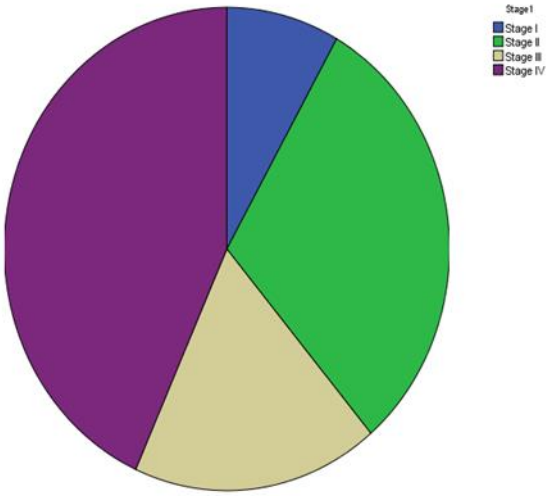

Figure 6. Stage of the cancer at the time of diagnosis in patients with salivary gland cancers

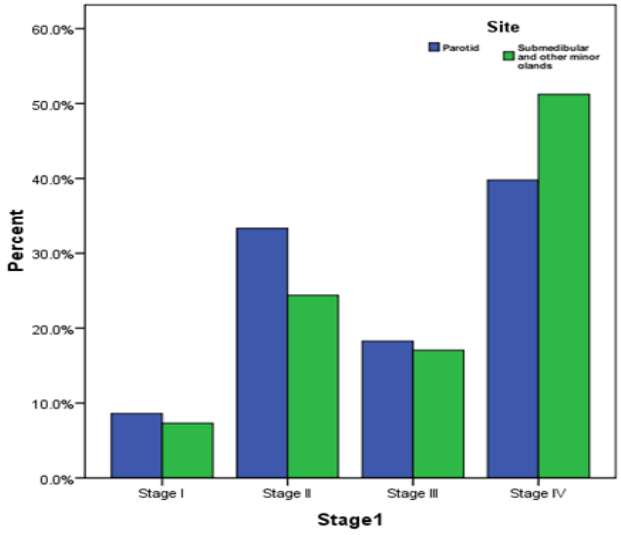

Figure 7. Stage of the cancer at the time of diagnosis: Parotid gland cancer versus submandibular and others

DISCUSSION

Salivary gland cancers are rare, in our study they account for 0.8% of all cancers. There was no significant association with age or gender was presented in our study. Parotid gland cancer was highly prevalent as compared to other salivary glands.

The Globocan 2020 has also reported salivary gland cancer as one of the least occurring cancers. The rarity causes lack of large scale studies and large clinical trials for recommendation of the evidence based

medicines. Recently, biological markers have been recommended to be studied so that treatment based on molecular classification can be advised for better clinical outcome(5).

The previously reported studies have suggested age and gender dominance, however there was only non-significant difference seen in our study(6). This may be geographical variation or may be linked with prevalence of head and cancers in other regions as radiotherapy and ionizing radiation as salivary gland cancers have shown significant association with previous history of both(7). Though there are studies where there is no significant difference is observed, but the results remained inconsistent (8).

Mucoepidermoid type was predominant in our series, this was consistent with other studies(9). Other studies have also reported the same with second most common histological types being adenoid cystic carcinoma(6). Previously a study reported from Finland including children (n=20), also showed parotid as the most common site with seven out of ten cancers and mucoepidermoid carcinoma as the most common histological types seen in five out of ten cases(10). There was no significant association of grade is observed. Similarly stage also did not show any significant association with site of the cancer. There is limited data available for comparison.

There is limited literature available on salivary gland cancers from Pakistan. This was also a retrospective analysis of an institutional database which is considered as the limitation of this study. Further large scale studies with large sample size is recommended. Further analysis of the molecular pattern is also recommended.

CONCLUSION

Salivary gland cancers are relatively rare cancer where parotid gland is the predominant site of the salivary gland cancer. There is no predominant pattern of association with age and gender observed. Further large scale prospective studies are recommended.

Conflict of interest:

Authors declare no conflict of interest

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Review Article

TRANSPLANT GLOMERULOPATHY: A BRIEF NARRATIVE REVIEW IN THE LIGHT OF INFORMED LITERATURE

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ABSTRACT

Transplant glomerulopathy is characterized by a post-transplant morphologic insult. It occurs as a consequence of chronic, periodic injury of endothelial cells triggered by chronic hepatitis C virus (HCV) infection, donor-specific antibodies (DSA), cell-mediated injury or thrombotic microangiopathy. The disease might not be overt; however, it often presents with obvious symptoms, such as proteinuria (nephrotic range), hypertension, and deteriorating glomerular filtration rate (GFR). Histopathology is the mainstay diagnostic tool for transplant glomerulopathy patients and electron microscopy serves the purpose to early diagnosis. The classic histopathological feature of the disease is glomerular basement membrane thickening with reduplication on light or electron microscopy without immune complex deposits. Conventional therapeutic regimens include intravenous immunoglobulins (IVIG), plasmapheresis and splenectomy, and lately rituximab, eculizumab and bortezomib. Transplant glomerulopathy patients usually have positive history for acute rejection, resulting mostly in antibody-mediated rejection. DSAs against class II is specifically linked with transplant glomerulopathy. Unfortunately, the prognosis of kidney allografts is dismal even under existing immunosuppressive regimens.

Key Words: Kidney transplantation, Transplant granulopathy, Early diagnosis and treatment

INTRODUCTION

Transplant glomerulopathy is characterized by a post-transplant morphologic insult, often linked with antibody-mediated allograft rejection. The classic histopathological feature of the disease is glomerular basement membrane thickening with reduplication on light or electron microscopy without immune complex deposits. Transplant glomerulopathy ensues as a consequence of chronic, periodic injury of endothelial cells triggered by chronic hepatitis C virus (HCV) infection, donor-specific antibodies (DSA), cell-mediated injury or thrombotic microangiopathy. Most prominent etiology is antibody-mediated rejection. Transplant glomerulopathy, in some cases, might not be overt, identifiable on biopsy, or present with obvious symptoms, such as proteinuria (nephrotic range), hypertension, and deteriorating glomerular filtration rate (GFR). It is one of the key causes of decreased allograft survival (1).

Transplant glomerulopathy is linked with worse kidney allograft outcomes (2-4). Several risk factors have been reported in the literature to be associated with transplant glomerulopathy which include age, DSA, HCV infection and post-transplant acute rejection beyond 3 months (5, 6). The diagnosis of transplant glomerulopathy post-transplantation is 1 and 5 years in 4% and 20% of the patients, respectively, suggested a study (5). However, few studies have also demonstrated lesser overall incidence of transplant glomerulopathy (6-9). Earlier study of 16 years of retrospective analysis reported transplant glomerulopathy in 4% of the transplanted patients (10). It is possible that the true prevalence of transplant glomerulopathy might be underestimated due to infrequent practice of performing protocol histology testing and most

probably missing the subclinical transplant glomerulopathy (10). In biopsies, the average time duration from kidney transplantation to transplant glomerulopathy diagnosis is 2 to 9 years (6, 10-13). Lately, a study suggested that post-transplant proteinuria during 1 year might forecast the development of transplant glomerulopathy in 5 years in highly sensitized patients (3). Ironically, transplant glomerulopathy is one the less studied transplant diseases that needs considerable attention and therefore the present paper presents the brief narrative review of transplant glomerulopathy based on informed literature.

DIFFERENTIAL DIAGNOSIS:

The causes for transplant glomerulopathy can be generally classified into immunological and non-immunological. The first category includes antibody-mediated rejection such as presence of HLA-antibodies, particularly DSA against Class II, T-cell mediated rejection and thrombotic microangiopathy. Non-immunological causes include age, hepatitis C virus positive serology, drug toxicity such as cyclosporine and previous acute rejection above 3 months of post-transplantation (14, 15).

Based on Clinical Presentation:

Based on common clinical presentations of elevated creatinine levels, proteinuria and hypertension in patients with transplant glomerulopathy, following differential diagnosis are possible:

- IgA Nephropathy
- Focal Segmental Glomerulosclerosis
- Membranous Nephropathy
- Membranoproliferative Glomerulonephritis
- Anti-Neutrophil Cytoplasmic Antibody-associated Glomerulonephritis
- Systemic Lupus Erythematosis (SLE)

Based on Histopathology:

Based on only morphology with glomerular basement membrane duplication, differential diagnoses will include

- Membranoproliferative Glomerulonephritis
- HCV Infection-related Glomerulonephritis
- Lupus Nephritis
- Cryoglobulinemia
- Thrombotic Microangiopathy

Usually, the diagnosis is straightforward in case if ultrastructural immunofluorescent assessment of the kidney allograft histology is performed. However, often anti-phospholipid syndrome, hemolytic-uremic syndrome or antibody-mediated chronic thrombotic microangiopathy can pose challenge in the diagnosis of transplant glomerulopathy, if there is negative C4d staining.

Investigations:

Histopathology is the mainstay diagnostic tool for transplant glomerulopathy patients. Transplant glomerulopathy possesses unique structural insult identified by thickness of glomerular basement membrane and duplication. In 1999, Racusen and team suggested the Banff 97 categories of kidney allograft pathology to identify the severity of double contours on grounds of transplant glomerulopathy grading (16). Other major histopathological changes include diffuse endothelial and mesangial swelling with narrow capillary loops (17), rise in cellularity, mesangial matrix and segmental scars (18), often with intracapillary thrombi of fibrin and cellular crescents (17).

Electron microscopy serves the purpose to diagnose early transplant glomerulopathy, by looking for the endothelium segregation from glomerular basement membrane, widening of subendothelium, new glomerular basement membrane development +/- cellular processes interpositioning (19, 20). However, there is no fixed criteria for diagnosis of transplant glomerulopathy through ultrastructural examination (21). The changes in ultrastructure also impact peritubular capillaries along with glomerular capillary loops

culminating in multiple layering of glomerular basement membrane (22), and has been reported in 90% of transplant glomerulopathy patients (23).

Endothelial staining of C4d, which include peritubular capillaries, is crucial to assessing the kidney allograft transplant biopsies. The C4 is a part of complement system pathway and is triggered by C1q activation through antigen–antibody complexes or sometimes directly initiated by bacterial polysaccharides (24). Now-a-days, it is a universal clinical practice to stain all the kidney allograft biopsies for C4d to investigate antibody reactivity with the help of complement fixating antibodies. This can be done through direct immunofluorescence microscopic examination on frozen tissue sample or through immunohistochemistry on fixed tissue (1).

Treatment Options:

Transplant glomerulopathy is a frequent reason for late kidney allograft rejection and loss, without availability of any efficacious treatment owing to long-standing and irrevocable nature of the condition (25). Conventional therapeutic regimens include intravenous immunoglobulins (IVIG), plasmapheresis and splenectomy, and lately rituximab, eculizumab and bortezomib (25, 26). In actuality, present treatment strategies are based on clinical experience, rather the randomized controlled trials or level 1 evidence (14). However, a handful of research studies have been carried out to explore new treatment regimens and their effectiveness for transplant glomerulopathy and have been summarized below.

A study by Sablik et al. (2016) treated transplant glomerulopathy patients with IVIG and pulse methylprednisolone (MPDN). The study found significant decrease in proteinuria from 0.62g/L/year to 0.11g/L/year (P=0.003) and concluded that IVIG and MP therapy is correlated with about 50% decline in loss of eGFR post-treatment within the first year (27). Similar research group cited decrease in progression of eGFR deterioration and improvement in proteinuria in over 60% of their transplant glomerulopathy patients administered with IVIG + MPDN (28). Abreu et al. (2017) in their retrospective analysis reported number of treatment regimens in their study for transplant glomerulopathy; Rituximab + IVIG, increase of immunosuppression, Plasmapheresis + Rituximab + IVIG, Rituximab + IVIG + Tacrolimus, Rituximab + MPDN, MPDN + increase of immunosuppression, Everolimus, Plasmapheresis + Rituximab + IVIG + Tacrolimus + increase of immunosuppression, Rituximab + IVIG + MPDN + Everolimus, Rituximab + IVIG + MPDN + increase of immunosuppression, Rituximab + IVIG + MPDN + Tacrolimus, MPDN + Tacrolimus + increase of immunosuppression, Rituximab + Tacrolimus + increase of immunosuppression, IVIG + MPDN and Rituximab. However, based on the results, neither of the treatment options improved the long-term survival of kidney allograft in transplant glomerulopathy patients (29). Another study also suggested no efficacy of IVIG + Rituximab treatment in severe cases of transplant glomerulopathy and was found to be related with high rate of adverse events (30).

The present treatment options are grounded on preventive recommendations, for instance, surveillance of donor specific antibodies (DSA), preventing antibody mediated allograft rejection and emphasizing medication adherence (31, 32). The use of anti-proteinuric agents (e.g., ACE and ARB) is currently ongoing (33). Desensitization protocols have also been utilized in highly sensitized transplant patients at risk for developing antibody mediated rejection (32, 33). These strategies in chronic antibody mediated rejection and/or transplant glomerulopathy patients have been used without any evidence of significant improvement (34). A study did not show significant findings with the use of rituximab in transplant glomerulopathy patients (35).

Bortezomib has been shown to be effective in cases of chronic antibody mediated rejection based on their mechanism of action of B-cells depletion in experimental studies (36-39). A randomized controlled trial is currently under process to examine whether bortezomib deters transplant glomerulopathy in patients with high levels of post-transplantation DSAs (NCT01349595 on ClinicalTrials.gov). The pathogenesis of transplant glomerulopathy also involves complement pathway; the C4d deposits in kidney biopsies of transplant glomerulopathy patients (14). Therefore, studies are determining the role of breaking this complement

pathway to prevent the development of transplant glomerulopathy. Eculizumab, an anti-C5 humanized monoclonal antibody, is the new drug currently under investigation for transplant glomerulopathy. Stegall et al. (2012) demonstrated that eculizumab reduced the antibody mediated rejection in highly sensitized patients (40).

Prognosis:

As mentioned earlier, transplant glomerulopathy is associated with decline in kidney allograft survival. Transplant glomerulopathy, unfortunately, has particularly poor prognosis in terms of allograft loss (41). Research literature also indicates poor prognosis linked with transplant glomerulopathy. Nair et al. (2010) reported poor allograft outcome in patients with acute transplant glomerulopathy mediated by antibody-mediated allograft rejection (42). Gloor and colleagues suggested that subclinical transplant glomerulopathy affects long term allograft kidney outcomes (5). Another study highlighted that kidney allografts after a decade of transplantation encountered 1/3rd decline in allograft survival in comparison with nearly 2/3rd in the matched control cohort (10). Other studies have also attested these findings (3, 7). One study mentioned post-diagnosis median allograft survival of 43 ± 7 months (43). The reported death censored allograft 5 years survival was 16.7% (4), whereas death censored allograft 10 years survival was 56% (44). Studies have also demonstrated high frequency of allograft loss in a very short time frame; Maryniak et al. (1985) cited 77% allograft failure within 3 years of diagnosis (17). Briner et al. (1993) documented 60% of the allograft loss within 6 months of diagnosis of transplanted glomerulopathy (45). A handful of research studies have tried to determine the prognostic value of diverse clinical and histological variables of transplanted glomerulopathy, in order to identify subpopulation with slower disease process, establishing a cohort for therapeutic adjustment. Literature communicates that severity of double contour in glomerular basement membrane substantially affects the allograft function and survival (43). Moreover, a study cited that transplant glomerulopathy severity is directly proportional to worse allograft survival (57% allograft incidence in grade I versus 87.5% in grade III (44). This result was also held by another study (46). Moreover, C4d is regarded as an independent predictor of decreased allograft survival in transplant glomerulopathy (43), and studies have shown that transplant glomerulopathy with C4d is associated with reduced allograft function (47).

DISCUSSION:

Transplant glomerulopathy is a pathologic diagnosis of kidney allograft that was first identified almost four decades back (48). It is mainly documented as a pathological insult of chronic kidney rejection. Transplant glomerulopathy is diagnosed in the classification of chronic allograft nephropathy (CAN) with chronic allograft kidney rejection in the Banff 97 classification, and of chronic antibody-mediated rejection in the Banff 05, 07 and 09 classifications (49).

The risk of transplant glomerulopathy is greater in patients with previous transplantation, acute rejection, presence of HLA antibodies and antibody-mediated rejection. A study by Sis et al. suggested increase incidence of antibody-mediated rejection (54%) in their biopsy-proven transplant glomerulopathy cases (50). Few other research studies communicated that about 45% of cases with antibody-mediated rejection subsequently progressed to transplant glomerulopathy in comparison with 6% of kidney recipients without rejection (51, 52). Transplant glomerulopathy manifests as progressive deterioration of kidney function, evidenced by high creatinine levels and proteinuria. In initial stages, the patients may manifest mild sub-nephrotic range proteinuria and unexplained moderate decline of kidney allograft function (1).

The interstitial and glomerular inflammation and thickening of the basement membrane of the peritubular capillaries are obvious in the progression of the transplant glomerulopathy, which indicates chronic and active inflammatory immune response in the glomerular basement membrane and microvasculature. This is also supported by presence of double contours and patent capillary loops with highly thickened basement membranes, endothelial and epithelial cells swelling and mild mesangial expansion. Chronic transplant glomerulopathy can have varied manifestations such as presence of focal and segmental glomerulosclerosis, glomerular basement membrane splitting, segmental sclerosis and hyalinosis, mesangial interposition and

increased lucency of the lamina rara interna and infrequent interposition, with no immune complexes (53). These manifestations have been illustrated in Figure (53). Earlier studies have confirmed the presence of glomerular, interstitial and peritubular capillary inflammation (5, 12, 54). Gloor et al. (2007) documented that transplant glomerulopathy is associated with glomerular inflammation and advances to duplication of the glomerular basement membrane. They also highlighted that progression of transplant glomerulopathy is also associated with consistent peritubular capillary inflammation (5). Sis et al. (2007) reported glomerulitis and peritubular capillary inflammation in 35% and 70% of the biopsy specimen of transplant glomerulopathy patients, respectively (50). Sun et al. (2012) found 94% glomerulitis and 90% peritubular capillary inflammation in transplant glomerulopathy patients (55). With reference to basement membrane thickening of the peritubular capillary, Aita et al. (2007) proposed that it could be a promising diagnostic biomarker of chronic allograft rejection. They suggested that peritubular capillary basement membrane thickening score calculated through light microscopy could reflect peritubular capillary basement membrane multilayering evidenced by electron microscopy (56).

Previous studies have frequently labeled transplant glomerulopathy manifestation as late, appearing after few years of kidney transplantation. In fact, it was seldom reported within one year of transplantation (57, 58). Sis et al. (2007) reported early diagnosis (3.8 months) of transplant glomerulopathy in their study (50). Studies have mentioned that the occurrence of transplant glomerulopathy is quite high, troubling 4% of the transplant patients after one year of transplantation (51, 59). Gloor et al. (2007) reported both early (4 months) and late (21 months) diagnosis of transplant glomerulopathy (51).

Peritubular capillary deposition of C4d has been observed previously in 57% of the biopsy specimens, which included 45% diffuse staining (C4d3), 11% focal staining (C4d2) (49). A handful of studies suggested that C4d deposition in the peritubular capillaries is strongly correlated with transplant glomerulopathy, and most of them possess DSAs (60, 61). Conversely, studies have also mentioned C4d-negative peritubular capillaries in transplant glomerulopathy cases with DSAs (5, 6, 50). Sis et al. (2007) reported that the occurrence of C4d deposition in transplant glomerulopathy was lesser than the presence of DSAs; C4d might be negative or varying, indicating that C4d-negative did not essentially ruled out the antibody-mediated glomerular injury (23). Diffuse C4d deposition has also been seen in the glomerular capillaries in studies (49). Gloor et al. (2007) documented that 32% of glomerular capillaries were covered with C4d in transplant glomerulopathy patients (51). Sijpkens et al. communicated that 91% of the transplant glomerulopathy biopsy specimens had segmental glomerular capillary wall staining with C4d (6).

Numerous research studies have shown that prognosis of transplant glomerulopathy is poor in the presence of DSAs, particularly against class II (62-64). Sis et al. (2007) also concluded that presence of anti-HLA, especially against class II might play a key role in advancing of transplant glomerulopathy and eventually poor outcomes (5).

CONCLUSION

In conclusion, transplant glomerulopathy is associated with glomerular and peritubular capillary basement membrane thickening along with presence of anti-HLA antibodies and often C4d deposition in the peritubular capillaries in immunostaining. Transplant glomerulopathy patients usually have positive history for acute rejection, resulting mostly in antibody-mediated rejection. DSAs against class II is specifically linked with transplant glomerulopathy. Unfortunately, the prognosis of kidney allografts is dismal even under existing immunosuppressive regimens.

Conflict of interest:

Authors declare no conflict of interest

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Review Article

INFLUENCE OF VARIANTS MUTATIONS ON THE PROGRESSION AND OUTCOME OF COVID-19 IN PAKISTAN

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 pandemic (COVID-19) has put millions of people at risk in an increasing number of countries, suggesting a serious threat to global public health. The first identification in late 2019, the strain has undergone several changes that have resulted in several genetically different variations that are cause for concern. By comprising the Delta, Gamma, Beta, and Alpha variations, each of which has shown evidence of increased virulence, transmissibility, or capacity for immune evasion as compared to ancestral strains. Their advancement over ancestral strains in transmission was discovered by genomic surveillance, highlighting the vital necessity for monitoring the evolution of SARS-CoV-2. Remdesivir and other treatments showed promising results in reducing the duration of the disease; however, the development of antivirals to prevent the emergence of new variations is still an important goal. The vaccine also provides hope, although its effectiveness against new genotypes needs to be evaluated. Pakistan is also facing the implications of COVID-19. The months-long closing of colleges and universities affected education as well. As SARS-CoV-2 continues to evolve, creating efficient antiviral therapies and guaranteeing vaccination accessibility continue to be critical concerns.

Key Words: SARS-CoV-2; variant; COVID-19; Pakistan

INTRODUCTION

After the SARS-CoV-2 (formerly known as 2019-nCoV) infection was initially discovered in Wuhan, China, in December 2019, it quickly spread throughout the world, resulting in approximately 14 million active cases and 582,000 fatalities as of July 2020 (1). The principal mode of transmission of the virus between humans are through contact and respiratory droplets (2). A variety of birds and bats have been associated with coronaviruses, which are thought to be their natural hosts. Analysis of coronaviruses using molecular observation studies indicates that these viruses have a common ancestral history since 10,000 years ago (3). The genetic labyrinth of bats and other terrestrial animals is related to overflow and interaction of evolution, where the genetic mutation has no boundaries. Acute respiratory distress syndrome (ARDS), immunological dysfunction, and multi-organ failure are among the varied clinical manifestations of COVID-19. SARS-CoV, MERS-CoV, and SARS-CoV-2 are examples of human coronaviruses that have developed defence

mechanisms to block or inhibit the production of interferon, which can occasionally cause host inflammatory reactions resulting in ARDS (4). Suffering from SARS-CoV-2, carriers play a significant role in the transmission of COVID-19, although at least 41% of household infections of SARS-CoV-2 were caused by pre-symptomatic and asymptomatic transmission (5). High transmissibility, international travel, and population density all enhanced the pandemic's global spread. Serious public health concerns have been raised in several countries where inadequate medical systems and socioeconomic disparities have contributed to an increase in the number of cases and mortality ratio (6). Globally, governments have adopted flexible strategies. By closing schools and businesses, physically separating people, and using face masks, non-pharmaceutical measures aim to reduce contact. Furthermore, isolation and quarantine prevent further transmission, testing and screening programs were the additional measures (7). However, difficulties persist because of the public's resistance and the emergence of novel variations. Our methods need to change along with the variants. These days, genomic surveillance monitors the effects of evolving lineages such as Alpha, Beta, and Gamma. Although vaccination campaigns have given hope, but the concerns about immunity variations and protection duration must be addressed (8). Science and public health collaborate to reclaim the initiative by remaining adaptive and promoting international cooperation against ever-changing virus variety (9).
Some variations, in particular, have raised concerns since they may impact several aspects of the pandemic, including as transmissibility, the severity of the disease, and the effectiveness of therapies and vaccinations. This pattern has major implications for both preventing the outbreak and maintaining the integrity of public health procedures (10). Some of the notable variants are the Omicron variant (B.1.1.529) with an extensive spike protein mutation pattern that caused nervousness around the world, the Beta variant (B.1.351) with mutations affecting immunity, the Gamma variant (P.1) with transmissibility and immune escape concerns, the Delta variant (B.1.617.2) with significantly increased transmissibility, and the Alpha variant (B.1.1.7) with elevated transmissibility (11). A graphical presentation of all corona virus variants is presented in Figure 1.

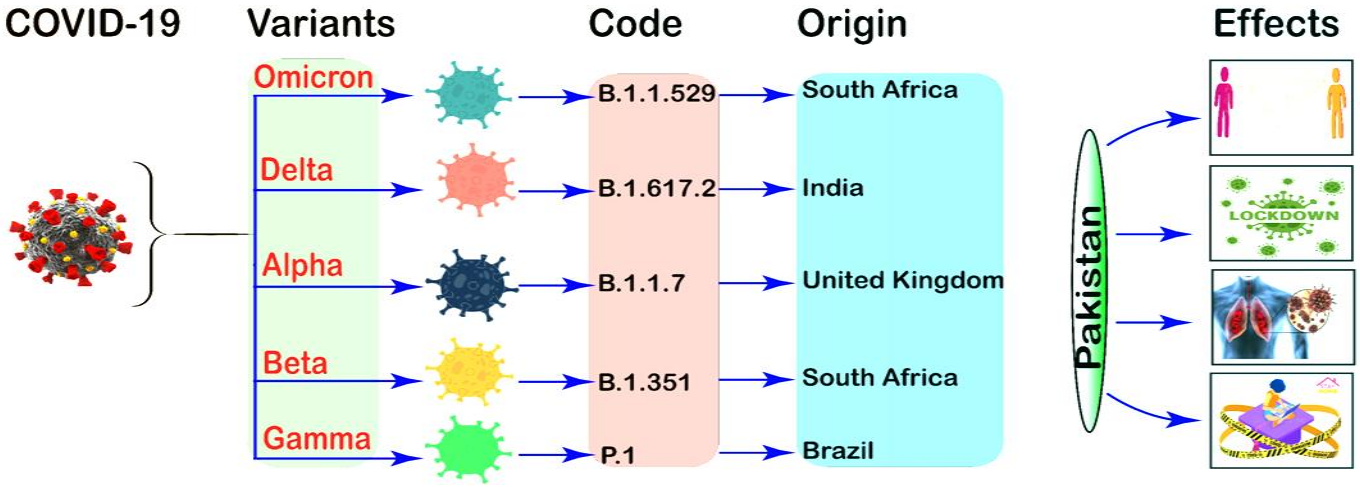

Figure 1: Graphical abstract showing the types of coronavirus which give rise to multiple genetically distinct variants such as Alpha, Beta, Gamma, and Delta variants.

Pakistan had entered into a complex public health and economic crisis following the emergence of the COVID-19 pandemic (12). By late 2021, the number of cases approached 1.2 million, placing an impact on the nation's healthcare infrastructure. Additionally, there was a severe lack of PPE, critical equipment, and ICU capacity in hospitals. Lockdowns and limits were implemented widely in an attempt to stop the virus's spread, but they had an adverse effect (13). The economy was affected by the closure of non-essential businesses, a decline in trade, and restrictions on mobility. Millions of people lost their jobs in the formal and unofficial sectors, further destroying the poor communities. The GDP of Pakistan dropped as a result of declining exports, manufacturing, and rising unemployment. For the benefit of communities that were

affected, the government put in place extensive social protection measures. Emergency money was made available to homes in need through the large-scale Ehsaas Emergency Cash project. Additionally, vaccination campaigns were boosted; by autumn 2021, over 75 million doses had been administered (14). Despite challenges, Pakistan demonstrated resilience and innovation in its multi-sectoral response. Future health strategies will depend on strengthening collaboration between the public and private health sectors and developing a defence strategy against novel variations. Pakistan is committed to resolving this persistent issue (15).

VARIANTS OF COVID-19

The term "variant of COVID-19" describes a particular strain or lineage of the SARS-CoV-2 virus that causes the COVID-19 illness. Variants are produced when a virus's genetic material (RNA) mutates which changes the virus's characteristics (16). Variations in transmission, sickness severity, immunological response, and even therapy or vaccination efficacy can all be indicators of these alterations. Viruses, including SARS-CoV-2, constantly undergo mutations as part of their natural evolution. The behaviour of the virus is mostly unaffected by most changes. However, certain mutations might give rise to new variations when they compound and improve like enhanced transmissibility or immunity evasion. Potential impacts of these variants on public health, such as the possibility of increased disease severity, easier spread, escape of immunity from prior infections or vaccinations, or decreased efficacy of diagnostic procedures, therapeutic interventions, and vaccines, need to be closely monitored and investigated (17). To monitor and classify these variations, virus samples from affected patients are genetically sequenced. To track the appearance and spread of new variations, several nations and organizations keep databases and systems up to date. This information is used to direct research and public health initiatives (18). A summary of Corona virus variants is given in Table 1.

IMPACT OF VARIANTS AND MUTATION ON TRANSMISSION DYNAMICS

Businesses, factories, and exports were forced to close as a result of nationwide lockdowns and social distancing measures. Throughout the mysterious path of SARS-CoV-2 evolution, some strains showed stability, raising concerns about increased transmissibility. Delta version (B.1.617.2) displayed more severe health and life-related complications. Millions of people were pushed into poverty as a result of the decline in formal and informal job opportunities. The Pakistani government tried a number of measures to prevent the virus's spread and minimize its socioeconomic consequences such as the Ehsaas Emergency Cash Program and giving priority to COVID-19 vaccinations, which resulted in the administration of more than 75 million doses by October 2021(19-24). The Omicron variant (B.1.1.529) made its first appearance in South Africa and displayed innovative resistance to specific antibodies (25). Here, the immune system takes on the role of a mystery as the powerful opponent of variable adaptability confronts the former protectors' immunity to diseases and immunizations. Some of the variations reveal a new aspect of the altered domain of disease severity (26). The Omicron variant has additional spike protein mutations, the majority of which occur in the receptor binding site. These mutations boost the variant's transmissibility while reducing the response to antibodies and vaccinations (27).

MODE OF TRANSMISSION

SARS-CoV-2 utilized an integrative approach to propagate from the beginning. Its principal weapon makes use of respiratory droplets released during breathing, coughing, and sneezing. The virus also showed alarming airborne capabilities. Its capacity to travel in small airborne suspensions and remain contagious in still indoor air for hours has been demonstrated by studies. Another channel for transmission is close touch. It was determined that surface contact was insufficient for the extensive diffusion (34). However, living together with an infected person increases danger, particularly in poorly ventilated interior spaces. Aerosols bearing SARS-CoV-2 are also produced during medical procedures that cause coughing or introduce high-flow oxygen (35). The WHO noted that these conditions might allow for airborne distribution. By clarifying its flexible approaches, we gain benefits. Recommendations ranging from masks to ventilation to distance-

keeping interrupt different approaches. Our comprehension of mechanisms changes as new varieties appear. Approaches to a multifaceted enemy must be similar. Transmission obstruction on all fronts can be achieved with a well-coordinated, multifaceted worldwide campaign. We might get closer to controlling this pandemic if we strategically outmanoeuvre SARS-CoV-2 (36). Mode of transmission of corona virus is presented in Figure 2.

Table 1. Different types of SARS-CoV-2 with description and disease

CORONAVIRUS TYPE	DESCRIPTION	DISEASE	REFERENCES
SARS-COV-2	Novel coronavirus that causes COVID-19	COVID-19	(28, 29)
SARS-COV	Severe acute respiratory syndrome coronavirus	SARS	(29)
MERS-COV	Middle East respiratory syndrome coronavirus	MERS	(30)
HCOV-229E	Human coronavirus 229E	Common cold	(31)
HCOV-OC43	Human coronavirus OC43	Common cold	(31)
HCOV-NL63	Human coronavirus NL63	Common cold	(31, 32)
HCOV-HKU1	Human coronavirus HKU1	Common cold	(33)

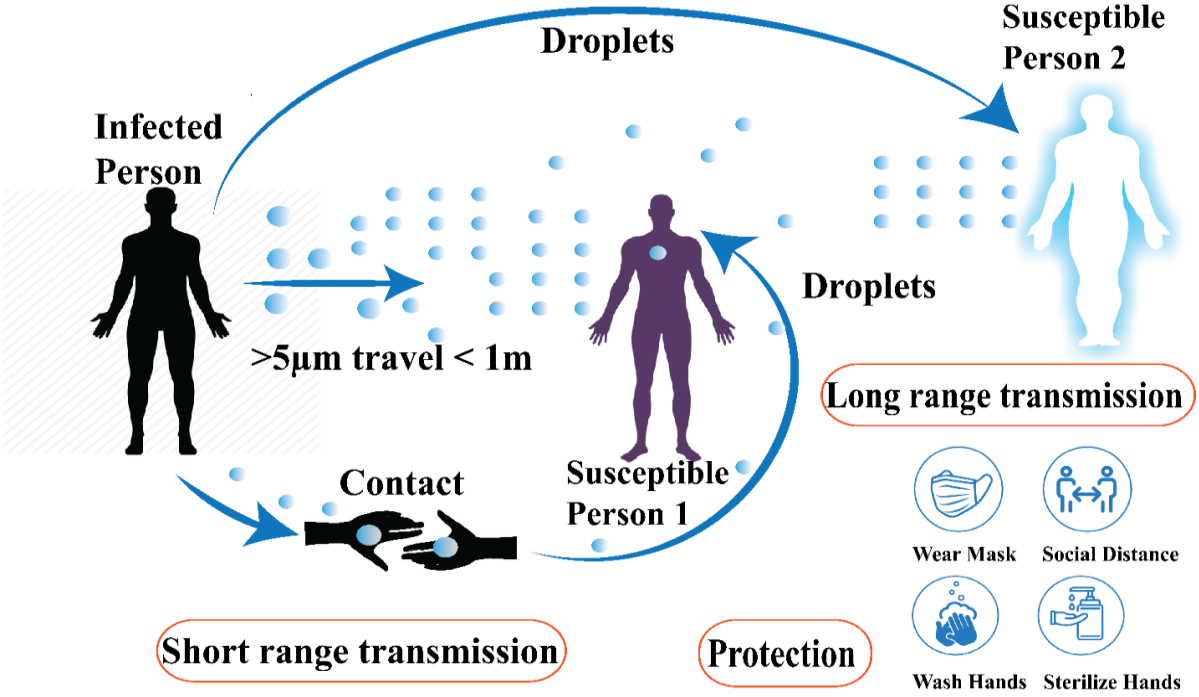

Figure 2. Short-range and long-range transmission of Coronavirus among the people. It is mostly spread from physical contact and droplets. There are some safety measures that can help to protect from Corona such as wearing a mask, social distancing, and washing hands with sterilizer.

FACTORS INFLUENCING COVID-19 PROGRESSION

There are increased risks for critical COVID-19 in certain patient groups. Poorer respiratory outcomes and mortality are frequently correlated with advanced age (37). The outlook gets worse for pre-existing medical conditions including diabetes and cardiovascular disease. These traits increase the likelihood of experiencing severe symptoms (38). The progress of the disease is influenced by the immune response itself. An excessive cytokine response can cause multi-organ damage and severe pulmonary inflammation. Initial viral load size

may influence severity since greater burdens are associated with more severe disease. Because of their phenotypic changes, variants of concern present further risks (39). Although antivirals attempt to stop replication, their efficacy varies according to the target virus. Activity varies by coronavirus strain, however, it is helpful for some. The coronavirus family includes not only the ordinary cold but also the potentially fatal SARS, MERS, and COVID-19 viruses (40). Results are influenced by a variety of fundamental parameters, including virus characteristics, immunological function, and patient health. It is nevertheless essential to understand these complex connections to optimize preventative and therapeutic approaches (41).

ANTIVIRAL MEDICATIONS AND THEIR EFFECTIVENESS

The purpose of antiviral drugs is to stop viruses from replicating and spreading throughout the body. They may work differently against different virus strains or species, but they may work against some viruses more effectively than others (42). The common cold and more serious respiratory conditions like Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and COVID-19 belong to the coronavirus family of viruses (43). A summary of Corona Viral medicines is given in Table 2.

Table 2. Various types of antiviral medicines with mechanism of action, dosage and approval

Antiviral medication	Mechanism of action	Clinical trials	Efficiency	Side effects	Dosage	Administration	Availability	FDA approval
Remdesivir	Nucleotide analogue	Yes	Moderate	Nausea,vomiting, elevated liver enzymes	IV infusion	Hospital setting	EUA in the United States	Yes
Favipiravir	RNA polymerase inhibitor	Yes	Moderate	Nausea, diarrhea, elevated uric acid levels	Oral tablets	Outpatient treatment	Japan, Russia, and India	Yes
Molnupiravir	RNA polymerase inhibitor	Yes	Promising	Nausea, headache, abnormal liver function tests	Oral capsules	Outpatient treatment	EUA pending in the United States	No
Ivermectin	Antiparasitic drug with antiviral properties	Mixed results	Inconclusive	Nausea, dizziness, diarrhea	Oral tablets	Outpatient treatment	Off-label use, available in some countries	No
Baricitinib	Janus kinase (JAK) inhibitor	Yes	Promising	Increased risk of infections, blood clots	Oral tablets	Hospital or outpatient treatment	EUA in combination with United States	Yes
Tocilizumab	IL-6 receptor antagonist	Yes	Promising	Increased risk of infections, liver enzyme elevations	Intravenous infusion	Hospital setting	EUAin several countries	Yes
Sotrovimab	Monoclonal antibody against spike	Yes	Promising	Hypersensitivity reactions,	Intravenous infusion	Hospital or outpatient treatment	EUAin the United States	Yes

	protein			infusion-related reactions				
Casirivimab/imdevimab	Monoclonal antibodies against spike protein	Yes	Promising	Hypersensitivity reactions, infusion-related reactions	Intravenous infusion	Hospital or outpatient treatment	EUAin several countries	Yes
Camostat mesylate	Serine protease inhibitor	In progress	N/A	N/A	Oral tablets	Outpatient treatment	Investigational use	No
Lopinavir/ritonavir	Protease inhibitors	Mixed results	Inconclusive	Gastrointestinal side effects, liver toxicity	Oral tablets	Hospital or outpatient treatment	Not recommended by some guidelines	No

EFFECT OF SARS-COV-2 PANDEMIC IN PAKISTAN

Pakistan has demonstrated extraordinary adaptability and resilience in the face of the worldwide pandemic. The country's response has been outstanding in terms of public health, the economy, and society (44). The healthcare system persisted and effectively treated an increase in COVID-19 patients. Healthcare workers coordinated efforts for testing, tracing, and treatment, demonstrating a consistent commitment to patient care. Strategic lockdowns and proactive health initiatives were implemented by the competent government of Pakistan. The community's support for cleanliness standards expressed a shared commitment to safety; while social distancing measures, business closures, and gathering restrictions created a protective barrier. The duration of this period demonstrates Pakistan's ability to overcome difficulties and grow even stronger. This chapter discusses cooperation, creative thinking, and the human spirit's unwavering determination to weather adversity and serve as an inspiration to others (45).

The pandemic has caused a significant negative economic impact on several industries. Lockdowns and limitations caused business interruptions, especially for small and medium-sized businesses, which in turn led to job losses and financial distress for families (46). As a quick reaction, the government launched economic stimulus plans and relief measures, helping impacted companies and individuals. Pakistan demonstrated its commitment to public health by stepping up testing and immunization efforts at the same time (47). The dissemination and accessibility of immunizations have significantly reduced the impact of the virus. Vaccination initiatives aim to protect more vulnerable populations and drastically reduce the severity of COVID-19 outbreaks globally.

CONCLUSION

The COVID-19 pandemic, which originated from the SARS-CoV-2 virus, has presented complex issues on a global scale. Since its discovery in 2019, this virus has undergone mutations and given rise to a variety of forms, including Alpha, Beta, Gamma, and Delta. In comparison to the original strain, these variations are more virulent, transmissible, and immunologically resistant. It is important to monitor the evolution of SARS-CoV-2 as genomic surveillance highlights their advantage. Although therapies such as remdesivir show potential, it is still critical to resist novel variations. Vaccines are promising, but more research is needed to determine how well they work against newly emerging strains. Pakistan, having 200 million people, had difficulty dealing with the effects of COVID-19. Patient care, tracing, and testing were challenges for the healthcare system. Lockdown situations led to unemployment and economic hardship. Schools suffered from

the closure of their facilities. Antiviral development and increasing vaccine accessibility continue to be priorities. Getting back stability and preparing for new waves are the goals of Pakistan's coordinated scientific and public health initiatives. Future health outcomes will be shaped by an international collaborative effort.

FUTURE PERSPECTIVE

Novel strains of SARS-CoV-2 will probably persist in emerging. Understanding these variations' effects on transmissibility, severity, and vaccine efficacy would require careful observation and research. Controlling upcoming outbreaks brought on by novel variations would need the implementation of public health strategies like focused therapies, fast testing, and genomic surveillance. It might spread throughout the population at lower densities, or become endemic. This could lead to recurring epidemics that resemble the seasonal flu. To reduce the effect of future outbreaks, public health initiatives will emphasize adaptive measures like vaccination campaigns, targeted testing, contact tracing, and the promotion of hygienic behaviors. Pakistan has also been badly hit, with an overburdened healthcare system and an incoherent economy. Although, there are causes for optimism. As effective vaccines and antiviral medications are being developed, the pandemic may be contained. Pakistan is also working to enhance its healthcare infrastructure and enhance its capacity to prepare for potential pandemics. The most likely possibility is that COVID-19 will spread throughout Pakistan and become endemic, which would mean that fewer people will get sick or die from the virus than it does currently. Still, there is a chance that a fresh pandemic wave could emerge, so it's critical to be alert, get vaccinated, and receive booster shots.

Conflict of interest:
Authors declare no conflict of interest
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Case Report

SUCCESSFUL MANAGEMENT OF ASPERGILLOSIS IN POST RENAL TRANSPLANT PATIENT: A CASE REPORT

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ABSTRACT

This case describes a 60-year-old man with a history of diabetes and hypertension who underwent a kidney transplant in September 2023. The patient presented with fever, cough for 5 days, empirical antibiotics were started but complaints were not resolved so fungal markers were sent, which showed elevated galactomannan levels, necessitating the initiation of voriconazole therapy. X Ray chest showed patch along with cavitatory lesion which was further confirmed by CT Scan Chest which confirmed a cavitatory lesion. This case report emphasizes the importance of considering fungal infection as a differential diagnosis in immunocompromised patients and achieving good results with appropriate treatment.

Key Words: Aspergillosis, kidney transplant, immunocompromised

INTRODUCTION

Immunocompromised patients, such as organ transplant recipients, are at greater risk for opportunistic infections, including fungal infections(1). If left untreated, fungal infections can cause life threatening illness leading to death. The purpose of this report is to highlight the importance of identifying fungal infections early in post transplant patients and to start appropriate antifungal treatment at the earliest (2).

Case Presentation

A 60 yrs old male patient, who underwent kidney transplant in september 2023, (was declared end stage renal disease secondary to diabetes and hypertension), presented to us with complains of fever and productive cough since past 5 days, he was admitted in hospital, baseline investigations were sent and was started on antibiotic therapy empirically.

His initial laboratory workup was within normal limits, only C Reative Protein (CRP) was raised and X- Ray chest showed right lower lobe patch and cavity formation, when after approximate bacterial coverage his symptoms not recovered then his fungal markers were sent and Computed Tomography Scan (CT) Chest with contrast was done. Galactomanan and BD Glucan were raised and CT Chest showed right lower lobe cavitatory lesion. His sputum for AFB smear was checked and HIV serology was done it was negative then he was started on voriconazole and BAL was done , samples of BAL were sent for galactomannan they also came out be positive, hence the diagnosis of aspergillosis was made. Patient responded to antifungal therapy and his respiratory symptoms were improved. Patient got discharged and continued on followed up.

DISCUSSION

People who have been immunologically suppressed, especially those who have received organ transplants, are at high risk of developing opportunistic infections which include viral, fungal and rare bacterial infections(1). Fever and respiratory symptoms in such patients, as seen in this report, should always raise suspicion of opportunistic infections. Early diagnosis and prompt initiation of appropriate therapy are critical in improving patient outcomes. When clinical suspicion is high for opportunistic infections always fungal markers should be sent (3). Additionally, radiographic studies such as chest CT scans can provide important information about the extent and nature of the disease (4). Pulmonary aspergillosis presents with a variety of clinical forms including invasive pulmonary aspergillosis, chronic necrotising aspergillosis (subacute invasive pulmonary aspergillosis), aspergilloma, chronic cavitary pulmonary aspergillosis (CCPA) and allergic bronchopulmonary aspergillosis(6). Differential diagnosis among these forms is sometimes difficult due to overlapping presentations. The case presented here was an aspergilloma, and met the definition of simple aspergilloma instead of CCPA , since the patient had only one pulmonary cavity, few symptoms and no radiological demonstration of progression.

In our case we screened our patient for fungal invasion for which we performed MRI Brain with contrast to rule out dissemination in brain(5),as it was limited disease so it responded well to voriconazole (2).

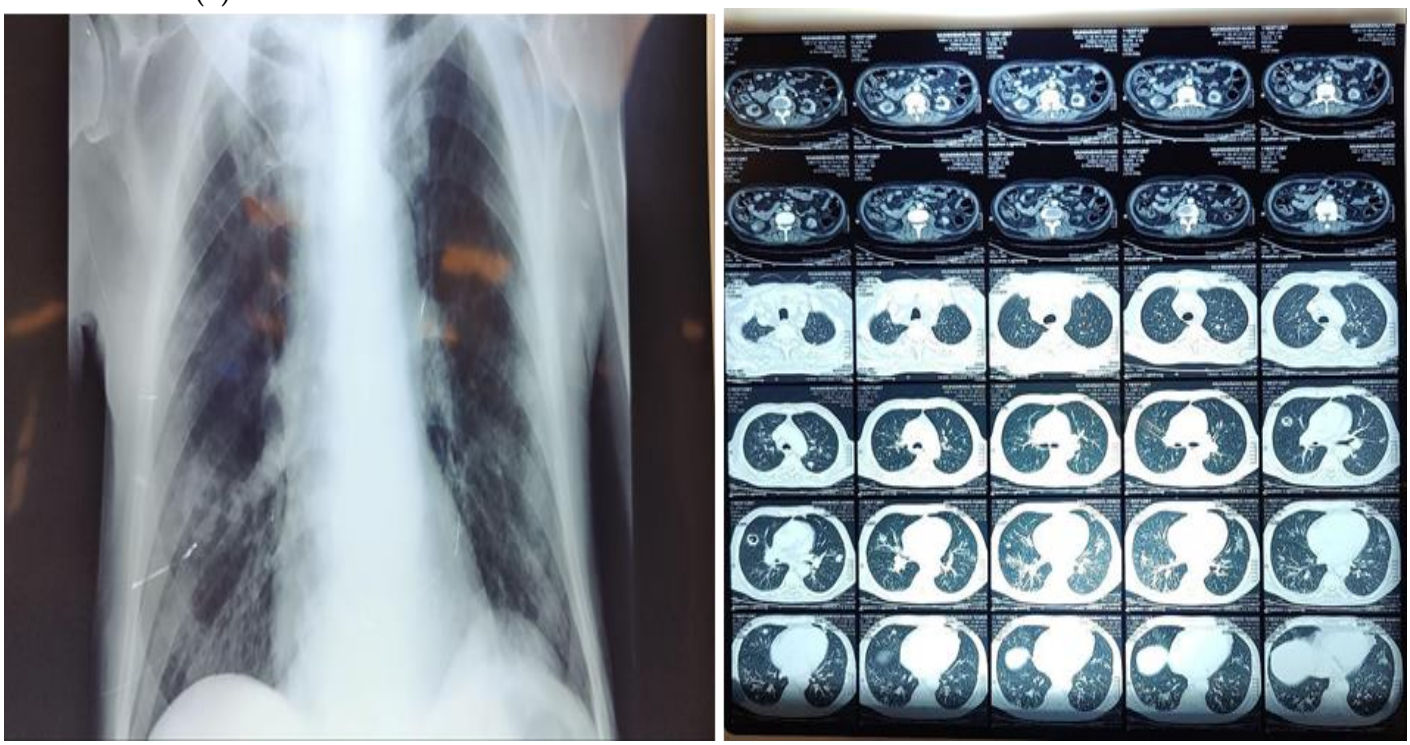

Figure 1. X-ray chest PA view and Computed Tomography of patient presenting with Aspergellosis after renal transplant

CONCLUSION

This case report highlights the importance of identifying fungal infections in immunocompromised patients and initiating appropriate antifungals as soon as possible. High galactomannan levels and signs of fungal infection on lung tomography raised suspicion of fungal infection. Initiation of voriconazole therapy resulted in a good response and relief of symptoms. Early recognition and

appropriate treatment of fungal infections is important to improve patient outcomes in immunocompromised individuals.

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Case Report

AUTOIMMUNE COMPLEXITY: A CASE REPORT OF IMMUNE THROMBOCYTOPENIA (ITP) WITH COEXISTENT POSITIVE AUTOIMMUNE MARKERS AND MULTIDISCIPLINARY CHALLENGES

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ABSTRACT

This case study explores a complex clinical presentation of an 85-year-old male diagnosed with immune-mediated thrombocytopenia (ITP) with coexistent positive autoimmune markers. A multidisciplinary approach was crucial in managing the patient, involving hematology, rheumatology, urology, and respiratory specialists. Despite tailored treatments, including platelet transfusions, immunosuppressive therapies, and antibiotics, the patient developed complications including hospital-acquired pneumonia, pulmonary infection, and acute kidney injury. Extensive discussions with the family led to the decision to transition the patient to comfort care, emphasizing the challenges in managing complex cases and the importance of a comprehensive care plan.

Key Words: Immune Thrombocytopenia, Autoimmune Markers, Multidisciplinary Challenges, Elderly Patient, Haematological Disorders

INTRODUCTION

Immune-mediated thrombocytopenia (ITP) is the condition when the immune system mistakenly targets and destroys platelets in the body due to a recognition error (1). The exact cause of ITP remains unclear, but it is believed to involve the misrecognition of platelets by the patient's immune system, leading to their premature destruction (2). Recent population-based cohort studies indicate a heightened incidence of systemic lupus erythematosus among individuals with immune thrombocytopenic purpura (ITP) (3).

Case Scenario

An 85-year-old male patient with a history of the aforementioned medical conditions presented to Aga Khan University Hospital (AKUH) with a three-day history of per rectal, per oral bleeding, and mild bleeding from bed sores. Upon admission, a comprehensive clinical assessment and laboratory workup showed a low platelet count. This prompted a provisional diagnosis of immune-mediated thrombocytopenia.

In response to the ITP diagnosis, the patient received a tailored treatment regimen, which included platelet transfusions, intravenous fluids, IV tranexamic acid, and topical epinephrine to manage and control the bleeding episodes. Hematology specialists were actively involved in the patient's care, and further investigations led to a pivotal step in the diagnostic process.

A bone marrow biopsy was performed under local anesthesia. The final biopsy report suggested normocellular bone marrow with peripheral platelet destruction, indicating a potential immune-mediated origin of thrombocytopenia. In the light of this finding, the patient underwent a series of treatments, including three doses of pulse IV Methylprednisolone, which were subsequently

transitioned to hydrocortisone as per hematology recommendations. Eltrombopag, a thrombopoietin receptor agonist, was also initiated to stimulate platelet production. A summary is given in Table 1 and 2.

Given the complex clinical presentation and the presence of positive antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-ds-DNA), rheumatology specialists were consulted to explore potential autoimmune associations (Table 3). Although the patient developed hematuria during hospitalization, the urology team advised against immediate intervention.

During the patient's stay, additional complications emerged, including hospital-acquired pneumonia, necessitating the optimization of antibiotic therapy. Expressly, tracheal cultures indicated a heavy growth of Klebsiella and Acinetobacter, leading to the initiation of IV Piperacillin-Tazobactam (Pip/taz). Furthermore, urine cultures identified E. coli, prompting adjustments to the antibiotic regimen to include IV Meropenem, IV Vancomycin, and IV Colistin. The patient's respiratory status deteriorated, leading to increased oxygen requirements and persistent desaturation. As a result, the patient was transitioned to Bilevel Positive Airway Pressure (BIPAP) therapy and transferred to the Special Care Unit for closer monitoring. A repeat chest X-ray revealed a worsening of bilateral perihilar patchy airspace opacities, indicative of a superimposed pulmonary infection.

Final blood culture reports identified Stenotrophomonas Maltophilia, necessitating further optimization of antibiotic therapy. The patient received multiple platelet transfusions throughout the hospital course and underwent three packed red blood cell (PCV) transfusions. Notably, the patient experienced acute kidney injury (AKI), which responded positively to gentle hydration.

Amidst the evolving medical challenges, detailed discussions were made with the patient's family regarding prognosis and treatment options. Ultimately, the family decided to transition the patient to comfort care. Considering the ongoing drop in platelet counts, rheumatology specialists discussed the potential use of intravenous immunoglobulin (IVIG) with the family, ensuring comprehensive communication and decision-making.

Following extensive dialogue with the patient's family, it was decided to discharge the patient with a structured nursing care plan. This included oral prednisolone, eltrombopag, and weekly complete blood counts (CBC) monitoring. The family was also advised to seek immediate medical attention at AKUH-ER in the event of any bleeding episodes or emergent medical concerns.

Associated Diagnosis/Significant Comorbid

The patient under consideration exhibits a medically intricate profile featuring Chronic Liver Disease (CLD) of non-B and non-C origin, a known history of hypertension, a previous diagnosis of benign prostatic hyperplasia (BPH) successfully managed with transurethral resection of the prostate (TURP), a prior non-ST-segment elevation myocardial infarction (NSTEMI) with a preserved ejection fraction (EF) of 55% under medical management, a documented abdominal aortic aneurysm, concurrent peripheral artery disease (PAD), and a pre-existing motor neuron disease.

Discussion

This patient's presentation of ITP, characterized by a low platelet count and positive autoimmune markers (ANA and anti-ds-DNA), is intriguing. ITP is known for its autoimmune pathophysiology, where the immune system erroneously targets and destroys platelets. The co-occurrence of ITP with positive autoimmune markers raises questions about potential associations with systemic autoimmune disorders. Recent studies have shed light on the management of complex cases of ITP.

In a study in 2020, the authors explored the clinical characteristics and outcomes of ITP associated with COVID-19, emphasizing the importance of a multidisciplinary approach in managing patients with both autoimmune conditions and infectious diseases (5). Furthermore, it was updated with international consensus guidelines for investigating and managing primary ITP, providing valuable insights into the diagnosis and Treatment of this condition (4). The positive ANA and elevated dsDNA levels suggest potential autoimmune involvement beyond ITP (3).

Table 1. Pharmacological Treatment of the patient after Discharge (Take Home Medicines)

Drug	Dose	Route	Frequency	Duration of treatment
Tab Calcium with Vitamin D	1 Tablet	Chewable	Once a day (4)	Continue till next follow-up
Bag Ceftazidime 1000 mg/bag	2000 mg	IVPB piggyback	Every 12 hours	Continue till 07/09/2023
vl Ipratropium bromide 500 mcg/2ml	500 mcg	Nebulizer	Every 08 hours	Continue till next follow-up
Tab Eltrombopag 50 mg/tab	100 mg	Oral	OD	Continue till the next follow-up
Cap Itopride Hydrochloride 150 mg/cap	150 mg	Oral	OD	Continue till next follow-up
Tab Atorvastatin Calcium 10 mg/tab	10 mg	Oral	At bed time	Continue till next follow-up
Tab Metoclopramide HCL 10 mg/tab	10 mg	Oral	Before meals	Continue till next follow-up
Sch Polyethylene glycol (peg) 1 sac	1 sac	Oral	OD	Continue till next follow-up
Tab Prednisolone 5 mg/tab	15 mg	Oral	Two times a day	Continue till next follow-up
Cap Omeprazole 20 mg/cap	40 mg	Oral	Before breakfast	Continue till the next follow-up

Table 2.0. Laboratory Investigations of the Patients in the Internal Medicine Department

Date	20/8/23	21/8/23	22/8/23	23/8/23	24/8/23	25/8/23	26/8/23	27/8/23	28/8/23
Hb	8.9	8.9	9.4	8.6	10.0	9.1	9.6	9.1	9.1
HCT	28.1	28.2	29.5	28.0	31.4	29.1	29.9	30.2	30.2
MCV	91.2	92.2	92.8	93.3	92.1	93.6	93.2	93.8	98.1
WBC	26.1	27.8	27.3	30.5	31.9	26.8	23.0	26.6	27.3
Neu	93.4	96.1	95.2	96.3	94.3	92.9	95.5	95.9	95.6
Lym	1.1	0.9	1.1	1.3	1.3	2.2	1.2	1.1	0.9
Platelets	14	09	43	26	49	38	17	22	19
BUN	47	47	53	60		78		74	74
Creatinine	1.4	1.2	1.2	1.4		1.8	1.9	1.7	1.5
Na		142	143	145		148	150	153	146
K	4.3	4.4	4.8	4.5	4.2	4.3	4.1	5.0	4.6
Cl		107	106	106	109	109	113	114	109
BIC		26.2	26.5	26.1		27.4	26.2	28.9	24.9
Ca						8.3	8.3		
Mg						3.0			

Alb						3.3			
CRP							17.2		12.3

Table 3. Autoimmune profile of the patient

Lab Investigation	Value
Coomb's Test	2+
ANA	Positive
dsDNA	37.6

Conclusion

This case underscores the complexities inherent in managing elderly patients with immune thrombocytopenia and concurrent autoimmune markers, especially in the presence of multiple comorbidities. The coexistence of ITP with positive antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-ds-DNA) raises questions about potential associations with systemic autoimmune disorders. The multidisciplinary approach proved pivotal in addressing evolving medical challenges, but despite interventions, the patient's deteriorating health necessitated a transition to comfort care. This emphasizes the need for ongoing research and guidelines to navigate the intricate landscape of autoimmune complexities in the elderly population, ensuring comprehensive and personalized patient care.

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