

SHORT ARTICLES

Self-Esteem of Patients Receiving Chemotherapy Treatment in a Tertiary Care Hospital Karachi, Pakistan

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ABSTRACT

Background: Cancer is becoming serious and emerging health concern around world. In Pakistan, 8% of all deaths are due to cancer as one of the major cause. Treatment of cancer consists of surgical management, radiation, chemotherapy, hormone therapy, immunotherapy, and combined therapy. Psychological imbalance is observed during treatment and cause altered self-esteem, which requires psychological modification. The study aimed to assess the alteration in self-esteem after receiving chemotherapy.

Methods: This research study was cross-sectional study, in which fifty individuals were selected between ages of 18-80 years from oncology unit of tertiary care hospital. A self-administered questionnaire consist of Rosenberg self-esteem scale assessing the self-esteem was administered. Teaching sessions for developmental change towards giving education on increasing self-esteem of patients were conducted for oncology nurses. Results were analyzed using SPSS version 22.

Results: Since, 44% male and 56% female were participated. In all surveyed individuals 96% participants identified with average normal self-esteem, 3% participants had low self-esteem, whereas, only 1% participants had high self-esteem.

Conclusion: This research study revealed that most of the patient suffering cancer had average self-esteem. Therefore, there is need to work on strategies to promote psychological well-being of patients, aiming to uphold and rehabilitate emotional aspects of cancer patients.

Keywords: Body Image; Quality Of Life; Concept; Cognition; Anti-neoplastic Agents.

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INTRODUCTION

Cancer is becoming serious problem in public health. Cancer influences psychological well-being and cause stress¹⁻⁴. Cancer is becoming the second source of death. In 2018, 9.6 million deaths have occurred. In Pakistan, 8% of all deaths are due to cancer as one of the major cause^{5,6}. Cancer alters individual's physical and psychological well-being but also social and occupational aspects, which effects family life⁷⁻⁹. Spiritual well-being an important aspect that is the fundamental base of human health relates with positive self-esteem and attitudes¹⁰. Research Studies have identified that patients who receive chemotherapy cognitive

pattern are altered which includes memory retention, attention and processing of information in daily life functions^{11,12}.

Chemotherapy is used as an "adjuvant therapy" It results in a number of consequences encompassing nausea, vomiting, reduced craving for food and hair loss these side effects alter perception of body image and lessens quality of life. Different quantitative studies have recognized that guilt and sadness occurs when self-esteem is lost because of hair loss, which then results in minimizing social activities and meeting people¹³⁻¹⁵. Antineoplastic chemotherapy delivers abundant advantage to patients of malignant and nonmalignant cancers⁵.

It is observed that during treatment the most emphasizing part is psychological imbalance due to stress related to effects of chemotherapy, which results in altered self-esteem¹. It is seen in breast cancer patients that due to change in physical appearance with mastectomy self-esteem is impaired which then influences negatively on body^{16,17}. The study aimed to assess the alteration in self-esteem among patients after receiving chemotherapy.

METHODS

This research study was cross-sectional and conducted in an oncology facility in tertiary care hospital located in Karachi, Pakistan. Through consecutive sampling, data was obtained from fifty patients who attended the oncology facility for chemotherapy. Data was collected from May to June 2017. A quantitative study questionnaire was used including variables of socio-demographic regarding receiving chemotherapy and patients' approach to life¹⁸. Before administering questionnaire to patients' instrument was refined and fulfilled by three oncology nurses. The assessment of self-esteem levels was measured by selecting the Rosenberg Self-Esteem Scale (RSES). Rosenberg established it and the original tool was in English, which was later interpreted and validated in 2001 for Portuguese¹⁹.

In determining self-esteem RSES scale is used in research. It contains total 10 items with five positive and five negative feelings, items are constructing on Likert scale evaluating four points from a range of strongly agree to strongly disagree. According to RSES the score above 30 is labeled as high self-esteem, score "between" 20-30 is labeled as average self-esteem whereas score less than 20 is low self-esteem¹⁹. Furthermore, teaching sessions for oncology nurses were conducted to capture developmental change by giving education on increasing self-esteem of patients. Oncology nurses were provided with teaching material consisting of ways to boost self-esteem during receiving chemotherapy treatment and to discuss it with patients while dealing patients receiving chemotherapy. A sustainability tool was formed consisting of behavioral attributes of oncology nurses after session, development attributes of staff

after session and nursing interventions on self-esteem. The sustainability tool was handed over to HOD oncology department to evaluate the needs with completion of form every fifteen days of session commencement and then submitted to head nurse of oncology department. Regular follow-ups were maintained by meeting with HOD and head nurse of oncology staff. Results were analyzed using SPSS version 22.

RESULTS

Table 1 reveals the frequencies of numerical characteristics of study individuals. Out of 50 participants, 44% (22) remained male participants whereas 56% (28) were female participants. In pertinent to age, most of the participants 66% were in age group of 51 and above years while a small number 12% of respondent lied in age group of 20-30. As for as monthly income status concern, participants having monthly income 10,000- 30,000PKR were 38%, while 31,000-60,000PKR were 38% and 91,000 PKR and above income were 5%.

Table 1: Demographic characteristics of the participants.

Demographic Variables	(n)%
Gender	
Male	22(44%)
Female	28(56%)
Age	
20 - 30	6(12%)
31 - 40	3(6%)
41 - 50	8(16%)
51 - Above	33(66%)
Income	
10,000 - 30,000	19(38%)
31,000 - 60,000	19(38%)
61,000 - 90,000	7(14%)
91,000 - above	5(10%)

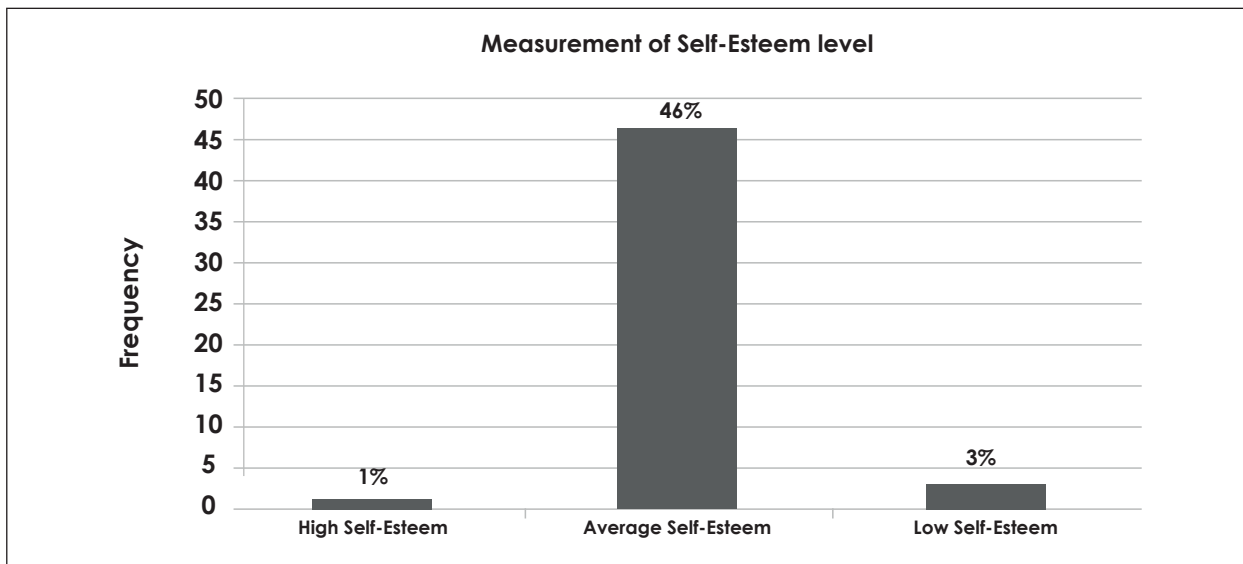
Table 2: Self-esteem level of study participants.

Questions based on self esteem	Strongly Agree	Agree	Disagree	Strongly Disagree
1. On the whole I am satisfied with myself	50%	4%	4%	2%
2. At times I think I am not good at all	8%	8%	28%	26%
3. I feel that I have a number of good qualities	46%	4%	10%	0%
4. I am able to do things as well as most other people	58%	6%	14%	2%

5. I feel that I do not have much to be proud of	12%	2%	28%	28%
6. I certainly feel useless at times	6%	0%	18%	46%
7. I feel that I am a person of worth	62%	0%	6%	2%
8. I wish I could have more respect for myself	52%	8%	2%	8%
9. All in all I am inclined to feel that I am a failure	8%	2%	20%	60%
10. I take a positive attitude towards myself	78%	4%	4%	4%

Table 2 revealed self-esteem level of study participants 10 questions related to self-esteem were asked from participants. Out of all 52% wished, they could have more respect for themselves. On asking, "I feel that I am a person of worth" participants revealed strongly agree 62%. Graph 1 is representing the measurement self-esteem levels

with high, average and low self-esteem. Among various types of cancer in the study participants, the higher frequency 12% was found in breast cancer while, 8% with ovarian cancer and only 4% with oral cancer. Majority of the participants (46%) were with average self-esteem.



Graph 1: Presenting frequency of self-esteem level.

DISCUSSION

The current research was carried out to analyze self-esteem among the cancer patients receiving chemotherapy. The results of the present research study presented generally, the average self-esteem with 96% in contrast one study was conducted in Brazil has revealed 28% with average self-esteem¹. Another cross sectional study conducted in Brazil by Chavaz and colleagues with a sample of 100 participants has recognized average self-esteem level was 7.07%²⁰.

In different studies conducted in patients receiving chemotherapy identified that religious people have greater capacity in dealing adverse circumstances related to ill health with using of spirituality, thus spirituality plays a major role in improving quality of life. Moreover, another important factor hope

is identified worldwide as one of the major mechanism in crucial states of life especially when suffering terminal illness where it is observed as average self-esteem²¹⁻²⁴. There are two important components, which need to be understood for the development of healthy personality and includes self-image and self-esteem. It is the highly need of individuals to have positive self-esteem respect and value while dissatisfaction with body image can cause low self-esteem which is found as 77.6%^{25, 26}. While in current study, 3% were found with low self-esteem. Factors associated with low self-esteem were found to be psychological includes anxiety, weakness, irritability, guiltiness, hopelessness and socially increase burden in daily living circumstances. Another cross-sectional study with 178 participants receiving chemotherapy conducted in Egypt has found 84.83% of low self-esteem while receiving chemotherapy²⁷.

A study conducted in Brazil explores that psychologically wellbeing can bring high positive levels of self-esteem, self-assurance, emotional steadiness and good thoughts²⁸. Consistent to this finding another study conducted in Brazil in an oncology department with 156 participants identified patients with higher frequency of self-esteem 76% while receiving chemotherapy¹, while in this study only 1% of patient was found with high self-esteem in receiving chemotherapy where finding are consistent with another study that revealed high level of self-esteem with 57.2%²⁹.

CONCLUSION

We concluded that patients with cancer receiving chemotherapy evaluated in this research study showed average self-esteem. Therefore, self-esteem in patients suffering cancer undergo chemotherapy is multifaceted; subsequently this study includes specific features of each person, which could help in approaching cancer patients and their management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

The research committee of Ziauddin Hospital and College of Nursing provided the approval for conducting the study.

PATIENT CONSENT

Patient was informed and written consent was taken prior to study participants.

AUTHORS' CONTRIBUTION

DJ was primary author and contributed thoroughly from selection until completion. SA analyzed and interpreted the data. FP and AN were the supervisors for the study.

REFERENCES

1. Leite M, Nogueira D, Terra. Evaluation of self-esteem in cancer patients undergoing chemotherapy treatment. *Rev Lat Am Enfermagem*. 2015; 23(6):1082-1089.
2. Sercekus P, Besen D, Gunusen N, Edeer A. Experiences of Family Caregivers of Cancer Patients Receiving Chemotherapy. *Asian Pac J Cancer Prev*. 2014;15(12):5063-5069.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108.
4. World Health Organization [Internet]. Cancer Fact Sheet; 2018 [updated 2018 September 12, cited 2020 June 10]. Available from: <https://www.who.int/news-room/fact-sheets/detail/cancer>
5. U.S. Department of Health & Human Services [Internet]. Global health Pakistan - leading causes of death in Pakistan ischemic heart disease, cancer, and lower respiratory infections are the top 3 killers in Pakistan, a South Asian country; 2019 [updated 2011 September 12, cited 2020 June 12]. Available from: <https://www.cdc.gov/global-health/countries/pakistan/default.htm>
6. World Health Organization [Internet]. Noncommunicable diseases (NCD) country profiles; 2018. p.223.
7. Abbasian F, kia N, Mirmohammad khani M, Ghahremanfard F, Ghods E. Self-esteem and spiritual health in cancer patients under chemotherapy in Semnan University of Medical Sciences in 2014. *Health Spiritual Med Ethics*. 2016;3(4):29-37.
8. Bevan M, Sternberg E. Caregiving burden, stress, and health effects among family caregivers of adult cancer patients. *JAMA*. 2012;307(4):398-403.
9. Lkhoyaali S, Haj M, Omrani F, Layachi M, Ismaili N, Mrabti H, *et al*. The burden among family caregivers of elderly cancer patients: prospective study in a Moroccan population. *BMC Res Notes*. 2015;8(347): 1-4.
10. Zaidi A, Ansari T, Khan T. The financial burden of cancer: Estimates from patients undergoing cancer care in a tertiary care hospital. *Int J Equity Health*. 2012;11(60):1-6.
11. Neuss M, Polovich M, McNiff K, Esper P, Gilmore TR, LeFebvre KB, *et al*. 2013 updated American society of clinical oncology/oncology nursing society chemotherapy administration safety standards including standards for the safe administration and management of oral chemotherapy. *J Oncol Prac*. 2017;9(2):1-13.
12. Gokal K, Munir F, Wallis D, Ahmed S, Boiangiu I, Kancherla K. Can physical activity help to maintain cognitive functioning and psychosocial well-being among breast cancer patients treated with chemotherapy? A randomised controlled trial: study protocol. *BMC Public Health*. 2015;15(414):2-8.
13. Guy Jr GP, Ekwueme DU, Yabroff KR, Dowling EC, Li C, Rodriguez JL, *et al*. Economic burden of cancer survivorship among adults in the United States. *J Clin Oncol*. 2013;31(30):3749-3757.
14. Kim I, Cho J, Choi E, Kwon I, Sung Y, Lee J, *et al*. Perception, attitudes, preparedness and experience of chemotherapy-induced alopecia among breast cancer patients: a Qualitative Study. *Asian Pacific J Cancer Prev*. 2012;13:1383-1388.
15. Lemieux J, Maunsell E, Provencher L. Chemotherapy-induced alopecia and effects on quality of life among women with breast cancer: a literature review. *Psychooncol*. 2008;17: 317-328.

16. Ha EH, Cho YK. The mediating effects of self-esteem and optimism on the relationship between quality of life and depressive symptoms of breast cancer patients. *Psychiatry Investig*. 2014;11(4):437-445.
17. Enache R. The relationship between anxiety, depression and self-esteem in women with breast cancer after surgery. *Procedia Soc Behav Sci*. 2012; 33, 124-127.
18. Joscelyne A, Knuckey S, Satterthwaite M, Bryant R, Meng M, Qian M, *et al*. Mental health functioning in the human rights field: findings from an international internet-based survey. *PLoS ONE*. 2015;1-12
19. Tinakon W, Nahathai W. A comparison of reliability and construct validity between the original and revised versions of the Rosenberg self-esteem scale. *Psychiatry Investig*. 2012;9:54-58.
20. Mata L, Chávez G, Faria B, Antunes A, Silva M, Oliveira P. Self-esteem and distress in patients undergoing cancer surgery: a correlational study. *Braz J Nurs*. 2016;15(4)664-647.
21. Courneya K, McKenzie D, Gelmon K, Mackey J, Reid R, Yasui Y, *et al*. A multicenter randomized trial of the effects of exercise dose and type on psychosocial distress in breast cancer patients undergoing chemotherapy. *AACR*. 2014;1-17.
22. Mesquita A, Chaves E, Avelino C, Nogueira D, Panzini R, Carvalho E. The use of religious/spiritual coping among patients with cancer undergoing chemotherapy treatment. *Rev Latino-Am Enfermagem*. 2013;21(2):539-545.
23. Broadhurst K, Harrington A. A mixed method thematic review: the importance of hope to the dying patient. *J Advan Nurs*. 2015;72(1):18-32.
24. Phelps A, Lauderdale K, Alcorn S, Dillinger J, Balboni M, Wert M, *et al*. Addressing spirituality within the care of patients at the end of life: perspectives of patients with advanced cancer, oncologists, and oncology nurses. *J Clin Oncol*. 2012;30(20):2538-2544.
25. Balboni M, Sullivan A, Amobi A, Phelps A, Gorman D, Zollfrank A, *et al*. Why is spiritual care infrequent at the end of life? Spiritual care perceptions among patients, nurses, and physicians and the role of training. *J Clin Oncol*. 2013;31(4):461-467.
26. Lettnin G, Dohms K, Mendes A, Stobaus C, Mosquera J, Neves S. Evaluating self-esteem levels of brazilian and portuguese adolescents. *Sci Res Publish*. 2015;6:314-322.
27. Sherief LM, Kamal NM, Abdalrahman HM, Youssef DM, Alhady MA, Ali AS, *et al*. Psychological impact of chemotherapy for childhood acute lymphoblastic leukemia on patients and their parents. *Medicine*. 2015;94(51):1-6.
28. Ferrari E, Petroski E, Silva D. Prevalence of body image dissatisfaction and associated factors among physical education students. *Trends Psychiatry Psychother*. 2013;35(2):119-127.
29. Bidstrup P, Christensen J, Mertz B, Rottmann N, Dalton S, Johansen C. Trajectories of distress, anxiety, and depression among women with breast cancer: Looking beyond the mean. *Acta Oncol*. 2015;54(5):789-796.



SHORT ARTICLES

High Incidence of Dengue Fever in Karachi and the Benefits of Ultrasound for Diagnosis of Complications

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ABSTRACT

Background: Dengue fever presentations are from asymptomatic fever to most serious complications. Acute high-grade fever, muscle and joint pain, myalgia, skin rashes, hemorrhage and circulatory shock are the common symptoms. There has been reported increase in number of dengue fever and last few years. Urgent diagnosis is critical to avoid complications like liver injury, cardiomyopathy, pneumonia, orchitis, oophoritis, seizures and encephalopathies. This study aimed to determine the complication caused by dengue fever after the diagnosis by clinical and laboratory investigation by performing ultrasound abdomen and thorax.

Methods: In this study one hundred and twenty one (121) patients diagnosed with dengue fever and laboratory investigations done from Jinnah Medical Hospital (JMH) and Pathological and Molecular Laboratories from August to November 2019. Ultrasound of abdomen and thorax was performed for diagnosing complications such as ascites, pleural and pericardial effusion through Xario-100 sonography machine.

Results: Out of one hundred and twenty one (121) patients diagnosed with dengue fever forty-three (43) were found to have developed the complications such as ascites, pleural and pericardial effusion by performing ultrasound abdomen and chest.

Conclusion: We found 35 % patients with dengue fever complications by ultrasound, which is most convenient diagnostic tool. Therefore, by performing early ultrasound scan of abdomen and thorax in these patients can reduce the risk of morbidity and mortality.

Keywords: Dengue; ELISA; Antigen; Ultrasound; Ascites; Pleural; Pericardial Effusion.

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INTRODUCTION

This study was performed to find out the significance of ultrasound imaging in evaluating the complications caused by dengue fever (diagnosed on clinical and laboratory findings) to determine the severity. High grade fever is the initial symptom caused by dengue virus which gets transmitted by Aedes mosquito's sting. Clinical findings include from high grade fever to most serious symptoms life

threatening hemorrhages and shock. Along with fever, muscular pain, joint pain, myalgia, rashes are the common symptoms^{1,2}. Dengue has emerged as a worldwide life-threat to public health, affecting population in more than hundred countries. Thus, the physician should be aware about the symptoms of this viral fever and ensure an early and adequate treatment plan. The incidence of dengue has increased dramatically in the last few years. The infection is now endemic in various countries.

Patients develop dengue hemorrhagic fever who were previously infected with one subspecies of the dengue virus develop severe capillary permeability and bleeding after being infected with another subspecies of the dengue virus³. The dengue virus is an arthropod-borne virus transmitted by *Aedes aegypti* having four serotypes (DENV1-4)². Dengue was first introduced in Pakistan at Karachi port through the importation of tiers containing eggs of infected mosquitoes. Thus dengue fever spread rapidly all over the country in the last few years. This infection is endemic in various countries worldwide and diagnosis of dengue virus done by ELISA^{3,4}. Study has also shown high incidence of dengue fever, and complication in 45% patients out of which 100 % patients shown gall bladder wall thickness and hepatomegaly⁵. The other complications of dengue fever are liver injury, complications of dengue virus cardiomyopathy, pneumonia, orchitis, oophoritis, seizures, encephalopathy, and encephalitis⁵. The complications of dengue fever such as abdominal complications such as liver injury, ascites, also cardiomyopathy, and pleural effusion may get confirmed diagnosis by performing ultrasound scan⁶. This study was performed to find out the significance of the role of ultrasound in the diagnosis of complications associated with dengue fever (diagnosed on clinical and laboratory findings) to determine the severity.

METHODS

In this study One hundred and twenty one (121) dengue fever patients having clinical findings along

with Laboratory investigations were done (CBC, ICT-MP, Dengue NS-1 by ELISA) from blood samples collected from patients visiting Medicare cardiac and General Hospital and Dr. Rubina Ghani molecular and pathology Laboratory. Oral consent was taken from the patients. Blood samples were collected for complete blood count (CBC) and ICT (immunochromatographic assay) was performed to rule out the presence of malarial parasite. Oral informed consent was taken from the patients and ERC of Jinnah Medical and Dental College took ethical approval.

The ethical review committee, Jinnah Medical and Dental College approved the use of human samples in the study protocol referenced above from 01-08-2019 to 01-11-2019. ELISA was performed by using MyBioSource Elisa Kit by adding 50µl/well of sample diluent (containing secondary antibody) for Dengue NS 1 ELISA into each of necessary ELISA³. All the diagnosed cases of dengue fever had ultrasound examinations done by using Xario-100 sonography machine using probe with frequency 2.5-3.5 MHZ to for determine the complications associated with dengue fever. All abdominal ultrasounds were performed after 6-8 hours of proper fasting to allow better distension of gallbladder, also examined liver, spleen, kidneys, for ascites. Thoracic ultrasound was performed in sitting posture. Steps for ELISA as given in following Flow chart (Figure 1) by Pal et al., Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits using clinical samples³.

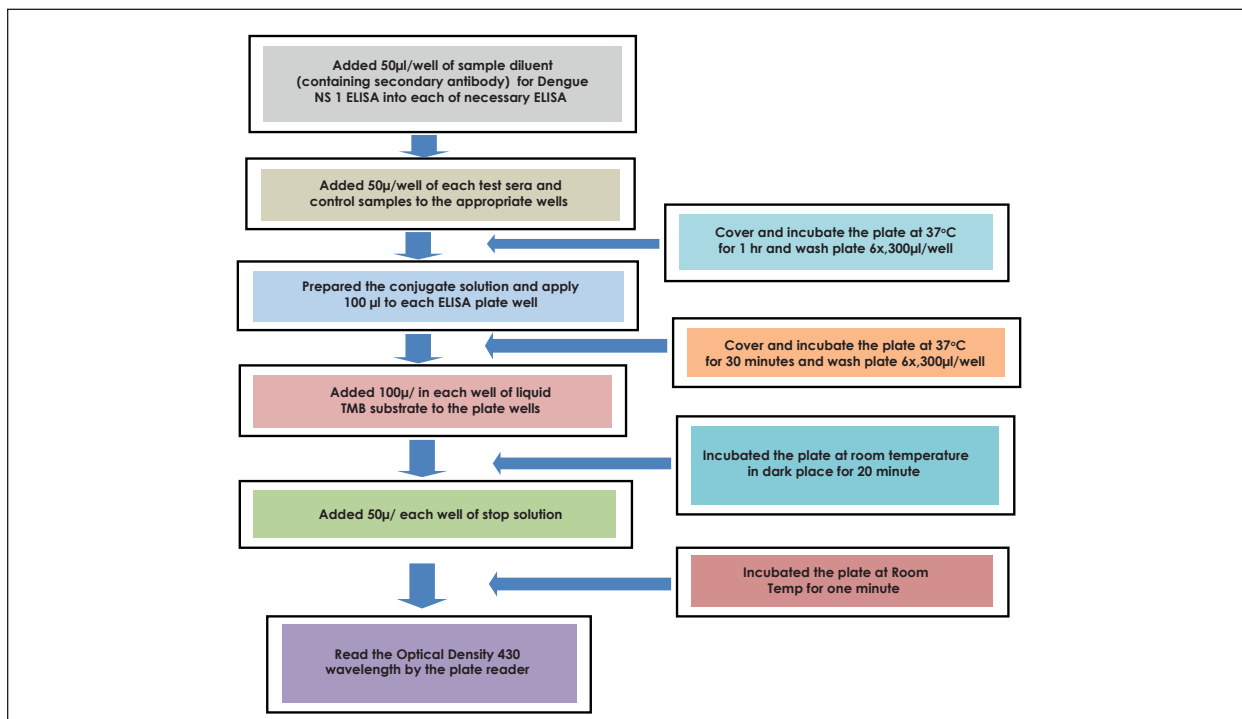


Figure 1: Enzyme-linked immunosorbent assays (ELISAS) targeting NS1 antigen (Ag).

RESULTS

Out of total of 121 patients diagnosed with dengue fever (with fever, headache, myalgia, dizziness and vomiting), 43 were found to have developed the complications. These include hepatomegaly, splenomegaly, ascites and pleural effusion by performing ultrasound abdomen and chest. After diagnosis of dengue fever by clinical signs and symptoms, ELISA was performed. Ultrasound abdomen was performed after 6-8 hours fasting.

We found Gall bladder wall thickness in 43 consistent finding in all serologically positive cases. Hepatomegaly was finding in 5 and splenomegaly was finding in 5, mild to moderate ascites in 30 cases (Table 1). Thoracic ultrasound performed in sitting posture, one patient with pericardial and bilateral mild to moderate pleural effusion was seen in 25 patients with dengue fever. Ultrasound images of abdomen in patient with dengue fever show gall bladder wall thickness, ascites, and pericardial effusion shown in Figure 2a-c.

Table 1: Ultrasound findings of the patients diagnosed with Dengue fever.

No.	Ultrasound of patients with dengue fever (N=121)	No. of cases with various complications (n=43)	Percentage (%) of patient with specific complication
Abdominal and Pelvis Ultrasound			
1	G.B Wall thickness	43	36%
2	Hepatomegaly	5	4%
3	Splenomegaly	5	4%
4	Ascites	30	25%
Ultrasound Chest			
5	Pericardial effusion	01	1%
6	Pleural effusion	25	20%

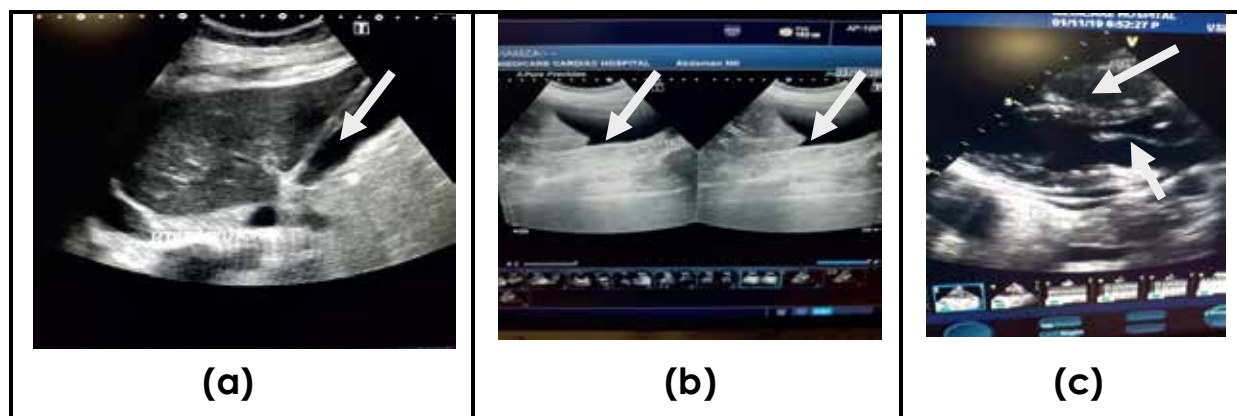


Figure 2: (a) Ultrasound findings of abdomen in patient with dengue fever, arrow show thickened Gall Bladder wall. (b) Ultrasound findings of abdomen in-patient with dengue fever show ascites. (c) Ultrasound findings of thorax inpatient with dengue fever show pericardial effusion.

DISCUSSION

In this study 43 (forty three) patients were found to have developed the complications such as ascites, pleural and pericardial effusion was confirmed by performing ultrasound abdomen and chest. Dengue fever (DF) can present as an asymptomat-

ic infection or simple high grade fever or dengue hemorrhagic fever (DHF) with multi-organ failure, due to increase in capillary permeability with leakage of fluids during the critical phase of dengue fever⁷⁻¹². A study done in teaching hospital of Sri Lanka during epidemic of dengue fever in 2016. The age range 12-51 years, comprising six

females and four males. The group of patients included seven cases of hemorrhagic fever, three of dengue fever and two patients with primary dengue infections had severe bleeding into gut. Other life threatening problems were acute severe hepatitis, severe septic shock, myocarditis, intracranial bleeding, and diarrhea and decompensated dengue shock. Blood transfusions and other empirical therapeutic methods were used along with fluid replacement to manage each patient. Bedside ultrasound scanning helped early detection of critical phase. All of these patients recovered completely¹³. In another study done to determine utility of ultrasound for patients with dengue fever symptoms lasting for a weeks, had reported gall bladder wall thickness in all patients, hepatomegaly (21%), splenomegaly (7%), ascites (96%) pleural effusion (87%). Hemorrhagic fever and shock syndrome are the most severe complications of dengue infection. Acute renal failure is one of the complications of severe dengue infection (2-5%) of cases and is associated with hypotension, rhabdomyolysis, or hemolysis. Mesangial proliferation and immune complex deposition is the characteristic feature of dengue-associated glomerulonephritis¹⁴. Liver dysfunction is commonest in both dengue fever and with DF complaints of abdominal pain, nausea, vomiting and anorexia.

Also a study shown that due to direct effects of the virus or host immune response on liver cells, circulatory compromise, metabolic acidosis and/or hypoxia caused by hypotension or localized vascular leakage inside the liver are possible cause of liver dysfunction^{15,16}. Bleeding is common complication both dengue fever and dengue hemorrhagic fever. Pathology of the dengue fever is not very well understood. Thrombocytopenia is most common in the laboratory finding in this viral fever. Abnormal platelet functions results in rapid peripheral destruction of platelets^{17,18}. A study also suggests that bleeding is more common in patients with severe thrombocytopenia. Such patients having platelet count less than 20,000/cumm and high risk of bleeding require platelet transfusion whereas patients with platelet count 21-40,000/cumm required platelet transfusions only with hemorrhagic episodes¹⁷⁻¹⁸.

Study done in Sri Lanka on dengue fever patients had shown high incidence of cardiac complications during dengue fever outbreak¹⁹. Studies have reported neurological complications associated with DF and DHF^{20,21}. A retrospective cross-sectional done at Dr. Soliman Fakeeh Hospital in Jeddah, Saudi Arabia between January 2010 and June 2014, five hundred and sixty seven patients had been diagnosis of dengue fever or dengue hemorrhagic fever/shock syndrome. Also there were associate complications of thrombocytopenia, plasma leakage syndrome (pleural effusion or ascites) or bleeding²². Furthermore, thrombocytopenia occurs maybe due to altered

megakaryocytopoiesis causing malfunction of platelets thus leading to hemorrhages²³⁻²⁸.

The Dengue vaccine (DV) attacks the monocytes, dendritic cells and macrophages. In addition, the DV non-structural protein (NS1) causes damage to endothelial cells and platelets. Moreover, abnormal activity of T-cells results in increase in vascular permeability²⁸⁻²⁹. Most serious complications of DF /DHF include hepatic dysfunction leading to acute hepatic failure, muscle disorder such as myositis, rhabdomyolysis, gastrointestinal and intracranial bleeding. Also may include endocrine disorders such as Diabetes ketoacidosis and Guillain-Barre syndrome³⁰. Early and proper diagnosis of dengue fever and complications has improved the survival but still more efforts for prevention and appropriate management of such patients. As found in the study done in Pakistan, due to overpopulation and poor sanitation the incidence this viral disease is very high in larger cities³⁰.

This indicated high incidence of dengue fever in last few months and evolved appropriate diagnosis of dengue and effective ultrasound scans had been able to diagnose complications such as ascites, pericardial and pleural effusion enabling better patient care to reduce mortality.

CONCLUSION

The study had shown high incidence of dengue fever in Karachi, Pakistan. If fever develops early diagnosis is most important in reducing complications and mortality. By determine the complication by ultrasound scan in dengue fever, which is most convenient diagnostic tool, early and appropriate management, can reduce the risk of morbidity and mortality.

ACKNOWLEDGMENTS

We are highly thankful to Medicare Cardiac and General hospital and patients to provide us approval for carrying out this study.

CONFLICTS OF INTEREST

The authors declared no conflict of interest.

ETHICS APPROVAL

The ethical review committee of Jinnah Medical and Dental College approved the use of human samples in the study protocol referenced above from 01-08-2019 to 01-11-2019.

PATIENT CONSENT

Verbal consent was obtained from all the patients.

AUTHORS' CONTRIBUTION

SI and SK contributed in providing ultrasound findings, SPK contributed in the concept of study design and article writing and RG contributed for providing samples of blood test and ELISA.

REFERENCES

- Hasan S, Jamdar SH, Alalawi M, Al Beajji SMA. Dengue virus: A global human threat: Review of literature. *J Int Soc Prev Community Dent.* 2016; 6(1):1-6.
- Kurane I. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. *Comp Immunol Microbiol Infect Dis.* 2007;30:329-340.
- Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, *et al.* Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. *PLoS One.* 2014;9(11): e113411.
- Yousaf MZ, Siddique A, Ashfaq UA, Ali M. Scenario of dengue infection & its control in Pakistan: An up-date and way forward. *Asian Pac J Trop Med.* 2018;11(1):15-23.
- Sherin A. Dengue fever: A major public health concern in Pakistan. *KMUJ.* 2011;3(1):1-3.
- Sai VPK, Dev B, Krishan R. Role of ultrasound in dengue fever. *Br J Radiol.* 2005;78(929):416-418.
- Wilder-Smith A, Schwartz E. Dengue in travelers. *N Engl J Med.* 2005; 353:924-932.
- Kularatne SAM. Dengue fever. *BMJ.* 2015;351: h4661.
- Dalugama C, Ralapanawa U, Jayalath T. Dengue myositis and review of literature. *Clin Case Rep Res Trials.* 2017;2:16-18.
- Dalugama C, Gawarammana IB. Dengue hemorrhagic fever complicated with transient diabetic ketoacidosis: a case report. *J Med Case Rep.* 2017;11(1):302.
- Ralapanawa DM, Kularatne SA, Jayalath WA. Guillain-Barre syndrome following dengue fever and literature review. *BMC Res Notes.* 2015;8:729.
- Kularatne SAM, Ralapanawa U, Dalugama C, Jayasinghe J, Rupasinghe S, Kumarihamy P. Series of 10 dengue fever cases with unusual presentations and complications in Sri Lanka: a single centre experience in 2016. *BMC Infect Dis.* 2018; 18:674.
- Stephenson JR. Understanding dengue pathogenesis: implications for vaccine design. *Bull World Health Organ.* 2005;83:308-314.
- Lizarraga KJ, Nayer A. Dengue-associated kidney disease. *J Nephropathol.* 2014;3(2):57-62.
- Itha S, Kashyap R, Krishnani N, Saraswat VA, Choudhuri G, Aggarwal R. Profile of liver involvement in dengue virus infection. *Natl Med J India.* 2005;18:127-130.
- Kularatne SAM, Imbulpitiya IVB, Abeysekera RA, Waduge R, Rajapakse RPVJ, Weerakoon KGAD. Extensive hemorrhagic necrosis of liver is an unpredictable fatal complication in dengue infection: a postmortem study. *BMC Infect Dis.* 2014;14:141.
- Srichaikul T. Hematologic Changes in Dengue Hemorrhagic Fever. *J Hematol Transfus Med.* 2014; 24:47-56.
- Makroo RN, Raina V, Kumar P, Kanth RK. Role of platelet transfusion in the management of dengue patients in a tertiary care hospital. *Asian J Transfus Sci.* 2007;1:4-7.
- Kularatne SAM, Pathirage MMK, Kumarasiri PVR, Gunasena S, Mahindawanse SI. Cardiac complications of a dengue fever outbreak in Sri Lanka, 2005. *Trans R Soc Trop Med Hyg.* 2007;101(8):804-808.
- Puccioni-Sohler M, Soares CN, Papaiz-Alvarenga R, Castro MJ, Faria LC, Peralta JM. Neurologic dengue manifestations associated with intrathecal specific immune response. *Neurol.* 2009;73:1413-1417.
- Weerasinghe WS, Medagama A. Dengue hemorrhagic fever presenting as encephalitis: a case report. *J Med Case Rep.* 2019; 13: 278.
- Badreddine S, Al-Dhaheer F, Al-Dabbagh A, Al-Amoudi A, Al-Ammari M, Elatassi N, *et al.* Dengue fever. Clinical features of 567 consecutive patients admitted to a tertiary care center in Saudi Arabia. *Saudi Med J.* 2017; 38(10):1025-1033.
- World Health Organization. Dengue - guidelines for diagnosis, treatment, prevention and control; new edition. World Health Organization. 2009. p.1-21.
- Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, *et al.* Dengue: A continuing global threat. *Nat Rev Microbiol.* 2010;8(12Suppl):S7-S16.
- Linares EM, Pannuti CS, Kubota LT, Thalhammer S. Immunospot assay based on fluorescent nanoparticles for dengue fever detection. *Biosens Bioelectron.* 2013;41:180-185.
- San Martin JL, Brathwaite O, Zanbrano B, Solorzano JO, Bouckennooghe A, Dayan GH, *et al.* The epidemiology of dengue in the Americas over the last three decades: A worrisome reality. *Am J Trop Med Hyg.* 2010;82:128-135.
- Arshad I, Malik FA, Hussain A, Shah SA. Dengue fever: Clinico-pathologic correlations and their association with poor outcome. *Professional Med J.* 2011;18:57-63.
- Whitehorn J, Simmons CP. The pathogenesis of dengue. *Vaccine.* 2011;29:7221-7228.
- Simmons CP, McPherson K, Van Vinh Chau N, Hoai Tam DT, Young P, Mackenzie J, *et al.* Recent advances in dengue pathogenesis and clinical management. *Vaccine.* 2015; 33:7061-7068.
- Zubair M, Ashraf M, Ahsan A, Nazir N, Hanif H, Khan HA. Dengue viral infections in Pakistan and other Asian countries: a comprehensive review. *JPMA.* 2016;66(7):884-888.

REVIEW ARTICLE

Cross Infection in Dentistry and the Dental Aerosols - A Potential Health Hazard

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ABSTRACT

Many hazards are often associated with different occupations and known as occupational hazards. Dentistry is also one of those professions having many occupational risks. Such hazards are broadly categorized into chemical, biological, physical, mechanical and psychological factors. Cross infection is one of such hazards faced by the health care providers' particularly dental workers. Cross infection is the transmission of infectious agents between patient and staff in clinical environment. Control of cross infection has remained the major concern of the dental community for decades. Various vectors are involved in cross infections in Dentistry including patients, environment (water, air), instruments etc. Particularly among the environmental factors, airborne route is one of the major routes of cross contamination and so aerosols produced in dental environment carry greatest pathogenic potential. Production of aerosols and splatters occur because of many procedures carried out in mouth including crown preparation, ultrasonic scaling, caries excavation etc. Patients, dental instruments and dental unit waterlines (DUWL) are the important sources of contaminated aerosol production in dental operator. Use of personal Protective equipments, regular use of pre-procedural mouth rinses and high evacuation devices are few of the suggested methods to reduce risk of cross contamination especially through aerosols and splatters in the dental offices. The related articles were searched through PubMed and Google Scholar.

Keywords: Dental Aerosols; Cross Infection; Airborne Contamination; Dental Operator; Personal Protective Equipment; Saliva.

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INTRODUCTION

Certain employments are often associated with certain risks, which are known as occupational hazards¹. Such hazards may include any material, substance or process that predispose or itself cause accidents or disease at workplace². According to the estimation of WHO, there is annual exposure of about 3 million health care workers to blood borne viruses³.

Among different professions, Dentistry is also one of them which is surrounded by many occupational risks⁴. These hazards often cause different diseases in dental health care workers which sometimes develop and intensify with time⁵.

Majority of dental practitioners and public consider the profession of dentistry as extremely hazardous⁶. Hazards found in dentistry are broadly categorized as chemical, physical, biological, mechanical and psychological factors⁷. Physical hazards include cuts or puncture wounds from needles or other sharp instruments, eye injuries occurring from projectiles etc⁸. Hazardous radiation including ionizing (X-rays) and non-ionizing radiation (visible and UV light) can badly affect the lives of dental health care providers⁹.

Hearing problems are usually caused by noise producing devices used in dental setup including hand pieces, ultrasonic scalers etc¹⁰. Mechanical hazards may include different musculoskeletal problems arising as a result of wrong body postures

or repeated body movements¹¹. While chemical hazards can be organic (gases, resins, solvents), inorganic (mercury toxicity), caustics (hydrogen peroxide, formaldehyde) or Latex glove allergy (contact dermatitis)¹². Stress, excessive work load and lack of job satisfaction can give rise to different psychological problems⁶. Biological hazards can occur due to cross contamination, infections and allergens of biological origin¹³.

DISCUSSION

Bernadino Ramazzini, known as the "Father of Occupational Medicine" recognized the role of occupation in health and disease in the 18th century. Occupational hazard is defined as risk to a person arising out of employment and can refer to any material, substance or situation that predisposes or itself causes accidents or disease at a work place¹⁴. Cross infection is one of the greatest health risk and occupational hazard confronted by the dental health care providers¹⁵.

1. Cross Infection in Dentistry

Cross infection is the transmission of infectious agents between patient and staff in clinical environment¹⁶. The world's health care system is facing a serious threat because of large number of cases of hospital-acquired infections¹⁷. Dental community is also combating this issue and so the control of cross infections is one of its biggest target¹⁸. The dental community's main concern for decades has been the spread of infection and its control in the clinical environment¹⁹. Accordingly a study by Mahboobi et al., dental practitioners among all health care workers is greatly exposed to infectious agents²⁰. On the other hand, it was reported in a study that five patients got HIV infection from an American dentist and this issue raised the concern of infection control among the dental communities²¹. The factors, which are predisposing dentists or patients to microbial infections, are the surgical nature of the dental practice and close proximity to patient's mouth²². Various vectors are involved in the spread of infections in dentistry including instruments and air²³. Particularly patient's blood, saliva and respiratory secretions are most significant vectors involve in cross infection²⁴. Cases have been reported regarding the exposure of dental workers to infectious materials, including contaminated equipment, body substances, environmental surfaces, water, or air²⁵. The major biological risk is those microorganisms that are present in non-sanitized or contaminated surfaces, biological matrices (blood, saliva and gingival fluids), dental unit water lines, or emitted by patients²⁶.

Percutaneous Exposure Incidents

Health care workers including dentists are greatly affected by percutaneous incidents including needle stick and sharp injuries¹⁴. Restricted field of

vision and limited access in the working area make dentists more prone to such sharp injuries¹⁴. Percutaneous exposure incidents facilitate the spread of blood borne infections including HIV, HEP B and HEP C²⁴.

Aerosol – A Potential Vector of Cross Contamination

Many studies reported that many infectious agents are airborne and so the hospital air has been found to be a potential route of transmission of hospital-acquired infections. Broad spectrum of microbial species have been isolated from hospital air whose source of origination can either be hospital staff, patients and their visitors, ventilation and air conditioning system etc¹⁷.

Cross contamination in dentistry can also occur as a result of various vectors including contaminated instruments, body substances, equipments, environmental surfaces, water and aerosols containing different pathogens²⁷. Formation of aerosols and splatters occur as a result of many dental procedures carried out in mouth containing many pathogens²⁸. Cross contamination through aerosols has remained a main concern for the dental community²⁹. Health care providers including dental personnel are at greater risk of exposure to pathogenic microorganisms found in these aerosols because they frequently perform aerosols producing procedures³⁰.

2. What are Aerosols?

Significant work done by Micik and colleagues in the domain of Aerobiology enabled them to use the two terminologies that are aerosol and splatter in the dental environment³¹. Aerosols are solid and liquid particles with particle size 50µm or less and suspended in air by humans, instruments or machines³². The generation of aerosols by human occurs because of breathing, talking, sneezing or coughing³³. Most pathogenic aerosols are considered to be those having particle size less than 50 µm³². Studies have reported that these aerosols can contaminate surfaces in range of one meter (3ft.). Respiratory passages and lungs are easily penetrated by small aerosol particles carrying the greatest pathogenic risk³⁴. Certain factors increase the chances of getting infections including: the host's immune response and dose, virulence; and pathogenicity of the micro-organism³³.

Pathogenic Potential of Aerosols

Mycobacterium tuberculosis, Streptococcus pyogenes, Corynebacterium diphtheriae and Neisseria meningitides are the main pathogens transmitted through air borne route and cause hospital acquired infections. 10–20% of such infections are caused by air borne bacteria¹⁷. Dental aerosols have been found to contain those microorganisms associated with various diseases such as staphylococcal infection, viral infections, tuberculosis, skin infections, conjunctivitis etc³².

When discussing infection control, consideration of bacterial aerosols is at top of list because of having greater pathogenic potential³⁵. Because of their smaller size, aerosols can remain airborne for longer duration and studies reported the survival of microorganisms in the aerosols for a week³⁶. Aerosols smaller particles carry the greatest pathogenic potential and can easily invade the respiratory passages²⁹. Staphylococcus and Micrococcus species are predominant microorganisms isolated from bio aerosols in dental clinics (Table 1)³⁷.

Table 1: Diseases spread by aerosols.

DISEASES KNOWN TO BE SPREAD BY DROPLETS OR AEROSOLS	
DISEASES	METHOD OF TRANSMISSION
Pneumonic Plague	Patient to patient without the usual insect vector (flea); apparently by inhalation of the causative bacteria
Tuberculosis	Droplet nuclei expelled from the patient by coughing; once considered an occupational disease for dentists
Influenza	Apparently associated with coughing but may require direct contact with the patient
Legionnaires' Disease	Aerosolization of Legionella pneumophila has been associated with air conditioning systems and hot tub spas
Severe Acute Respiratory Syndrome	Spread by direct contact and aerosolized droplets

From Harrel and Molinari, 2004³¹.

Composition of Dental Aerosols

Variations in the composition of dental aerosols is reported in studies with the operative site and patients³¹. Dental aerosols have been found to contain components of nasopharyngeal secretions, saliva, plaque, blood, tooth components, restorative or other materials used in the dental procedure³⁸.

Formation of Aerosols

The use of compressed air and water by high-powered devices result in production of aerosols³⁹.

Cross Infection through Aerosols

The potential sources of microbes found to contaminate dental aerosols are the dental unit water lines, saliva, plaque/calculus, blood, contaminated equipments etc³⁰. *Mycobacterium tuberculosis*, *streptococci*, *Pneumococci*, *staphylococci* are frequently reported bacteria to be present in dental aerosols. Viruses found to be present in aerosols include influenza virus, common cold viruses, Cytomegalovirus, hepatitis virus, herpes simplex virus and Epstein-Barr virus³². Presence of fungi is also reported in studies and found to be an important risk for health care workers³⁷. Some studies reported the exposure of dental health care workers to up to 1.86 E + 05 bacteria/m³ of air while according to other studies; it could be up to 4.3 E + 05 bacteria/m³, generated during dental procedures³⁷. Studies reported higher levels of aerosols for cavity preparation (24-105 CFU/m³) and for ultrasonic scaling (42-71 CFU/m³), and lower levels were reported for

extraction (9-66 CFU/m³) and for oral examination (24-62 CFU/m³)³⁷. After conclusion of dental treatment ,time taken by bio aerosols to return to baseline is approximately 2 hours³⁷(Figure 1).

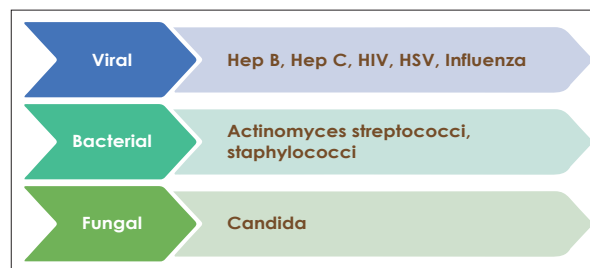


Figure 1: Common transmissible infections in dentistry.

3. Airborne Contamination During Dental Treatment

Airborne route has been documented in studies as one of the greatest potential source of spreading infection in clinical settings⁴⁰. Studies reported the spread of different microorganisms in dental office as a result of different procedures⁴¹. Many procedures that are performed in dental offices result in production of aerosols and splatters including crown preparation, ultrasonic scaling ,caries excavation⁴² etc. The use of certain equipments such as ultrasonic scalers, air polishers, dental hand pieces and air abrasion units produce most visible and viable bio aerosols³⁰. Patients, dental instruments and dental unit waterlines (DUWL) are the important sources of aerosol production in dental operatory⁴³.

Saliva and Respiratory Sources of Contamination

Numerous bacteria are harbored by the oral cavity from the dental plaque, the respiratory tract and oral fluids⁴³. Air borne contamination through these microorganisms can easily occur when saliva is aerosolized by mechanical instrumentation³¹.

Contamination from the Operative Site

Bio aerosols are produced from the operating site because of different dental procedures using mechanical instrumentation including ultrasonic scalers, hand pieces, air abrasion units, air polishing device etc¹⁵. The materials removed by these instruments from the operative site becomes aerosolized by the action of the rotary instrument, ultrasonic vibrations or the combined action of water sprays and compressed air³¹.

Aerosols Producing Instruments in Dental Operatories

Ultrasonic scalers, air-polishing devices, dental hand pieces and air abrasion units produce airborne particles by the combined action of compressed air, water sprays, organic particles, such as tooth dust and tissue, organic fluids such as saliva and blood from the site where instrument is used⁴³. Studies reported the greatest production of aerosols as a result of ultrasonic and sonic scalers,

air turbines, and air polishers⁴⁴. A water spray is required by these instruments to prevent heat production, lavage the working area and for cooling the working tip. A potentially pathogenic aerosol is formed when this water spray mix with patient's saliva or blood²⁷. Studies reported that these instruments can result in about four fold increase of airborne bacteria in the dental operatory³⁹.

Contamination through Dental Unit Water Lines (DUWL)

Dental unit water lines are found to be an important source of contaminated aerosol production⁴⁵. Pathogens isolated from dental unit water are the *Legionella pneumophila* and *Pseudomonas* species and found to be aerosolizing by high powered devices including ultrasonic scalers and hand pieces⁴⁶.

4. Ultrasonic Scalers - Greatest Producers of Aerosols

Studies reported ultrasonic scalers and air polishers as the greatest producers of aerosols in dental operatory⁴⁷. Ultrasonic scaling produces considerable amount of aerosol spray, which can act as a vector for microorganisms and aid in spread of infection. These scalers when used produce a mixture of compressed air and water which spurts from the hand piece, further mixing with patient's saliva and blood forming a fine spray which ejects from the patient's mouth⁴⁸. Aerosols producing devices reduce the air quality in dental office due to increased air contamination³⁹. Aerosol from ultrasonic instrumentation always contains blood and lingers in the air for 30 min or longer in the dental offices or nearby areas³² (Figure 2).



Figure 2: Aerosol production from ultrasonic scalers.

5. Ways of Preventing Microbial Cross Infection

Strict aseptic principles are needed to be incorporated in the clinical practice in order to reduce microbial cross contamination⁴⁹. For minimizing cross contamination, different materials and procedures are recommended such as use of personal protective barriers, decontamination of surfaces, immunization of dental staff, sterilization of

instruments and pre-procedural mouthwashes⁴¹. Dental workers are highly recommended by Centre for disease control (CDC) for using personal protective equipment (PPE) (Masks, goggles and gowns) and barriers to cover clinical surfaces when performing dental treatments⁵⁰. Use of personal protective equipment is extremely important for dental health care providers as they have greater risk to be exposed to pathogenic microbes¹⁴.

6. Aerosols Reduction Ways

For contaminated aerosols reduction, Harrel and Molinari recommended three levels of defense which are as follows²⁹:

1. Use of Personal Protective equipments
2. Regular use of Pre-procedural mouth rinses
3. Use of high evacuation device³⁹.

To effectively minimize bio aerosol contamination, CDC has recommended the use of universal barrier techniques for all dental procedures⁴². Pre-procedural rinsing with effective mouthwashes mainly Chlorhexidine (CHX) in varying concentrations, have also found to be very effective in reducing aerosol contamination specially before ultrasonic scaling and periodontal surgeries⁵¹. For reducing cross contamination, the dental practitioners are strongly recommended to put into practice the control of bio aerosols contamination in their routine infection control protocol¹⁵. Use of expensive methods such as high-efficiency particulate air and ultraviolet chambers in the ventilation system have also been recommended¹⁵ (Figure 3).

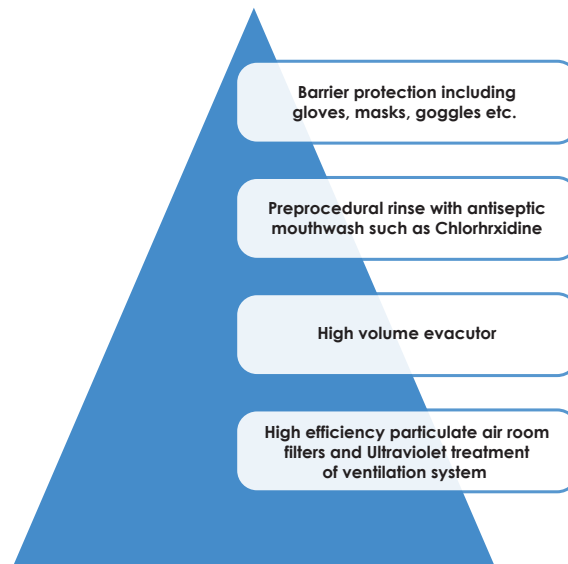


Figure 3: Methods of reducing airborne contamination.

In order to minimize the chances of cross infection, it is strictly recommended to incorporate aseptic principles in the clinical practice. Use of personal protective instruments (gloves, masks, gown and goggles), decontamination of surfaces, immunization of dental staff, sterilization of instruments are strictly recommended for reducing cross infection by Centre for disease control and prevention (CDC), American Dental association (ADA) etc. Pre-procedural rinsing with effective mouthwash (chlorhexidine (CHX), Povidone-iodine (PI), etc.), use of high evacuation device and some expensive methods like high-efficiency particulate air and ultraviolet chambers in the ventilation system has also been recommended to minimize cross contamination through aerosols in the dental operatory.

CONCLUSION

Dentistry is surrounded by many occupational perils. Cross infection through various routes are reported in many studies including air, blood, water etc. Airborne route is of prime concern when discussing the infection control programs in dentistry as this route carries the greatest potential to transmit infections. Aerosols and splatters have remained an important debate for the dental community whenever infection control is taken into consideration. Aerosols and splatter are produced because of many procedures performed in dental set up and so there is a greater chance of cross contamination through these airborne particles.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

AA did conceptualization of study and wrote the first draft. SAB and MH revised draft critically and Fakhruddin managed the literature searches.

REFERENCES

1. Agrawal N, Gupta N, Bey A, Garg AK, Sharma V. Occupational hazards in modern dentistry: A review. *Int J Med Health Res.* 2014;1:1-9.
2. Fasunloro A, Owotade FJ. Occupational hazards among clinical dental staff. *J Contemp Dent Pract.* 2004;5(2):134-152.
3. Hasanah NT, Kasuma N, Fitriana A. The Difference of Bacterial Aerosol Quantity Based on Blood Agar Plate Position. *UI Proceedings on Health and Medicine.* 2017;1:83-88.
4. Al-Amad SH, Awad MA, Edher FM, Shahramian K, Omran TA. The effect of rubber dam on atmospheric bacterial aerosols during restorative dentistry. *J Infect Public health.* 2017;10(2):195-200.
5. Mehta A, Gupta M, Upadhyaya N. Status of occupational hazards and their prevention among dental professionals in Chandigarh, India: A comprehensive questionnaire survey. *Dental Res J.* 2013;10(4):446-451.
6. Bennadi D, Reddy V, Thummala NR. Preventive and curative measures adopted by dentists to combat occupational hazards-a cross sectional study. *Int J Pharm Pharm Sci.* 2015;7(10):416-418.
7. Smith DR, Leggat PA, Walsh LJ. Workplace hazards among Australian dental students. *Aus Dent J.* 2009;54(2):186-188.
8. Ekmekcioglu H, Unur M. Eye-related trauma and infection in dentistry. *J Istanbul Univ Fac Dent.* 2017;51(3):55-63.
9. Aravind B, Joy ET, Kiran MS, Sherubin JE, Sajesh S, Manchil PRD. Attitude and awareness of general dental practitioners toward radiation hazards and safety. *J Pharm Bioallied Sci.* 2016;8(Suppl 1):S53-S58.
10. Qsaibati ML, Ibrahim O. Noise levels of dental equipment used in dental college of Damascus University. *Dent Res J.* 2014;11(6):624-630.
11. Kaul R, Shilpa P, Sanjay C. Musculoskeletal disorders and mental health related issues as occupational hazards among dental practitioners in the city of Bengaluru: a randomized cross-sectional study. *JDR Clin Trans Res.* 2015;4(1):589-598.
12. Ayatollahi J, Ayatollahi F, Ardekani AM, Bahrololoomi R, Ayatollahi J, Ayatollahi A, et al. Occupational hazards to dental staff. *Dent Res J.* 2012;9(1):2-7.
13. Yoo YJ, Kwak EJ, Jeong KM, Baek SH, Baek YS. Knowledge, attitudes and practices regarding methicillin-resistant *Staphylococcus aureus* (MRSA) infection control and nasal MRSA carriage rate among dental health-care professionals. *Int Dent J.* 2018;68(5):359-366.
14. Solanki J, Gupta S. Occupational Hazards among western indian private dental practitioners: a questionnaire-based descriptive study. *J Indian Assoc Public Health Dent.* 2017;15(1): 48-52.
15. Narayana T, Mohanty L, Sreenath G, Vidhyadhari P. Role of preprocedural rinse and high volume evacuator in reducing bacterial contamination in bioaerosols. *J Oral Maxillofac Pathol.* 2016;20(1):59-65.
16. Volgenant C, de Soet J. Cross-transmission in the dental office: Does this make you ill? *Curr Oral Health Rep.* 2018;5(4):221-228.
17. Mirhoseini SH, Nikaeen M, Khanahmad H, Hatazadeh M, Hassanzadeh A. Monitoring of airborne bacteria and aerosols in different wards of hospitals-Particle counting usefulness in investigation of airborne bacteria. *Ann Agric Environ Med.* 2015;22(4):670-673.
18. Yadav S, Kumar S, Srivastava P, Gupta KK, Gupta J, Khan YS. Comparison of efficacy of three different mouthwashes in reducing aerosol

- contamination produced by ultrasonic scaler: a pilot study. *Indian J Dent Sci.* 2018;10(1):6-10.
19. Al Maghlouth A, Al Yousef Y, Al Bagieh N. Qualitative and quantitative analysis of bacterial aerosols. *J Contemp Dent Pract.* 2004;5(4):91-100.
 20. Ramamoorthy A, Jeevakarunyam SJ, Janardhanan S, Jeddy N, Vasan SA, Raja A, *et al.* Survey on utility of yoga as an alternative therapy for occupational hazards among dental practitioners. *J Nat Sci Biol Med.* 2015;6(1):149-152.
 21. Yüzbasıoğlu E, Saraç D, Canbaz S, Saraç YS, Cengiz S. A survey of cross-infection control procedures: knowledge and attitudes of Turkish dentists. *J Appl Oral Sci.* 2009;17(6):565-569.
 22. Laheij A, Kistler J, Belibasakis G, Välimaa H, De Soet J, Workshop EOM. Healthcare-associated viral and bacterial infections in dentistry. *J Oral Microbiol.* 2012;4(1):17659.
 23. Merte JL, Kroll CM, Collins AS, Melnick AL. An epidemiologic investigation of occupational transmission of Mycobacterium tuberculosis infection to dental health care personnel: infection prevention and control implications. *J Am Dent Assoc.* 2014;145(5):464-471.
 24. Saini R. Chlorine dioxide: An ideal preprocedural mouthrinse in dental set-up. *Eur J Gen Dent.* 2015;4(3):113-116.
 25. Al-Maweri SA, Tarakji B, Shugaa-Addin B, Al-Shamiri HM, Alaizari NA, AlMasri O. Infection control: Knowledge and compliance among Saudi undergraduate dental students. *GMS Hyg Infect Control* 2015;10:1-10.
 26. Abichandani SJ, Nadiger R. Cross-contamination in dentistry: A comprehensive overview. *Chron Young Scientists.* 2012;2(1):3-9.
 27. Suresh SR, Manimegalai M, Sudhakar U. Comparison of efficacy of preprocedural rinsing with chlorhexidine and essential oil mouthwash in reducing viable bacteria in dental aerosols - a Microbiological Study. *Int Journal Contemp Dent.* 2011;2(6):1-2.
 28. Swaminathan Y, Toby Thomas J, Muralidharan N. The efficacy of preprocedural mouth rinse of 0.2% chlorhexidine and commercially available herbal mouth containing salvadora persica in reducing the bacterial load in saliva and aerosol produced during scaling. *Asian J Pharm Clin Res.* 2014;7(1):71-74.
 29. Gopalakrishnan D, Juluri R, Srihari J, Viswanathan V. Comparing the efficacy of two mouth rinses in reducing bacterial aerosol contamination. *J Dent Oral Health.* 2017;4:1-4.
 30. Ramesh A, Thomas JT, Muralidharan N, Varghese SS. Efficacy of adjunctive usage of hydrogen peroxide with chlorhexidine as preprocedural mouthrinse on dental aerosol. *Natl J Physiol Pharm Pharmacol.* 2015;5(5):431-435.
 31. Harrel SK, Molinari J. Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. *J Am Dent Assoc.* 2004;135(4):429-437.
 32. Afzha R, Chatterjee A, Subbaiah SK, Pradeep AR. Microbial contamination of contact lenses after scaling and root planing using ultrasonic scalers with and without protective eyewear: A clinical and microbiological study. *J Indian Soc Periodontol.* 2016;20(3):273-278.
 33. Zemouri C, de Soet H, Crielgaard W, Laheij A. A scoping review on bio-aerosols in healthcare and the dental environment. *PloS one.* 2017;12(5):e0178007.
 34. Singh A, Manjunath RS, Singla D, Bhattacharya HS, Sarkar A, Chandra N. Aerosol, a health hazard during ultrasonic scaling: a clinico-microbiological study. *Indian J Dent Res.* 2016;27(2):160-162.
 35. Bhuvanawari P. Aerosols-A concern for dentists. *Res J Pharm Technol.* 2014;7(8):9.
 36. Holloman JL, Mauriello SM, Pimenta L, Arnold RR. Comparison of suction device with saliva ejector for aerosol and spatter reduction during ultrasonic scaling. *J Am Dent Assoc.* 2015;146(1):27-33.
 37. Kobza J, Pastuszka J, Brągoszewska E. Do exposures to aerosols pose a risk to dental professionals? *Occup Med.* 2018;68(7):454-458.
 38. Manarte-Monteiro P, Carvalho A, Pina C, Oliveira H, Manso MC. Air quality assessment during dental practice: Aerosols bacterial counts in an university clinic. *Rev Port Estomatol Cir Maxilofac.* 2013;54(1):2-7.
 39. Sawhney A, Venugopal S, Babu GR, Garg A, Mathew M, Yadav M, *et al.* Aerosols how dangerous they are in clinical practice. *J Clin Diagn Res.* 2015;9(4):ZC52- ZC57.
 40. Raghunath N, Meenakshi S, Sreeshyla H, Priyanka N. Aerosols in dental practice-A neglected infectious vector. *Br Microbiol Res J.* 2016;14(2):1-8.
 41. Retamal-Valdes B, Soares GM, Stewart B, Figueiredo LC, Faveri M, Miller S, *et al.* Effectiveness of a pre-procedural mouthwash in reducing bacteria in dental aerosols: randomized clinical trial. *Braz Oral Res.* 2017; 31:e21.
 42. Jawade R, Bhandari V, Ugale G, Taru S, Khaparde S, Kulkarni A, *et al.* Comparative evaluation of two different ultrasonic liquid coolants on dental aerosols. *J Clin Diagn Res.* 2016;10(7):ZC53-ZC57.
 43. Acharya S, Priya H, Purohit B, Bhat M. Aerosol contamination in a rural university dental clinic in south India. *Int J Infect Control.* 2009;6(1):1-7.
 44. Kaur R, Singh I, Vandana K, Desai R. Effect of chlorhexidine, povidone iodine, and ozone on microorganisms in dental aerosols: randomized double-blind clinical trial. *Indian J Dent Res.* 2014;25(2):160-165.
 45. Mamajiwala AS, Sethi KS, Raut CP, Karde PA, Khedkar SU. Comparative evaluation of chlorhexidine and cinnamon extract used in dental unit waterlines to reduce bacterial load in aerosols during ultrasonic scaling. *Indian J Dent Res.* 2018;29(6):749-754.
 46. Petti S, Vitali M. Occupational risk for Legionella infection among dental healthcare workers: meta-analysis in occupational epidemiology. *BMJ open.* 2017;7(7):e015374.

47. Rao RM. Determination of efficacy of pre-procedural mouth rinsing in reducing aerosol contamination produced by ultrasonic scalers. *Nitte Uni J Health Sci.* 2015;5(3):52-56.
48. Devker N, Mohitey J, Vibhute A, Chouhan VS, Chavan P, Malagi S, *et al.* A study to evaluate and compare the efficacy of preprocedural mouthrinsing and high volume evacuator attachment alone and in combination in reducing the amount of viable aerosols produced during ultrasonic scaling procedure. *J Contemp Dent Pract.* 2012;13(5):681-689.
49. Umar D, Basheer B, Husain A, Baroudi K, Ahamed F, Kumar A. Evaluation of bacterial contamination in a clinical environment. *J Int Oral Health.* 2015;7(1):53-55.
50. Saccucci M, Ierardo G, Protano C, Vitali M, Polimeni A. How to manage the biological risk in a dental clinic: current and future perspectives. *Minerva stomatologica.* 2017;66(5):232-239.
51. Rani KR, Ambati M, Prasanna JS, Pinnamaneni I, Reddy PV, Rajashree D. Chemical vs. herbal formulations as pre-procedural mouth rinses to combat aerosol production: a randomized controlled study. *J Oral ResRev.* 2014;6(1):9-13.



REVIEW ARTICLE

Zinc α -2 Glycoprotein (ZAG) a Novel Biomarker for the Detection of Oral Squamous Cell Carcinoma

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ABSTRACT

Oral cancer, the most challenging and life threatening disease in the field of dentistry, may start as a reactive lesion due to constant stimulus from tobacco consumption, transform into a pre-malignant lesion (dysplastic lesion) and ultimately develop into a cancerous lesion (Invasive carcinoma). There is a fundamental revolution taking place in the analyzing methods; extraction of biological protein from the saliva rather than from tissues or blood. Several of the biomarkers have been studied with pro-carcinogenic effects like Interleukins (ILs), tumor necrosis factor (TNF) and leptin, but only a few have been stated in the literature, which show anti-cancer characteristics like adiponectin and zinc alpha-2 glycoprotein. This review explored the diagnostic and prognostic values of a biomarkers zinc alpha-2 glycoprotein (ZAG) in adults suspected of oral squamous cell carcinoma (OSCC). The PubMed, EMBASE and Google Scholar were searched for scientific studies reported on the potential mechanism of zinc alpha-2 glycoprotein. All the research articles were selected in which ZAG is applied solely or in conjunction with other biomarkers in oral cancer and other cancers. These literatures were carefully assessed to find out and compile the diagnostic and prognostic values and to inquire therapeutic action of ZAG in the process of carcinogenesis.

Keywords: Biomarker; Early Diagnosis; Oral Squamous Cell Carcinoma; Zinc-Alpha (2)-Glycoprotein.

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INTRODUCTION

Adipokines or adipocytokines are biologically active molecules, which function as a cell signaling protein and usually synthesize in the adipose tissues¹. These factors can be synthesized at other sites in the human body like saliva, plasma, urine and other fluids in a variety of circumstances. However, its mechanism of action will be different on other sites of secretion as compared to adipose tissue². Adipokines include a variety of pro-inflammatory peptides like tumor necrosis factor (TNF), leptin, resistin, adiponectin and zinc alpha-2 glycoprotein³. Several of the adipokines have been studied with pro-carcinogenic effects like ILs, TNF and leptin, but only a few have been stated in the literature, which shows anti-cancer characteristics like adiponectin and zinc alpha-2 glycoprotein⁴. The alteration in the level of such molecules fetches

changes in the human cells or tissue, which leads to the sequence of events from chronic inflammatory process to pre-cancerous state to cancerous growth⁵. There up-regulation or down-regulation in contrast to normal ranges will indicates that there must be suspicious changes or diseases undergoing in the human body⁶. These altered levels of biomarkers may be rendered as a valuable candidate for screening, diagnosis, prognosis and therapeutic intervention for specific disease⁷⁻⁸.

In the field of Dentistry, Oral cancer is the most challenging and life threatening disease which may start as a reactive lesion to a constant stimulus such as tobacco consumption, transform into a pre-malignant lesion (dysplastic lesion) and ultimately develop into a cancerous lesion (Invasive carcinoma)⁵. Oral squamous cell carcinoma (OSCC) is the most commonly occurring epithelial

derived malignant lesion (90-95%) of the oral cavity⁹. Oral squamous cell carcinoma (OSCC) is the most prevalent disease sixth highest globally and ranked second highest in Pakistan or Indian suncontinent¹⁰. The consumption of high risk factors like smoked or smokeless tobacco is very common amongst our population¹¹, which brings changes in proteomics and creates a microenvironment for the tumor initiation and proliferation^{12,13}. The majority of OSCC cases are diagnosed at an advanced stage along with nodal involvement and distant metastasis, this occurs usually due to patient's negligence to seek early treatment or either due to misdiagnosis of the suspicious lesion by the consultant^{14,15}. This ultimately leads to a poor prognosis (<50%) with high morbidity and mortality rate of this lethal disease^{16,17}.

The research articles were selected in which ZAG is applied solely or in conjunction with other biomarkers in oral cancer and other cancers. There were total 282 research articles available on the search engines out of which only four articles represented the association of zinc alpha-2 glycoprotein with oral squamous cell carcinoma from 1999 to 2017 which were included in this research. The other 85 articles stated the correlation of zinc alpha-2 glycoprotein and other type of cancers, out of which only those article were selected fall under 10 year period.

In this study, we have focused and analyzed about the action of zinc alpha 2 glycoprotein (ZAG) in the early diagnosis of OSCC in order to identify such lesions at its initial phase? Can it be useful in predicting the prognosis of OSCC patient and can it be used as a therapeutic molecule for the future researches? Is there any correlation of ZAG expression with the etiological variables, tumor differentiation and different clinical parameters of oral squamous cell carcinoma?

DISCUSSION

This review explored the diagnostic and prognostic values of a biomarker zinc alpha-2 glycoprotein in adults suspected of oral squamous cell carcinoma (OSCC). The searched for scientific studies reported on the novel adipokine zinc alpha-2 glycoprotein (ZAG).

Structural Framework of Zinc α -2 Glycoprotein

Zinc alpha-2 glycoprotein was first identified in the human serum by Burgi and Schmid in 1961 and belongs to a group of macroglobulin, which is associated with multiple functions in the human body²⁰. A soluble protein synthesizes in the human tissues and is secreted in the body fluids like serum, plasma, saliva in various metabolic diseases and cancerous processes²⁰⁻²³. Human serum ZAG is encoded by the AZGP-1 gene on Chromosome 7q22.1 of the liver²¹. Its molecular weight ranges

from 38-41kDa, with a single chain polypeptide. Araki et al. in 1988 had determined the first polypeptide sequence of ZAG consisting of 278 mature amino acids in a polypeptide chain²⁴. The Figure 1 represents the ribbon diagram of ZAG showing three domains (α 1, α 2, and α 3) and glycan chains are represented in ball and stick model²⁰. The α 1- α 2 super domains of ZAG adopt a fold that is symmetrical to MHC-1 but lacking binding to β 2M is slightly different from that in MHC-1⁴.

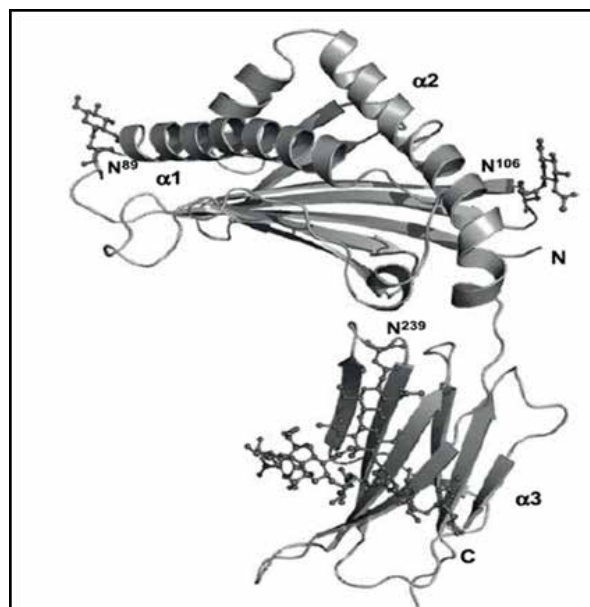


Figure 1: Structural framework of Zinc α -2 glycoprotein (Hassan et al., 2008).

Functional Role of Zinc α -2 Glycoprotein

Zinc alpha-2 glycoprotein functionally resembles a lipid mobilizing factor (LMF), so it has a strong role in cancer cachexia²⁵. Its lipolytic effect takes place by activation of the cyclic AMP pathway via β 3 adrenoreceptor²⁶. Felix et al. have reported elevated levels of zinc α -2 glycoprotein in the serum of pancreatic cancer cachexic patients and suggested that ZAG can be used as a reliable tool for early diagnosis of cancer cachectic patients in the clinical setting²⁷. In addition, ZAG possesses a high degree structural and functional similitude with the MHC-1 molecule and a large groove analogous to MHC-1 peptide binding groove²⁰. The configuration of the binding groove reflects its primary role in immune response and lipid catabolism²³ (Figure 2). The uniqueness of ZAG molecule is exhibited by alteration in the residue of peptide binding groove and because of this alters the molecule binds with different proteins, antigens and ligands²⁰. Vidotto et al. reported that zinc α -2 glycoprotein possibly reflects immune responses to tumor antigen for killing the tumor cells as well as mucosal break down through proteolytic actions to prevent tumor invasion in Head and Neck Squamous Cell Carcinoma^{28,29}.

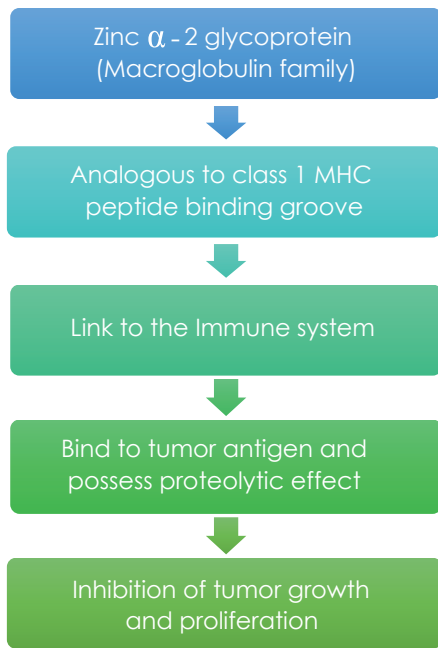


Figure 2: Relationship of ZAG with immune system.

Zinc α-2 Glycoprotein Role in Carcinogenesis

The consumption of smoked or smokeless tobacco is a prevalent factor behind the causation of OSCC¹³. These products trigger an inflammatory reaction in the tissue or body fluids which creates an oxidative stress and activates redox-sensitive transcription factors such as NF-kappaB, which

leads to release of Inflammatory cytokines/adipokines such as IL-6, TNF-α and ZAG¹⁸ (Figure 3). The high expression of these factors in the majority of circumstances correlates with the tobacco-related diseases¹⁹. Thus, may be high or low expression of ZAG can link to the initiation, progression or suppression of OSCC²⁰. According to Chukkris et al. betel nut chewing, tobacco use and alcohol consumption were highly correlated with the increased expression of ZAG with statistically significant (p < 0.001) outcomes in OSCC cases as compared to cancer free controls⁸.

Similar to the above study, Tsai et al. also found a strong association of smoking with increased expression of ZAG protein in smokers as compared to non-smokers (p < 0.0001)²¹. Their results showed that smoking can be an independent risk factor causing a variation in ZAG levels in the human plasma. Luisa Airoidi et al. published a research in which there was a 2.5 fold down-regulation of ZAG protein in the urine of chronic smokers as compared to non-smokers¹⁹. Cigarette smoke elevated levels of TNF-α in the circulation, which might decrease ZAG expression in some body compartments. These statistics indicate towards the role of ZAG in the process of carcinogenesis but through this review, we attempt to summarize its relative part in the oral cancer progression.

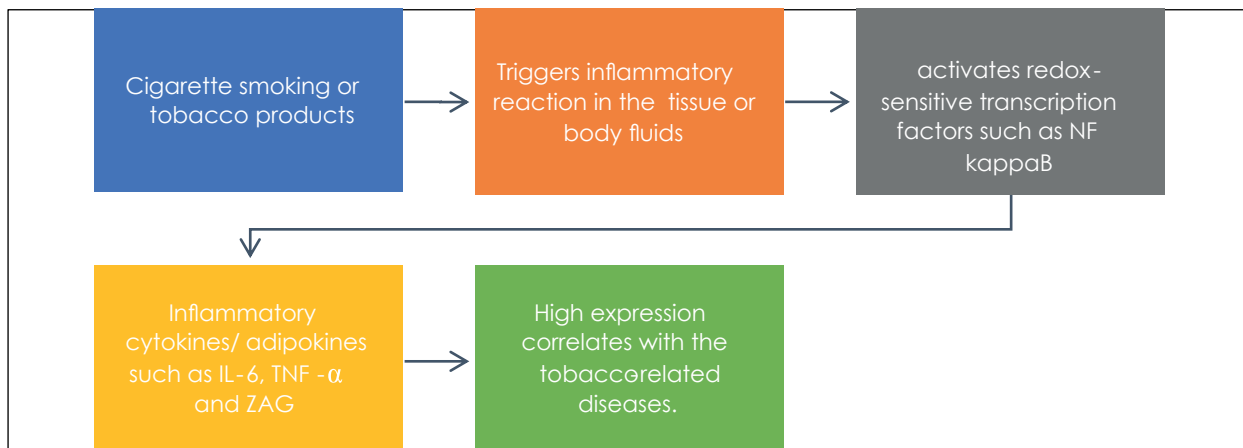


Figure 3: Mechanism of tobacco-related disease instigation.

Zinc α-2 Glycoprotein and Signaling Pathways

Zinc alpha-2 glycoprotein holds anti-cancer effect against the tumor cells as its high expression down-regulates the Cyclin dependent kinase 2 (cdc-2) enzymatic activities in the cell cycle (Figure 4) and RNase activity eliminate the mutated or damaged RNAs ultimately hinder tumor cell growth³⁰. Similarly, Brysk et al. reported that zinc a-2 glycoprotein mRNA expression was lower in oral squamous cell carcinomas (Tu-138) cell line than the adjacent non-tumor tissue and Tu-138 cells

growth was inhibited on ZAG matrix for the first 2 days and then proliferated at a reduced rate³¹. According to the literature, the high expression of ZAG binds to hydrolases and inhibits the enzyme-mediated tumor invasion indirectly activates apoptosis³². Yi Xu et al. described that knockdown of ZAG would promote epithelial-mesenchymal transition, tumor invasion and reduced apoptosis by TGFβ1-ERK2 signaling pathway³³. It suggests that high expression of ZAG being associated with down-regulation of mesenchymal marker

(N-Cadherin) and up-regulation of epithelial marker (E-Cadherin) by inhibiting the TGF- β 1/ERK 2 signaling pathway (Figure 5). However, they proposed

that the up-regulation of ZAG may have an anti-proliferative and pro-apoptotic function in human hepatocellular.

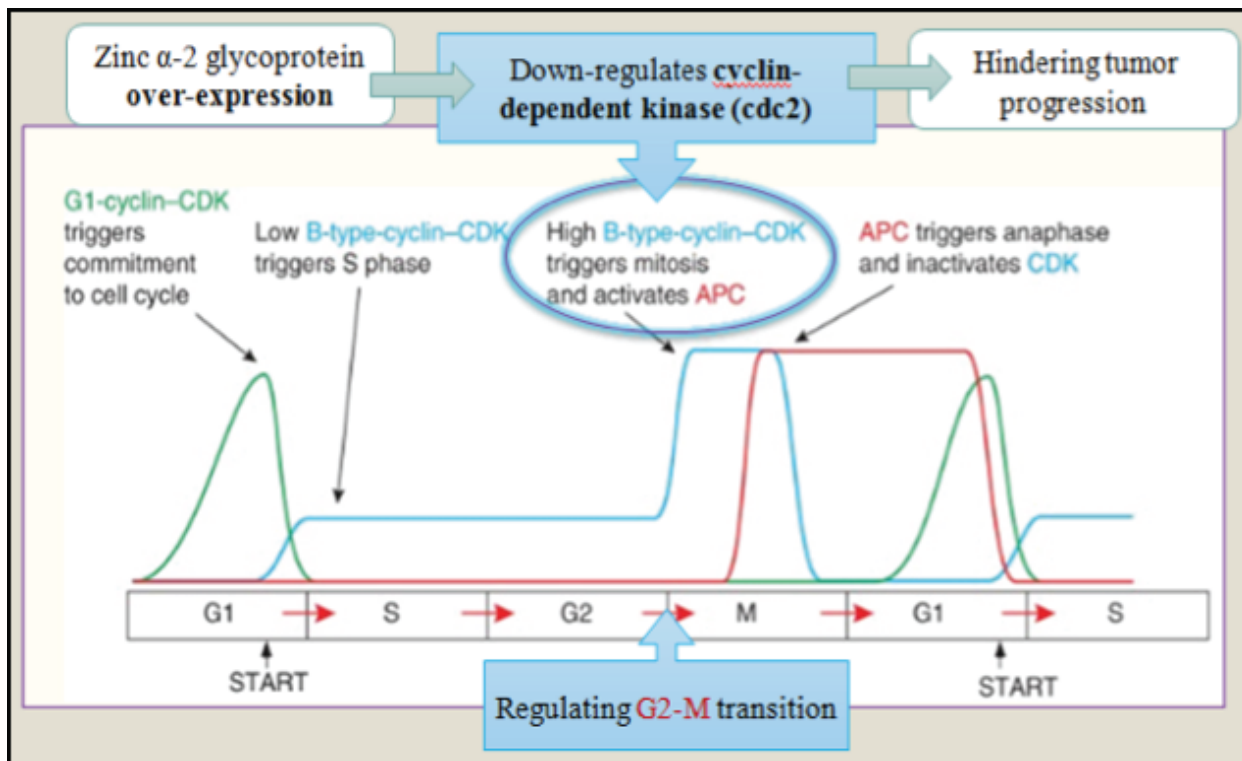


Figure 4: Represents the effect of ZAG on the cell cycle.

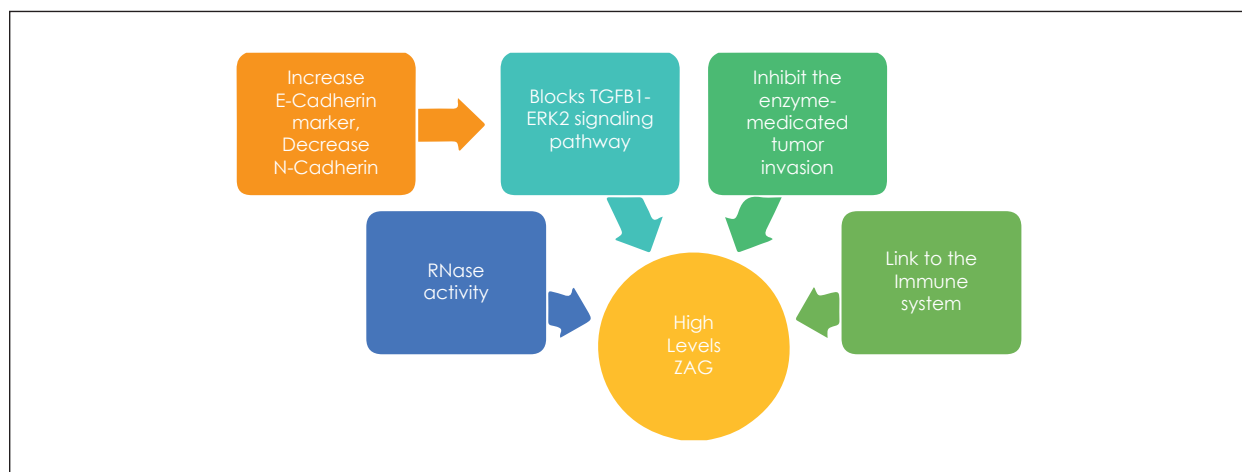


Figure 5: Anti-cancerous effects of ZAG at an early phase of cancers.

Expression of Zinc α -2 Glycoprotein in OSCC

Zinc alpha-2 glycoprotein is a biochemically important protein and gene, which is directly or indirectly involved in various chronic diseases and cancers including oral squamous cell carcinoma (OSCC)³⁴. However, very little work has been done to find an association between zinc alpha-2 glycoprotein and oral cancers by using different methods of analysis. Chukkris et al. have reported the significant up-regulation of zinc alpha-2 glycoprotein in the saliva of

OSCC patients as compared to controls with relative intensity of <1 in controls and 0-15 in OSCC cases⁸. In this study the expression of ZAG was significantly up-regulated in lesional cells than oral exfoliated cells ($p < 0.001$), and this result was consistent with the salivary results. Its increased level in the early phases of OSCC might render as an early diagnostic marker with 85% sensitivity and 100% specificity.

Another study conducted by Chen et al. showed higher levels of zinc α -2 glycoprotein in the saliva samples of OSCC patients at early stages and suggested that it can be used as novel diagnostic biomarkers for the oral cancers⁹. Similarly, Vidotto et al. has also reported an increased expression of zinc alpha-2 glycoprotein in saliva specimens of head and neck cancer (HNSCC) up-to 1.5 fold²⁸. They correlated that the up-regulation of ZAG in early stages of oral cancer may reflect an immune response against the tumor cell surface antigen and prevent the enzyme-mediated invasion of tumor cells by binding of macroglobulin into hydro-lases.

Lei et al. and Brysk et al. have evaluated the role of zinc α -2 glycoprotein in inhibiting proliferation of oral squamous cell carcinoma cell lines they found the down-regulation of Cyclin dependent kinase 2 (cdc2) activities in the cell cycle which is an important rate limiting enzyme to regulate G2-M phase for the growth of cells³⁰⁻³². These statistics recommended that ZAG could be used as a new early diagnostic or therapeutic target for the analysis and its unique behavior in tumorigenesis help to secure a better prognosis for OSCC patients.

Expression of Zinc α -2 Glycoprotein in Other Malignancies

Many studies had been conducted to identify an association between zinc alpha-2 glycoprotein and different type of malignant tumors³⁵⁻³⁷. In this regard, Jung reported that the over-expression of AZGP-1 protein was seen in the well-differentiated prostate cancer tissue as compared to a poorly differentiated tumor on immunohistochemistry (IHC)³⁸. They also stated that the expression of ZAG decreases as the tumor differentiation increases and higher the expressions better the chances of patient survival. AZGP-1 can be an independent prognostic marker in clinical setting for localized cancer, but it may also be used to stain adjacent cancerous field^{37,38}. Similarly Dubois et al found higher expression of ZAG proteins in well-differentiated breast cancer tissue (81%) and even higher in normal tissue immediately adjacent to breast tumor (91%) through IHC, which may indicate its crucial role in the initial stages of carcinogenesis and it might encompass some anti-carcinogenic activity³⁹.

A study conducted by Hua Xiao et al. showed a significant difference between the expressions of AZGP1 in the saliva of healthy controls and a lung cancer group; controls showed the low relative intensity of ZAG in comparison to cases⁴⁰. However, this study showed no significant association of smoking with the expression of AZGP-1 in the healthy controls and lung cancer cases. The expression of AZGP-1 mRNA in the cancerous process may be affected by chromatin remodeling by histone acetylation, which regulates gene activi-

ty by amending the chromatin conformation⁴¹. Chun-Yu Huang et al. showed a strong correlation between ZAG levels and histological grade, tumor location and tumor invasion in the gastric tumor⁴². Another study by Hong Tang et al. showed down-regulation of ZAG expression in the poorly differentiated tissues of ESCC and this down regulation of ZAG might indicate ESCC progression, aggressive tumor behavior, and poorer clinical outcome¹⁰.

In a research conducted by Yingming Xue et al., showed high expression of AZGP1 mRNA (57.1%) was seen in the colon cancer tissue with at least a two-fold rise compared to paired normal colonic mucosa tissue⁴³. The high levels of AZGP-1 signified more advanced progression of the disease with worsen clinical outcome in colorectal cancer patients. Fei Wang et al. showed up-regulation of ZAG mRNA and protein expression in Hepatocellular carcinoma and liver cirrhosis compared to normal liver tissue with statistically significant results ($p < 0.01$)⁴⁴. This significant expression may result due to modification in the protein levels caused by the infective risk factors for hepatocellular carcinoma, but there is a scope for future research to find an exact relationship between ZAG expression and carcinomas. All the above statistics are conferring that ZAG can be applied as an early diagnostic, tumor suppressive, prognostic marker and promising therapeutic target for oral cancer and other cancers in the clinical capacity³⁶⁻⁴⁵ but we cannot refute that there is also necessitated for further clinical based studies to conform these functions of ZAG in the carcinogenesis.

CONCLUSION

As the time changes, many advancements and evolution are enchanting in the field of research and medicine. However, there is still a vast scope for further clinical experimentation to establish potential biomarkers for the mass screening of high risk individuals of OSCC or other cancers. It is essential to detect such lesion in the initial phase of its malignant transformation to ensure better prognosis of the patient and provide good health. There is a fundamental revolution taking place in the analyzing methods; extraction of biological protein from the saliva rather than from tissues or blood. In this review, some promising mechanism of action of ZAG has been recognized by analyzing the previous researches, but there is requisite for further researches to be conducted on its diagnostic and therapeutic values in the saliva. It is necessary to discover the exact mechanism of zinc α -2 glycoprotein in the pathogenesis of precancerous lesions and oral squamous cell carcinoma.

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CONFLICT OF INTEREST

There was no conflict of interest among the authors.

AUTHORS' CONTRIBUTION

MF did the conceptualization of study, literature search and prepared the write up. MH and SB had done the proof reading. RA did the overall evaluation of the study and proof reading.

REFERENCES

- Lago F, Dieguez C, Gómez-Reino J, Gualillo O. Adipokines as emerging mediators of immune response and inflammation. *Nat Clin Pract Rheumatol*. 2007; 3(12):716-724.
- Dalamaga M. Interplay of adipokines and myokines in cancer pathophysiology: Emerging therapeutic implications. *World J Exp Med*. 2013; 3(3): 26-33.
- Mancuso P. The role of adipokines in chronic inflammation. *Immunotargets Ther*. 2016; 5:47-56.
- Ebadi M, Mazurak VC. Potential biomarkers of fat loss as a feature of cancer cachexia. *Mediators Inflamm*. 2015;2015:820934.
- Strimbu K, Tavel JA. "What are biomarkers?" *Curr Opin HIV AIDS*. 2010; 5(6): 463-466.
- Schaaij-Visser TB, Brakenhoff RH, Leemans CR, Heck AJ, Slijper M. Protein biomarker discovery for head and neck cancer. *J Proteomics*. 2010; 73(10):1790-1803.
- Wang Q, Gao P, Wang X, Duan Y. Investigation and identification of potential biomarkers in human saliva for the early diagnosis of oral squamous cell carcinoma. *Clin Chim Acta*. 2014; 427: 79-85.
- Heawchayaphum C, Pientong C, Phusingha P, Vatanasapt P, Promthet S, Daduang J, *et al*. Peroxiredoxin-2 and zinc-alpha-2-glycoprotein as potentially combined novel salivary biomarkers for early detection of oral squamous cell carcinoma using proteomic approaches. *J Proteomics*. 2018; 173: 52-61.
- Chen YT, Chen HW, Wu CF, Chu LJ, Chiang WF, Wu CC, *et al*. Development of a multiplexed liquid chromatography multiple-reaction-monitoring mass spectrometry (LC-MRM/MS) method for evaluation of salivary proteins as oral cancer biomarkers. *Mol Cell Proteomics*. 2017; 16(5): 799-811.
- Tang H, Wu Y, Qin Y, Wang H, Wang L, *et al*. Reduction of AZGP1 predicts poor prognosis in esophageal squamous cell carcinoma patients in Northern China. *Onco Targets Ther*. 2016; 10: 85-94.
- Sahaf R, Naseem N, Anjum R, Nagi AH, Path FR. A Study of 89 Cases of Oral Squamous Cell Carcinoma Presenting at Teaching Hospitals of Lahore, Pakistan. *J Pak Dent Assoc*. 2017; 26 (1): 26-31.
- Hannan MA, Rahman MA, Hossain S, Rahman QB. Role of habitual risk factors on oral squamous cell carcinoma. *UDCJ*. 2018; 8(1): 28-35.
- World Health Organization [Internet]. Global Adult Tobacco survey for Pakistan; 2014 [updated 2019 May 2, cited 2020 June 5]. Available from: <https://extranet.who.int/ncdsmicrodata/index.php/catalog/257>
- Mehrotra R, Yadav S. Oral squamous cell carcinoma: Etiology, pathogenesis and prognostic value of genomic alterations. *Indian J Cancer*. 2006; 43(2): 60-66.
- Mohiuddin S, Fatima N, Hosein S, Hosein M. High risk of malignant transformation of oral submucous fibrosis in Pakistani females: A potential national disaster. *J Pak Med Assoc*. 2016; 66(11): 1362-1366.
- Gupta N, Gupta R, Acharya AK, Patthi B, Goud V, Reddy S, *et al*. Changing Trends in oral cancer - a global scenario. *Nepal J Epidemiol*. 2016; 6(4): 613-619.
- Coelho KR. Challenges of the oral cancer burden in India. *J Cancer Epidemiol*. 2012; 1(1): 1-17.
- Vanni H, Kazeros A, Wang R, Harvey BG, Ferris B, De BP, *et al*. Cigarette smoking induces over-expression of a fat-depleting gene AZGP1 in the human. *Chest*. 2009; 135(1):1197-1208.
- Airoidi L, Magagnotti C, Iannuzzi AR, Marelli C, Bagnati R, Pastorelli R, *et al*. Effects of cigarette smoking on the human urinary proteome. *Biochem Biophys Res Commun*. 2009; 381(3): 397-402.
- Hassan MI, Waheed A, Yadav S, Singh TP, Ahmad F. Zinc α 2-glycoprotein: a multidisciplinary protein. *Mol Cancer Res*. 2008 6(6): 892-906.
- Tsai JS, Chen SC, Huang KC, Lue BH, Lee LT, Chiu TY, *et al*. Plasma zinc α 2-glycoprotein levels are elevated in smokers and correlated with metabolic syndrome. *Eur J Clin Investig*. 2015; 45(5): 452-459.
- Huang D, Mao X, Peng J, Cheng M, Bai T, Du M, *et al*. Role of adipokine zinc- α 2-glycoprotein in coronary heart disease. *Am J Physiol Endocrinol Metab*. 2019; 317(6):1055-1062.
- Marrades MP, Martínez JA, Moreno-Aliaga MJ. ZAG, a lipid mobilizing adipokine, is downregulated in human obesity. *J Physiol Biochem*. 2008; 64(1):61-66.
- Rydén M, Agustsson T, Andersson J, Bolinder J, Toft E, Arner P. Adipose zinc- 2-glycoprotein is a catabolic marker in cancer and noncancerous states. *J Intern Med*. 2012; 271(4): 414-420.
- Bing C, Bao Y, Jenkins J, Sanders P, Manieri M, Cinti S, *et al*. Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. *Proc Natl Acad Sci USA*. 2004; 101(8): 2500-2505.
- Sörensen-Zender I, Beneke J, Schmidt BM, Menne J, Haller H, Schmitt R. Zinc-alpha2-glycoprotein in patients with acute and chronic kidney disease. *BMC Nephrol*. 2013; 14:145.
- Felix K, Fakelman F, Hartmann D, Giese NA, Gaida MM, Schnölzer M, *et al*. Identification of serum proteins involved in pancreatic cancer cachexia. *Life Sci*. 2011; 88 (5-6): 218-225.
- Vidotto A, Henrique T, Raposo LS, Maniglia JV,

- Tajara EH. Salivary and serum proteomics in head and neck carcinomas: before and after surgery and radiotherapy. *Cancer Biomark.* 2010; 8(2): 95-107.
29. Chen Y, Azman SN, Kerishnan JP, Zain RB, Chen YN, Wong YL, *et al.* Identification of host-immune response protein candidates in the sera of human oral squamous cell carcinoma patients. *PLoS One.* 2014; 9(10): e109012.
30. Brysk MM, Lei G, Adler-Storhiz K, Chen Z, Brysk H, Tyring SK, *et al.* Zn- α 2-glycoprotein expression as a marker of differentiation in human oral tumors. *Cancer Lett.* 1999; 137(1): 117-120.
31. He N, Brysk H, Tyring SK, Ohkubo I, Brysk MM. Zinc-alpha (2)-glycoprotein hinders cell proliferation and reduces cdc2 expression. *J Cell Biochem Suppl.* 2001; 36:162-169.
32. Lei G, Brysk H, Arany I, Tyring SK, Srinivasan G, Brysk MM. Characterization of zinc-alpha (2)-glycoprotein as a cell adhesion molecule that inhibits the proliferation of an oral tumor cell line. *J Cell Biochem.* 1999; 75(1): 160-169.
33. Xu MY, Chen R, Yu JX, Liu T, Qu Y, Lu LG. AZGP1 suppresses epithelial-mesenchymal transition and hepatic carcinogenesis by blocking TGF β -ERK2 pathways. *Cancer Lett.* 2016; 374(2): 241-249.
34. Wang R, Yuan Y, Zhou Y, Zhang D, Zhang L, Zeng X, *et al.* Screening diagnostic biomarkers of OSCC via an LCM-based proteomic approach. *Oncol Rep.* 2018; 40(4): 2088-2096.
35. Zhao J, Fan YX, Yang Y, Liu DL, Wu K, Wen FB, *et al.* Identification of potential plasma biomarkers for esophageal squamous cell carcinoma by a proteomic method. *Int J Clin Exp Pathol.* 2015; 8(2):1535-1544.
36. Us Altay D, Keha EE, Ozer Yaman S, Ince I, Alver A, Erdogan B, *et al.* Investigation of the expression of irisin and some cachectic factors in mice with experimentally induced gastric cancer. *QJM.* 2016; 109(12):785-790.
37. Kristensen G, Berg KD, Tofft BG, Stroomberg HV, Nolley R, Brooks JD, *et al.* Predictive value of AZGP1 following radical prostatectomy for prostate cancer: a cohort study and meta-analysis. *J Clin Pathol.* 2019; 72(10):696-704.
38. Jung WY, Sung CO, Han SH, Kim K, Kim M, Ro JY, *et al.* AZGP-1 immunohistochemical marker in prostate cancer: potential predictive marker of biochemical recurrence in post radical prostatectomy specimens. *Appl Immunohistochem Mol Morphol.* 2014; 22(9): 652-657.
39. Dubois V, Delort L, Mishellany F, Jarde T, Billard H, Lequeux C, *et al.* Zinc-alpha2-glycoprotein: a new biomarker of breast cancer? *Anticancer Res.* 2010; 30(7): 2919-2925.
40. Xiao H, Zhang L, Zhou H, Lee JM, Garon EB, Wong DT. Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. *Mol Cell Proteomics.* 2012; 11(2): M111.012112.
41. Albertus DL, Seder CW, Chen G, Wang X, Hartojo W, Lin L, *et al.* AZGP1 Autoantibody Predicts Survival and Histone Deacetylase Inhibitors Increase Expression in Lung Adenocarcinoma. *J Thorac Oncol.* 2008; 3(11):1236-1244.
42. Huang CY, Zhao JJ, Lv L, Chen YB, Li YF, Jiang SS, *et al.* Decreased expression of AZGP1 is associated with poor prognosis in primary gastric cancer *PLoS One.* 2013; 8 (7):e69155/1-7.
43. Xue Y, Yu F, Yan D, Cui F, Tang H, Wang X, *et al.* Zinc- α -2-Glycoprotein: A candidate biomarker for colon cancer diagnosis in Chinese population. *Int J Mol Sci.* 2015; 16(1): 691-703.
44. Wang F, Geng Y, Zhang WM, Geng X. Identification of ZAG protein as a novel serologic biomarker candidate for liver cancer. *Adv Mater Res.* 2012; 340: 383-389.
45. Peng H, Chen R, Brentnall TA, Eng JK, Picozzi VJ, Pan S. Predictive proteomic signatures for response of pancreatic cancer patients receiving chemotherapy. *Clin Proteomics.* 2019; 16, 31.



KAP STUDY

Analysis of Current COVID-19 Situations in Dental Practice among Karachi Population

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ABSTRACT

Background: December 2019, marked the beginning of novel Coronavirus (COVID-19) from China (Wuhan), which hit the general population and healthcare systems, worldwide, as a pandemic. Karachi reported the first case of COVID-19 on February 26, 2020 in a student returning from Iran. Currently, World Health Organization has put forward specific guidelines for dental practitioners. The primary objective of the study was to determine the practices of dental practitioners in Karachi in following the standard guidelines set by the World Health Organization.

Methods: In this, cross sectional questionnaire-based study, we have collected data from 111 dental practices through random sampling for analysis across Karachi, Pakistan. Structured questionnaire was designed comprising questions regarding knowledge, equipment, financial burden and procedures performed. Using SPSS version 25, descriptive analysis was executed on these variables. A multiple regression test was used to detect any relationship between them.

Results: Majority (55.3%) of the dentists were found aware of the standard guidelines regarding dental practices and treatment of patients during COVID-19 outbreak but implementation in practice has been a major dilemma. Shortage of equipments as well as financial crisis was the main causative factors. Non-emergency procedures (5%) are being performed as well as inadequate protective measures are being adapted.

Conclusion: According to the world standards, dental practices in Karachi should follow the standard guidelines put forward by world-renowned health organizations. Majority ($p=0.44$) of the dentists are aware of the standard guidelines, but lack of resources and financial instability are the primary factors affecting their dental practice.

Keywords: Coronavirus; Dentists; Dental Practice; Precautions; Protective Equipment; Infection Control; Public Health.

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INTRODUCTION

December 2019, marked the beginning of the novel Coronavirus associated with pneumonia, called as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) reported in Wuhan, China. This later spread out of control and was labeled as pandemic by World Health Organization (WHO) on March 11 2020^{1,2}. Coronavirus, a major pathogen, is targeting human population, which is primarily affecting the

respiratory system. Previous outbreaks of Coronavirus include Middle East Respiratory Syndrome (MERS) happening in Saudi Arabia on April 2014 and Severe Acute Respiratory Syndrome (SARS) in 2002-2004, which posed a major challenge on the healthcare system of the affected nations³.

Interestingly, it has been suggested that this virus was found amongst Chinese horseshoe bats (*Rhinolophus sinicus*) and pangolins and passed on

to other mammalian hosts causing this pathology^{4,5}. Infectivity of novel Coronavirus is of utmost significance because through this tremendous information can be generated to help in the development of effective drugs and vaccine. COVID-19 interaction with angiotensin-converting enzyme inhibitor 2 (ACE-2) has been proposed as to be the primary factor of infectivity. This might be concerning for the patients currently on ACE inhibitors and Angiotensin Receptor Blockers (ARBs) therefore; guidance on its use is urgently required for such patients⁶.

The COVID-19 is believed to spread mainly by respiratory droplets such as sneezing and coughing from human to human being in direct close contact^{9,10}. It is also found to be airborne according to the new research⁹. Incubation period for COVID-19 has been stated to be anywhere between 2-14 days in which the patient may remain asymptomatic. The best estimate of incubation period has been found to be 5 days, after which the patient can present with symptoms¹¹. The infected patient may show flu like symptoms including high grade fever, dry persistent cough, sore throat, myalgia, anosmia, loss or altered sense of taste and stomach upset¹²⁻¹⁴. Generally, patients who are young and without any comorbidity may recover well. However, elderly people and those with comorbidities particularly those with compromised immune system, hypertension, diabetes and chronic respiratory disorders are significantly vulnerable and fall in the highest mortality group⁶. Although patients in this high-risk group usually suffer severe type of COVID-19 infection, cases of mortality have been reported in young individuals without any positive medical history¹⁵⁻¹⁷. Recently, patients as young as 7 weeks old have fallen victim to the pandemic¹⁸.

Regarding the current situation, dental practitioners should acquire knowledge necessary to treat their patients safely and be able to prevent cross infection. Dentists and their staff should comply with the standard social distancing protocols suggested by the Centers for Disease Control and Prevention (CDC) and should enforce their patients' likewise¹⁹.

In light of the exponential rise in the affected cases, lack of staff, equipment, financial crisis and capacity to withstand the steep upsurge faced by health-care professionals, there has been a dire need to adjust health care giving facilities to curtail their routine work to cope with the current crisis²⁰. This included cancelling elective procedures, redeployment of medical staff and minimizing any aerosol generating procedures. If necessary, aerosol-generating procedures need to be performed, then using high volume saliva ejector may reduce chances of COVID-19 transmission. This posed a challenge to the dentists due to the involvement of high-speed hand piece used in majority of the dental procedures causing aerosol generation and the close proximity with the patients^{21,22}. The WHO

has specifically laid down preventative measures for the practicing dentists to follow which includes performing emergency procedures only and following personal protective equipment guidelines^{23,24}.

Our objective for this study was to determine whether the dentists are aware of the Personal Protective equipment guidelines and how the dentists implementing them in their practice. It was also evaluated whether dentists are abiding by the standard guidelines in all areas of their practice and refraining from any procedures, which are classified as non-emergency.

METHODS

A cross sectional survey-based study was carried out by using a questionnaire, which consisted of questions regarding knowledge, equipments, financial burdens and procedures performed by the dental practitioners in this COVID-19 crisis. This questionnaire was circulated amongst different dental practices in Karachi, Pakistan. Particular emphasis was given to the availability of the personal protective equipment as well as performing procedures during COVID-19 outbreak.

Sampling method was random, 111 dental practitioner's data was collected and analyzed. Verbal and written consent was taken from each of the participants prior to filling out the questionnaire. Ethics review committee of Altamash Institute of Dental Medicine has approved this study (AIDM/EC/04/2020/01). Data was collected anonymously, and confidentiality was maintained throughout this study. A structured questionnaire was constructed using Google forms under the headings of demographic data, knowledge, equipment, financial burden and procedures.

Participation in this study was based on inclusion and exclusion criteria. This study comprised of current practising dentists only and those who were not currently practising in Karachi were excluded from the study. Our study consisted of the following variables in which demographic, knowledge, equipment and financial burden were independent variables whereas procedures performed being the dependant variable.

In our study we had 3 independent variables and 1 dependant variable. For statistical analysis we have used Multiple Linear Regression Technique to analyse our data using SPSS version 25. Each of these independent variables were analysed to look for any significant relationship with the dependent variable. Responses for knowledge, equipment, financial burden and procedures were given a mean and a p-value of 0.05 which was considered as statistically significant.

RESULTS

This study consisted of 111 dental practices located in Karachi. Majority of the dental practitioners belonged to the age group of 20-30 years with a small sample belonging to the 31-40 years age group category. Gender which was the most predominant in the study consisted of females. Three quarters of our sample size had a graduate level of education and post graduate education being the minor category. Predominantly, practitioners are working in the private sector with nearly half of them also practising dentistry in hospitals as well as private clinics.

When these variables of knowledge, equipment, financial burden and procedures were entered in multiple linear regression technique for statistical analysis, they suggested presence of relationship amongst them. The correlation of the procedures with knowledge, equipment and financial burden was found ($r=0.532$). In our study, knowledge (p-value 0.44) and financial burden (p-value 0.75) had no significant relationship with the procedures. However, equipment (p-value 0.00) had significant relationship with procedures as shown in Table 1.

Table 1: Significance of knowledge, equipment and financial burden in relation to procedures using multiple linear regression test.

Variables	Coefficient	Standard Error	t-test Value	p-Value
Knowledge	-0.066	0.120	-0.770	0.44
Equipment	0.561	0.103	5.824	0.00
Financial Burden	-0.031	0.122	-0.326	0.75

More than 3 quarters of dentists, amounting to a total of 85.1%, have agreed that they the sufficient amount of knowledge regarding procedures categorised as urgent as shown in Figure 1. Nearly all of them also agreed that regarding the current COVID-19 crisis, personal protective equipment (PPE) as stated by World Health Organisation (WHO) guidelines should be used when doing a procedure on a patient. In generality, participants chose gloves, n95 mask, goggles and surgical mask of being utmost important while being in contact with patients. From the data, it has been found that some dental practices disagreed for using hazmat suit, goggles, surgical cap and face shield. Majority of the dental practitioners have informed through their responses that they are implementing social distancing protocols in their respective dental practices whereas a few dental practitioners have reported lack of adherence social distancing protocols in their respective practices. Nearly all the dental practitioners reported that availability of PPE is a big issue during these times. They believed that the reason for shortage of PPE is due to the unethi-

cal hoarding of the equipment. Majority of the participant's viewpoint was that sterilisation of the equipment is more important now than it ever was before COVID-19 pandemic.

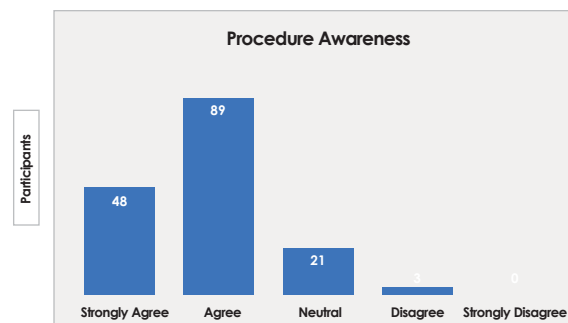


Figure 1: Dentists are aware of the procedures classified as emergency procedures as per the standard guidelines issued.

Correlation amongst demographics such as age, gender and education with procedures was carried out using Spearman's correlation test. A significant relation was found of gender with procedures (p-value 0.001). Although similar was not seen with age (p-value 0.117) and education (p-value 0.948) with procedures as shown in Table 2.

Table 2: Correlations of age, gender and education with procedures.

Parameters	Correlations	Age	Gender	Education	Procedures
Age	Correlation	1.00	-0.41	0.51	-0.150
	Sig. (2-tailed)		0.000	0.000	0.117
Gender	Correlation	-0.414	1.00	-0.238	0.319
	Sig. (2-tailed)	0.000		0.012	0.001
Education	Correlation	0.515	-0.238	1.00	-0.006
	Sig. (2-tailed)	0.000	0.012		0.948
Procedures	Correlation	-0.150	0.319	-0.006	1.00
	Sig. (2-tailed)	0.117	0.001	0.948	

Almost all the dental practitioners assumed that proper disposal of PPE is of unconditional importance to prevent chances of cross infection of COVID-19. Majority reported decrease in number of patients daily since the beginning of the COVID-19 pandemic. Many assumed that due to COVID-19 pandemic, financial issues have occurred. Preponderantly, participants deduced that they are experiencing deficit in availability of dental materials for their procedures.

Due to the economic crisis dental practitioners predominantly agreed to perform non-emergency dental procedures with a small number of dental professionals who abstained from performing non-urgent procedures. Multiple participants concluded that washing patient's hand with hand sanitizer to be the foremost step in preventing transmission of COVID-19. Myriad of dental practitioners denoted that performing aerosol generating procedures such as scaling, root canal treatment

and restorative procedures should be postponed indefinitely as shown in Figure 2. In generality, dentists in their practice decided to delay non-emergency procedures. From the sample size, it was concluded that more than half of the dentists were performing emergency procedures for patients who visited dental practice with COVID-19 symptoms. Nearly quarter of the dentists refused to perform treatment on a patient presenting to the dental practice with severe pain along with suspected COVID-19 symptoms.

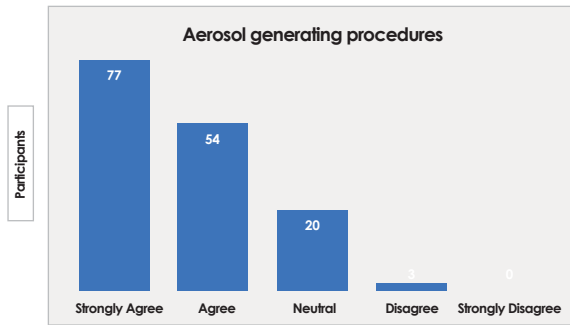


Figure 2: Aerosol generating procedures such as scaling, root canal treatment and restorative procedures should be deferred indefinitely.

DISCUSSION

With regards to the current COVID-19 pandemic, dental practice has been equally affected as compared to the other sectors present in the community. Amongst other healthcare professionals, dentists are struggling to follow guidelines released by world-renowned healthcare organizations. This study reflected how dental professionals were dealing with patients amidst COVID-19 outbreak in Karachi. Data has been gathered from 111 dental practices where majority of dentists are practicing in hospital-based setting whereas a quarter of them working in both hospital and clinic setting. Most of them are aware of the guidelines set forth by the world-renowned organizations but financial issues are one of the causative factors involved in non-compliance during these challenging times.

Current situation suggested that dentists have enough knowledge regarding PPE standard guidelines, but their use is varied throughout the Karachi. During this pandemic, healthcare professionals are experiencing distress and overloaded work regime. Fewer numbers of dentists are using the recommended personal protective equipment during COVID-19 outbreak. Some dental practitioners, working in the private sector, due to their current economic instability, are still performing non-emergency aerosol generating procedures. Due to current situation, lack of resources such as personal protective equipment was a major concern amongst the practicing dental surgeons. Prime reasons being financial instability and unethical hoarding of these equipment²⁵.

It has been found that dental practitioners have adequate amount of knowledge required for the procedures classified as urgent by ADA however, non-urgent procedures were also being carried out which should be deferred according to FDI^{26,27}. Social distancing has been categorically stated to be one of the primary methods for breaking the transmission cycle for COVID-19. Dental practitioners within their clinics are implementing these protocols.

In light of the current crisis, world renowned healthcare organizations such as WHO, Fédération Dentaire Internationale (FDI) and American Dental Association (ADA) recommended usage of necessary PPE when performing procedures on the patient. Although, it can be concluded that many dental practitioners were not implementing these crucial guidelines in their dental practices.

Lack of implementation of the guidelines and significant workload is leading to severe risk of COVID-19 spread. It is believed that awareness programs for dental practitioners and the community is an essential step to mitigate the risk of spread of the disease during this crisis.

In this modern era, social media has been playing pivotal role to close the gap in communication and can be utilized to convey the important messages as well as to form unified platform to update guidelines as per the evolving situation. We also note that there are reports of non-compliance to the guidelines in developed countries reflecting the magnitude of the current global pandemic crisis and its evolving nature²⁸. Although proper guidelines have been mentioned by many world-renowned health organizations, it was noticed that its implementation was a major hurdle in Karachi, Pakistan. Interestingly, in other countries such as UK, there are reports of non-compliance as mentioned on the General Dental Council (GDC) website²⁸.

It can be inferred that due to the rapid spread of the pandemic along with a lag in timely arrangements to adapt to the latest guidelines, which are also changing as the situation continues to evolve, it is difficult to conform to the guidelines. It was found that international guidelines published by ADA and FDI were helpful. Recommended guidelines of World Health Organization for PPE usage, American Dental Association, World Dental Federation (FDI) and GDC amidst the COVID-19 outbreak for the practicing dentists, certain measures are to be followed and implemented in practice. We concluded the following points as recommended by GDC, WHO, ADA and FDI. GDC recommended providing telephonic triage to assess the patients need and treatment planning accordingly. FDI suggests fourhanded technique is beneficial for controlling infection. The use of saliva ejectors with

low volume or high volume can reduce the production of droplets and aerosols²⁷.

Government should strictly implement rules where unethical hoarding of personal protective equipment must be stopped²⁹. Similarly, government needs to come up with a contingency plan to support financially in order to compensate for the financial loss occurred because of postponing the non-emergency procedures by the private dental practices. For every patient visiting the dental practice, screening should be performed in the form of checking temperature. Hand sanitizer should be available for the patients and all healthcare professionals, making sure it is used frequently³⁰.

CONCLUSION

The majority of the dentists are aware of the guidelines however, due to financial burden, lack of resources and rapidly evolving situation with frequent need of changing guidelines. Moreover, it is difficult for dentists in Karachi to adhere to the most recent WHO guidelines.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS APPROVAL

Ethics review committee of Altamash Institute of Dental Medicine has approved this study (AIDM/EC/04/2020/01).

PATIENT CONSENT

Written and verbal consent was taken from the participants in this study.

AUTHORS' CONTRIBUTIONS

AS collected data and was a major contributor in writing the manuscript. MK analysed data and was a major contributor in writing the manuscript. AL collected data and was a major contributor in writing the manuscript. SH collected data for analysis and produced figures. NA provided knowledge and guidance for this manuscript. MM provided intellectual support.

REFERENCES

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, *et al*. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. 2020;382(13):1199-1207.

2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*. 2020;382(8):727-733.
3. John S Ji. Origins of MERS-CoV, and lessons for 2019-nCoV. *Lancet Planet Health*. 2020;4(3):e93.
4. Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health*. 2020;25(3):278-280.
5. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *J Adv Res*. 2020;24:91-98.
6. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054-1062.
7. Sommerstein R, Kochen MM, Messerli FH, Gräni C. Coronavirus disease 2019 (COVID-19): Do angiotensin-converting enzyme inhibitors/angiotensin receptor blockers have a biphasic effect? *J Am Heart Assoc*. 2020;9(7):e016509.
8. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med*. 2020;8(4):e21.
9. Ijaz MK, Brunner AH, Sattar SA, Nair RC, Johnson-Lussenburg CM. Survival characteristics of airborne human coronavirus 229E. *J Gen Virol*. 1985;66(12):2743-2748.
10. Adhikari SP, Meng S, Wu Y, Mao Y, Ye R, Wang Q, *et al*. Novel Coronavirus during the early outbreak period: Epidemiology, causes, clinical manifestation and diagnosis, prevention and control. *Infect Dis Poverty*. 2020;9(29):1-12.
11. Cai J, Sun W, Huang J, Gamber M, Wu J, He G. Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020;26(6):1343-1345.
12. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-513.
13. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395(10223):470-473.
14. Greenhalgh T, Koh GC, Car J. Covid-19: a remote assessment in primary care. *Br Med J*. 2020;368:m1182.
15. Chang D, Lin M, Wei L, Xie L, Zhu G, Cruz CS, *et al*. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *J Am Med Assoc*. 2020;323(11):1092-1093.
16. Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, *et al*. Comorbidities and multi-organ injuries in the treatment of COVID-19. *Lancet*. 2020;395(10228):e52.
17. Xie J, Tong Z, Guan X, Du B, Qiu H. Clinical characteristics of patients who died of coronavirus disease 2019 in China. *J Am Med Assoc*. 2020;3(4):e205619.
18. NBC Universal Inc. [Internet] 7 Week Old Girl Dies

- of Coronavirus; New Cases Top 3500; 2020 [updated 2020 April 2; cited 2020 June 15]. Available from: <https://www.nbcconnecticut.com/news/coronavirus/coronavirus-in-ct-gov-lamont-to-provide-update-on-states-coronavirus-response-tour-sc-su-field-hospital/2248534/>
19. U.S. Department of Health and Human Services. [Internet] Social Distancing, Quarantine, and Isolation; 2020 [updated 2020 June 12; cited 2020 June 16]. Available from: <https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/social-distancing.html>
20. Guardian News and Media Limited. [Internet] Pakistan arrests doctors protesting over lack of virus safety equipment; 2020 [updated 2020 April 6; cited 2020 June 15]. Available from: <https://www.theguardian.com/world/2020/apr/06/pakistan-arrests-doctors-protest-lack-coronavirus-safety-equipment>
21. Sabino-Silva R, Jardim AC, Siqueira WL. Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. *Clin Oral Investig.* 2020; 24:1619-1621.
22. Cheung JC, Ho LT, Cheng JV, Cham EY, Lam KN. Staff safety during emergency airway management for COVID-19 in Hong Kong. *Lancet Respir Med.* 2020;8(4):e19.
23. NHS. [Internet] Issue 3, Preparedness letter for primary dental care; 2020 [updated 2020 March 25; cited 2020 June 16]. Available from: <https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/03/issue-3-preparedness-letter-for-primary-dental-care-25-march-2020.pdf>
24. World Health Organization. [Internet] Rational use of personal protective equipment for coronavirus disease 2019 (COVID-19); 2020 [updated 2020 February 22; cited 2020 June 17]. Available from: https://apps.who.int/iris/bitstream/handle/10665/331215/WHO-2019-nCov-IPCPE_use-2020.1-eng.pdf
25. The News [Internet]. Ban imposed on face mask hoarding, profiteering; 2020 [updated 2020 March 10; cited 2020 June 15]. Available from: <https://www.thenews.com.pk/print/626676-ban-imposed-on-face-mask-hoarding-profiteering>
26. American Dental Association [Internet]. ADA develops guidance on dental emergency, non-emergency care Recommendations part of dentists' response over COVID-19 concern; 2020 [updated 2020 March 18; cited 2020 June 16]. Available from: <https://www.ada.org/en/publications/ada-news/2020-archive/march/ada-develops-guidance-on-dental-emergency-non-emergency-care>
27. FDI World Dental Federation [Internet]. Journal of Dental Research publishes COVID-19 guidelines from researchers based in Wuhan, China; 2020 [updated 2020 March 17; cited 2020 June 15]. Available from: <https://www.fdiworlddental.org/news/20200317/journal-of-dental-research-publishes-covid-19-guidelines-from-researchers-based-in>
28. General Dental Council [Internet] COVID-19 latest guidance for England; 2020 [cited 2020 June 16]. Available from: <https://www.gdc-uk.org/information-standards-guidance/covid-19/covid-19-latest-information/covid-19-latest-guidance-for-england>
29. Pakistan Today [Internet]. Eighth Pakistani succumbs to corona, over 1,000 affected; 2020 [updated 2020 March 26; cited 2020 June 17]. Available from: <https://www.pakistantoday.com.pk/2020/03/25/coronavirus-claims-another-life-tally-crosses-1000/>
30. U.S. Department of Health and Human Services [Internet]. Hand hygiene recommendations guidance for healthcare providers about hand hygiene and COVID-19; 2020 [updated 2020 March 17; cited 2020 June 15]. Available from: <https://www.cdc.gov/coronavirus/2019-ncov/hcp/hand-hygiene.html>



KAP STUDY

Awareness, Attitude and Practices Related to COVID-19 Pandemic in General Public of Province Sindh, Pakistan

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ABSTRACT

Background: The outbreak of COVID-19 started from Wuhan, China and spread throughout the world, from the day it infected first person until now it has been the leading cause of death in 2020. It has changed the daily life routine of people and is responsible for the adaptation of practices as precautionary measures the current study was aimed "to assess the knowledge of the public about COVID-19 and to investigate their attitude and practices during lockdown".

Methods: It was a cross sectional study conducted by the Masters in Healthcare Management students of Ziauddin University Karachi, from 28 March to 5 April 2020 during COVID-19 Pandemic Lock down. The sample size was n=384. A questionnaire based on three forms was formulated to record the Knowledge Attitude and Practice of people about COVID-19 and data was analyzed.

Results: In the study, 53.5% (316) were male and 46.8% (282) were female participants, most of them were from Karachi 62.1% (374), 42.4% (255) were highly educated (masters) and 47% (283) were health care providers (Doctors, Nurses, and Paramedic Staff etc.). As far as, attitude is concerned people showed a positive response towards Government policies announced for this pandemic and they were following a good hygiene practice.

Conclusion: The participants showed much (76%) awareness regarding this pandemic i.e., COVID-19, its outbreak and basic knowledge about it. Most of them were satisfied by the measures taken by the Government of Province Sindh.

Keywords: Awareness; Attitude; Practices; COVID-19; Public.

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INTRODUCTION

Coronaviruses infections are emerging respiratory viruses and known to cause illness ranging from the common cold to severe acute respiratory syndrome (SARS)^{1,2}. The current outbreak of novel coronavirus (COVID-19) in Wuhan City, Hubei Province of China has emerged as a global outbreak and significant public health issue³. On 30 January 2020, the World Health Organization (WHO) named the disease Covid-19 caused by the corona virus. WHO has declared this pandemic a Public Health Emergency

of International Concern (PHEIC)^{4,5}. The disease is highly infectious and its main clinical symptoms include fever, dry cough, fatigue, myalgia, and dyspnea. Corona virus is zoonotic pathogens that can be transmitted via animal-to-human and human-to-human⁶.

There is no evidence right now to suggest that Covid-19 will disappear in summer, a senior WHO expert said, urging countries to fight the new virus decisively at current stage. "We do not know yet what the activity or behavior of the virus will be in

different climatic conditions". In Pakistan the first case was reported on February 26 in Karachi and till mid of March provisional government lock down the province of Sindh because of continuous rise in number of cases. People were also required to stay at home to avoid contacting with others, maintain social distancing and personal cleanliness. All the measures were taken according to WHO CDC prevention guidelines^{7,8}.

The government has imposed lock down so that the people stay at home and adopt social distancing. Educational institutions, government and private offices, mosques, shopping centers and bazaars are also closed across the country. So far, the death rate from the outbreak of COVID-19 has not been alarming in Pakistan but still the cooperation of the people and national unity must be shown in order to fight this virus with courage and determination. The current study was aimed to assess the knowledge of public about COVID-19 and to investigate their attitude and practices during lockdown. This survey has the potential to highlight the knowledge gaps and important elements which could be helpful to encourage Pakistani people and other authorities to play more active role not only in prevention and treatment and possibly to overcome this rapidly growing and highly contagious disease.

METHODS

It was a cross sectional study conducted by the Masters in Healthcare Management students of Ziauddin University Karachi, from 28 March to 5 April 2020 during COVID-19 Pandemic Lock down. The sample size was calculated based on the propor-

tion of 50% at bound of error 5% and confidence interval of 95%. Calculated sample size was n=384. A questionnaire based on three forms was formulated to record the Awareness, Attitude and Practice of people about COVID-19. The proforma was shared with the people by using Google form link that was sent to them using social media (WhatsApp, Facebook) and responses were recorded for 3 days. The Google survey started with participant consent and further assessment was performed after the consent. Data was analyzed using SPSS version 20. Results are expressed in frequency and percentages. Ethical approval for the project was taken from ERC of Ziauddin University.

RESULTS

Total 610 participants submitted their response in 3 days after that link was kept off for responses and data was analyzed. Thus, 12 participants did not answer for all the questionnaires and they were excluded from study. In this study, 53.5% (316) were male and 46.8% (282) were female participants, most of them were from Karachi 62.1% (374), and followed by other cities of province Sindh i.e. 47.9%. Educationally 46.7% (281) were graduate, 42.4% (255) were highly educated (masters), and 47% (283) were health care providers (Doctors, Nurses, and Paramedic Staff etc.). The major age group who participated in our study was 21-30 years (71.9%) followed by 31-40 years (14.8%). Most of the participants were aware about the disease outbreak, cause, mode of transmission, sign and symptoms and preventive measures. However, when asked about most susceptible age group for the infection 49.2% (296) as shown in Table 1.

Table 1: Awareness of participants regarding COVID-19 (n=598).

Participants awareness	Response in frequency n (%)				
	China	Italy	Iran	America	Others
1. The disease outbreak started from:	593 (98.5%)	3 (0.3%)	2 (0.2%)	1 (0.1%)	1 (0.2%)
2. Cause of disease	Bacteria	Virus	Fungi	Parasite	Other
	15 (2.5%)	582 (96.5%)	0 (0%)	0 (0%)	3 (0.5%)
3. Disease can be transmitted through: Frequency (%)	Air	Person to person contact	Contact with infected person and objects touched	Sexual route	Others
	37 (6.1%)	118 (19.6%)	440 (73.1%)	1 (0.2%)	7 (1.5%)
4. Most susceptible persons for the disease are:	Children	Young sters	Adult and older	Disease is not specific to any age group	
	16 (2.7%)	4 (0.7%)	296 (49.2%)	286 (47.5%)	

5. An infected person may show sign and symptoms of:	5 days	6-10 days	11-15 days	Up to 21 days	
	94 (15.6%)	95 (15.8%)	380 (63.1%)	32 (5.3%)	
6. Signs and symptoms of COVID – 19 are:	Dry cough, fever and breathlessness	Productive cough, fever and flu	Allergy, seizures and red spots on skin	Seizers, fever and cough	I don't know
	565 (93.9%)	28 (4.7%)	1 (0.2%)	2 (0.3%)	4 (0.7%)
7. If a person had a contact with infected person what should he/she do:	Isolate himself/herself and wait for sign and symptoms to appear	Immediately rush for COVID – 19 screening test	Live a normal routine life	Disinfect him/her self	
	426 (70.8%)	137 (22.8%)	1 (0.2%)	35 (5.8%)	
8. Is there any specific treatment/ vaccine available for COVID-19?	Yes	No	I don't know		
	27 (4.5%)	515 (85.5%)	58 (9.6%)		
9. What are the chances of recovery after being infected by COVID – 19?	5%	50%	80%	>95%	
	22(3.7%)	100 (16.6%)	179 (29.7%)	295 (49%)	
10. If someone is having symptoms and travelling history:	He/she hide it as it may require quarantine	He/she inform about all related history to doctor	Treatment of COVID – 19 is expensive it is better to hide the history from doctor	Self-medication is an effective way to deal with the symptoms	
	83 (13.8%)	491 (81.6%)	10 (1.7%)	13 (2.2%)	
11. The primary source of knowledge shared by you in above questionnaires is:	TV/News paper	Social media Apps (FB, WhatsApp)	Self -surfing on Internet	Others	
	188 (31.2%)	322 (53.5%)	75 (12.5%)	17 (2.8%)	

When asked about attitude (Table 2) and practices (Table 3) most of those positively on taking preventive measure and by the policies of the Sindh Government planned for this Pandemic. They also reported that negligence by the public in this

lockdown may worsen the situation and may be a cause of increase in number of cases and may provoke the incidence from hundreds to thousands.

Table 2: Attitude of participants regarding COVID-19 (n=598).

Questions based on participants attitude	Response in frequency (%)			
	Strongly Agree	Agree	Disagree	Strongly Disagree
1. Government has taken good measures and provided sufficient information COVID-19	497 (82.6%)	349 (58%)	53 (8.8%)	8 (8.3%)
2. We should stay at home avoid social communication as directed by government and healthcare professionals	497 (82.6%)	99 (16.4%)	4 (7%)	0 (0%)
3. We should avoid traveling to other cities during this pandemic	503 (83.6%)	88 (14.6%)	5 (8%)	5 (8%)
4. Do you think this lock down will be helpful in preventing the spread of infection?	393 (65.3%)	188 (31.2%)	18 (3.0%)	2 (0.3%)
5. Do you think Government policies are effective in this outbreak?	145 (24.1%)	383 (63.6%)	70 (11.6%)	3 (5%)
6. Despite lock down people are not staying at their homes	212 (35.2%)	339 (56.3%)	40 (6.6%)	8 (1.3%)
7. Non-Serious attitude of people is leading to an increase in number of cases of COVID-19 in Sindh	412 (68.4%)	171 (28.4%)	14 (2.3%)	2 (0.3%)

Thus, 98.9% participants responded "positive" when asked about practices of prevention followed by them as shown in Table 3. The role of social media, TV and other internet sources in spreading the knowledge and awareness is also acknowledge-

able as most of the participant's responses are highlighting that the primary source of information to them was social media followed by TV and other resources.

Table 3: Practices followed by participants (n=598).

Questions based on followed practices	Response in frequency (%)		
	Yes	No	I don't know
1. We should wash our hands frequently:	596 (99.0%)	4 (0.7%)	0 (0%)
2. We should avoid touching mouth, nose and eyes	587 (97.5%)	10 (1.7%)	4 (0.7%)
3. While going out of home, we should cover our face:	582 (96.7%)	15 (2.5%)	4 (0.7%)
4. We should keep hand sanitizer while going out to sanitize our hands:	585 (97.2%)	15 (2.5%)	2 (0.3%)
5. We should cover our face while sneezing or coughing:	595 (98.8%)	5 (0.8%)	2 (0.3%)
6. If we develop any of symptom of COVID-19 we should isolate ourselves	596 (99.0%)	2 (0.3%)	1 (0.2%)
7. It is our national responsibility to follow instructions provided by government	597 (99.2%)	1 (0.2%)	1 (0.2%)

DISCUSSION

Currently, Corona virus is the daily discussion topic among the public and media too, which resulted in increasing public attention towards their awareness and preventive measures towards covid-19. In this study, 76% participants showed the sufficient basic knowledge regarding COVID-19 as expected graduate participants and pharmacist/healthcare providers are more knowledgeable than others are and there was no significant difference in knowledge of COVID-19 when genders were compared. But this frequency is higher than the similar study conducted in Riyadh to evaluate the public awareness regarding swine flu and they reported only 51% respondents were knowledgeable about the viral breakout¹¹. However, the people have basic knowledge regarding COVID-19 that it is viral disease (96.5%), mode of transmission (73%), signs and symptoms of disease (93.9%) and vaccine/treatment availability (85.5%). However, the results regarding knowledge of susceptible age group signs and symptoms of COVID-19 are not satisfactory and it highlights the need to raise more awareness¹². As our study suggests, most of the participants reported the main source of their knowledge is Internet (53.5%) and TV (31%). For this reason, attention of higher authorities is required to improve these mediums to be used for health promotion specifically for such emerging infectious diseases¹³.

Survey results identified that 98% people who belongs to different professions, different age groups and different regions of Pakistan are following a good hygiene practice. These findings are parallel with the findings of previous study conducted to assess the public awareness in Saudi Arabia, their participants also showed high level of personal hygiene practices^{5,14,15}.

Generally, most of the public has a positive attitude towards the roles of guidelines provided for COVID-19 prevention. Another important finding is that more than 89% participants showed support for the government actions to control corona virus and 88% respondents find Government policies effective for this pandemic. Among which most participants are in the age group of 20-30 years old and it has been found that female gender has more believe in government abilities as compare to males. Whilst, 98% participants believe that they should stay at home and avoid social communications and these findings can be compare with a survey conducted in America for COVID-19 public awareness which reported 80% respondents are willing to stay at home and skip social events¹⁶.

There were some areas where public knowledge was relatively low, for example every 5 out of 10 respondents believed that the most susceptible persons for COVID-19 are adults and older age group and only 4 out of 10 respondents under-

stands that COVID-19 is not age specific. When asked about chances of recovery after getting infected by COVID-19 only 5 out of 10 respondents were aware that there are more than 97% chances of recovery after getting infected from COVID-19^{17,18}. Previously many studies have been conducted to demonstrate the level of knowledge regarding other infectious viral breakouts like Avian Influenza, influenza strain H1N1 and swine flu. There is also a survey conducted in India for COVID-19 awareness in healthcare workers^{19,20}. However, to the best of our knowledge, this is the first survey conducted in Pakistan to assess the knowledge and attitude of people toward COVID-19. Therefore, this study could be helpful for public health officials in planning healthcare related education or awareness programs for other emerging infectious disease^{21,22}. As it was lock down in province usage of technology was preferred, Google form based survey was conducted and questionnaire was filled by sending the link using social media apps so most of the enrolled participants were educated.

CONCLUSION

The participants of our study were much aware regarding this Pandemic COVID-19, its outbreak and basic knowledge. Most of them seemed to be satisfied by the measures taken by Government of Sindh and they showed that they are following hygiene practices as preventive measure as instructed by Health Care Professionals. The only misconception / gap that needs to be addressed are the knowledge regarding age of onset and signs and symptoms of disease.

ACKNOWLEDGEMENTS

The authors acknowledged the Ziauddin University for facilitating the study.

CONFLICT OF INTEREST

There was no any conflict of interest.

ETHICS APPROVAL

The Institutional Review Board of Ziauddin University approved this research.

PATIENTS CONSENT

Proper consents were obtained from the participants of the study.

AUTHORS' CONTRIBUTION

All the authors contributed equally in manuscript writing and analysis the final submitted copy was critically reviewed and approved by FA.

REFERENCES

1. Hu B, Zeng LP, Yang XL, Ge XY, Zhang W, Li B, *et al.* Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog.* 2017;13(11): e1006698.
2. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Medicine.* 2020 ;8(4):420-422.
3. Li F. Structure, function, and evolution of coronavirus spike proteins. *Annu Rev Virol.* 2016;3:237-261.
4. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report; 2020.p.67.
5. Erfani A, Shahriarirad R, Ranjbar K, Mirahmadzadeh A, Moghadami M. This paper was submitted to the Bulletin of the World Health Organization and was posted to the COVID-19 open site, according to the protocol for public health emergencies for international concern as described in Vasee Moorthy *et al.* The information herein is available for unrestricted use, distribution and reproduction in any medium, provided that the original work is properly cited as indicated by the Creative Commons. 2020:1-20.
6. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, *et al.* A new coronavirus associated with human respiratory disease in China. *Nat.* 2020;579(7798): 265-269.
7. World Health Organization [internet]. The COVID-19 risk communication package for health-care facilities; 2020 [updated 2020 March 10, cited 2020 June 2]. Available from: <https://iris.wpro.who.int/handle/10665.1/14482>
8. Qureshi UU, Saleem S, Khan A, Afzal MS, Ali MS, Ahmed H. Outbreak of novel Corona virus (2019-nCoV); implications for travelers to Pakistan. *Travel Med Infect Dis.* 2020;34:101571.
9. Balkhy HH, Abolfotouh MA, Al-Hathloul RH, Al-Jumah MA. Awareness, attitudes, and practices related to the swine influenza pandemic among the Saudi public. *BMC Infect Dis.* 2010;10(1):42.
10. Gautret P, Benkouiten S, Salaheddine I, Belhouchat K, Drali T, Parola P, *et al.* Hajj pilgrims' knowledge about Middle East respiratory syndrome coronavirus, August to September 2013. *Euro Surveill.* 2013;18(41):20604.
11. Almutairi KM, Al Helih EM, Moussa M, Boshaiqah AE, Saleh Alajilan A, Vinluan JM, *et al.* Awareness, attitudes, and practices related to coronavirus pandemic among public in Saudi Arabia. *Fam Community Health.* 2015;38(4):332-340.
12. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, *et al.* Presumed asymptomatic carrier transmission of COVID-19. *JAMA.* 2020;323(14):1406-1407.
13. Wen J, Aston J, Liu X, Ying T. Effects of misleading media coverage on public health crisis: A case of the 2019 novel coronavirus outbreak in China. *Anatolia.* 2020;31(2):331-336.
14. Zhong BL, Luo W, Li HM, Zhang QQ, Liu XG, Li WT, *et al.* Knowledge, attitudes, and practices towards COVID-19 among Chinese residents during the rapid rise period of the COVID-19 outbreak: a quick online cross-sectional survey. *Int J Biol Sci.* 2020;16(10): 1745-1752.
15. Albarrak AI, Mohammed R, Al Elayan A, Al Fawaz F, Al Masry M, Al Shammari M, *et al.* Middle East Respiratory Syndrome (MERS): Comparing the knowledge, attitude and practices of different health care workers. *J Infect Public Health.* 2019; S1876-0341(19)30239-4.
16. Everett JA, Colombatto C, Chituc V, Brady WJ, Crockett M. The effectiveness of moral messages on public health behavioral intentions during the COVID-19 pandemic. 2020;1-23.
17. Battagay M, Kuehl R, Tschudin-Sutter S, Hirsch HH, Widmer AF, Neher RA. 2019-novel Coronavirus (2019-nCoV): estimating the case fatality rate—a word of caution. *Swiss Med Wkly.* 2020; 150:w20203.
18. RTI Innovation Advosrs [Internet]. RTI Surveyed 1,000 Americans about Awareness, Perceptions of COVID-19; 2020 [updated 2020 March 12, cited 2020 June 16]. Available from: <https://www.rti.org/coronavirus-united-states-survey>
19. Modi PD, Nair G, Uppe A, Modi J, Tuppekar B, Gharpure AS, *et al.* COVID-19 awareness among healthcare students and professionals in Mumbai metropolitan region: a questionnaire-based survey. *Cureus.* 2020;12(4):e7514.
20. U.S. Department of Health and Human Services [Internte]. Novel coronavirus structure reveals targets for vaccines and treatments; 2020 [updated 2020 March 3, cited 2020 June 15]. Availbale from: <https://www.nih.gov/news-events/nih-research-matters/novel-coronavirus-structure-reveals-targets-vaccines-treatments>
21. Pakistan Today [Internet]. Calls for lockdown as corona cases cross 700; 2020 [updated 2020 March 23, cited 2020 June 15]. Availbale from: <https://www.pakistantoday.com.pk/2020/03/21/calls-for-lockdown-as-corona-cases-cross-700/>
22. Tribune [Internet]. Pakistan cannot afford complete lockdown over coronavirus fears, says PM; 2020 [updated 2020 March 20, cited 2020 June 16]. Availbale from: <https://tribune.com.pk/story/2180055/1-live-pakistan-reports-third-covid-19-death-astally-tops-450/>

CASE REPORT**Granulosa Cell Tumour – A Rare Presentation at Age Twenty****Munazza Anis¹, Shama Chaudhary¹, Rahima Rais², Rubina Hussain¹, Sayeda Naseer²**¹Department of Obstetrics and Gynecology, Ziauddin University Hospital, ²Student, Ziauddin University, Karachi, Pakistan.**ABSTRACT**

Granulosa cell tumor is a rare type of ovarian tumor, which arises from sex cord stroma. Histologically this tumor has two types and is named according to the common age group they affect; adult granulosa cell tumor (AGCT) and juvenile granulosa cell tumor. AGCT constitutes 2-5% of all ovarian cancers. Mostly present in women of age > 40 years. In this case report, we discussed the role of conservative surgery in young adult reported with granulosa cell tumor. An unmarried teenage girl presented at a private tertiary care hospital with abdominal pain and abdominal distention. Radiological examinations suggested a mass originating from the right ovary for which laparotomy was done and a ruptured cyst was found near the right ovary with a mass adherent to surrounding peritoneal viscera. Right ovarian cystectomy along with omental biopsy and left ovarian biopsy was performed. Rare presentation of this tumor will help clinicians to not categorize the type histologically with the age group.

Keywords: Granulosa Cell Tumor; Ovarian Mass; Laparotomy.**Corresponding Author:****Dr. Munazza Anis**Department of Obstetrics and Gynecology,
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INTRODUCTION

Majority of adult-type granulosa cell tumor cases present in early stages. Stage I has a favorable outcome and a low rate of recurrence as compared to late stages¹. These tumors are unilateral with no side predominance. Women of reproductive age reported with hirsutism and menstrual irregularities such as amenorrhea, intermenstrual bleeding and menorrhagia due to hormones produced by tumor; estrogen and androgen. Some patients also report abdominal distention, acute abdominal pain, nausea, and vomiting due to ovarian torsion or hypotension. FOXL2 mutation determination is recommended for primary diagnosis². Granulosa cell tumors are also associated with endometrial hyperplasia or carcinoma³. The study will not only discuss the different aspects of granulosa cell tumor in young adult but also strengthen the role of conservative surgery in management of young adults suffering from granulosa cell tumor.

CASE REPORT

A 19-year-old unmarried female with no known comorbidities presented to Out Patient Department (OPD) of Ziauddin University Hospital with complaints of lower abdominal pain for past two weeks and abdominal distention for past two weeks. She also reported to have had irregular menstrual cycles for last two years and had taken hormonal therapy for this. On abdominal examination, slight distention was noted with inverted umbilicus, abdomen was soft and slightly tender with a palpable mass below the umbilicus.

Initially, Ultrasound Pelvis (Figure 1a) was performed which revealed a large multilobulated solid lesion measuring 16.0x10.9cm with multiple cystic areas in mid of the pelvis. It also suggested the presence of mild to moderate ascites in the abdominopelvic cavity.

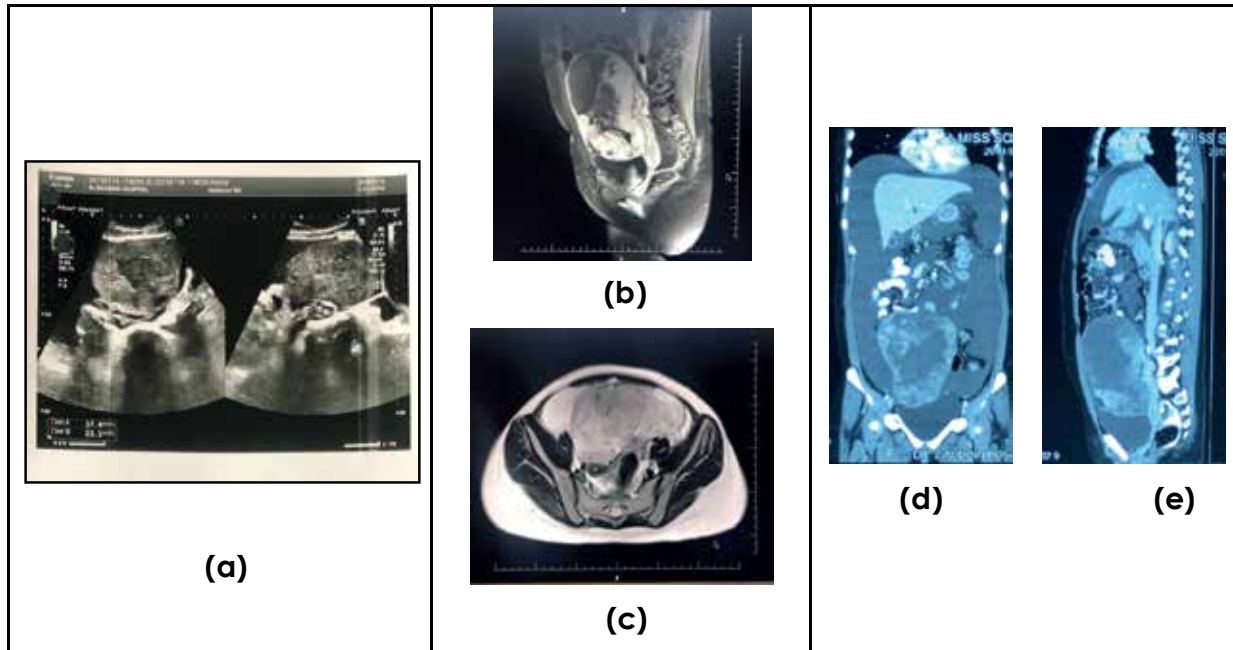


Figure 1: (a) Ultrasound of Pelvis showing multi lobulated solid mass in mid pelvis; (b, c) MRI of Pelvis showing oval shaped mass towards right adnexa; (d, e) CT scan of the abdomen showing solid mass extending to abdominal wall anteriorly, compressing the uterus posteriorly with moderate to severe ascites in abdominopelvic cavity.

Further evaluation of the disease was made by Magnetic resonance imaging (MRI) of Pelvis with contrast (Figure 1b, c) which showed a large well defined oval-shaped capsulated solid mass measuring (16.5 x 10.1 x 13.2)cm in craniocaudal (CC), anteroposterior (AP) and transverse (TV) dimensions in the center of the pelvis with predominant bulk towards right adnexa. Both ovaries were not separately identified completely from the lesion and probably the lesion was arising from the right ovary. Areas of necrosis and hemorrhage were noted inside the lesion. Omental thickening anterior to abdominal wall and free fluid in lower abdomen and pelvic peritoneal cavity was also noted. Normal size anteverted uterus with normal endometrium was seen.

The computerized tomography (CT) scan of whole abdomen (Figure 1d, e) was done which confirmed the previous findings. Anteriorly the mass is extending towards anterior abdominal wall. Posteriorly

extended mass is abutting and compressing the uterus. Posterolaterally, mass is abutting the terminal ileum on the right side and cecum on the left side of the sigmoid colon. Inferiorly the mass is abutting the dome of urinary bladder. Superiorly it is abutting and compressing the adjacent bowel loops. The mass is abutting and compressing the right ureter. Both ovaries are not separately visualized. Moderate to severe ascites seen in abdominopelvic cavity. Few lymph nodes of less than one centimeter are seen in abdomen, para-aortic and aortocaval location.

Her basic workup investigations were done which showed (Table 1) which shows hemoglobin 10.6 g/dl, total leucocyte count $9.80 \times 10^9/L$ platelets 364000 platelets/ μL , cancer antigen-125 1697 U/mL, lactic acid dehydrogenase 195 U/mL, beta human chorionic gonadotropin negative and alpha-fetoprotein 2.03 ng/mL (Table 1).

Table 1: Investigation profile of a patient.

Variables	Results	Reference Value
Hb	10.6	12.0 - 15.5g/dl
TLC	9.80	$4.5 - 10.5 \times 10^9/L$
PLT	364	150 - 450
CA125	1697	<46 U/mL
LDH	195	100 - 190U/L
bHCG	-VE	-
AFP	2.03	<10ng/mL

Hb: Hemoglobin; TLC: Total leucocyte count; PLT: Platelets; CA: cancer antigen; LDH: Lactic Acid Dehydrogenase; bHCG: Beta human chorionic gonadotropin; AFP: alpha-fetoprotein.

Patient was admitted and Laparotomy (Right ovarian cystectomy +Omental biopsy +Left ovarian biopsy + Adhesiolysis + Superficial bladder repair) was performed. Intraoperative findings (Figure 2a, b): right ovarian cyst, glistening surface, torsion of ovary, ruptured cyst adherent anteriorly to bladder.

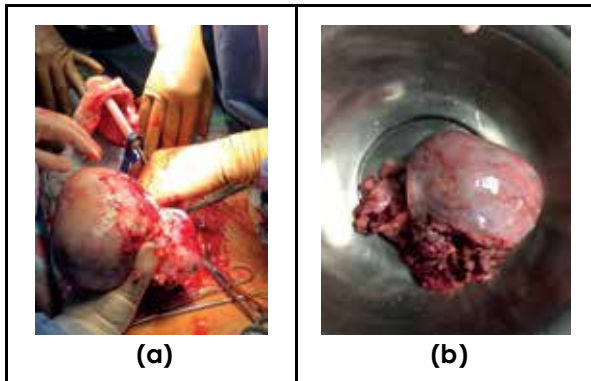


Figure 2a, b: Intraoperative findings showing right ovarian cyst with glistening surface, it was ruptured adherent anteriorly to bladder and right fallopian tube stretched out over cyst.

Frozen section was taken and sent for histopathology studies, showed adult granulosa cell tumor and calretinin was strongly positive on immuno-histochemistry. Case was discussed and further plan for endometrial sampling and chemotherapy was recommended. Endometrial biopsy showed disordered proliferative endometrium.

DISCUSSION

Granulosa cell tumors are uncommon sex stromal neoplasms with a slow progressing nature⁴. The granulosa cells produce the sex steroid and due to high levels of estrogen, cause hyperestrogenism⁵. Granulosa cell tumors are mainly of the following two types: Adult tumor and Juvenile tumor. We make this distinction on the basis of clinical and histopathological characteristics⁶. Adult type being the more common one and accounting for 95% of all granulosa cell tumors and 2%- 5% of all ovarian malignancies⁷.

The juvenile type as the name suggests is the form that affects mainly the prepubertal girls and women of age < 30 years while the adult type shows the highest incidence in ages of 50 to 55 years (postmenopausal period). It is the less rare type and represents about 5% of all Granulosa cell tumors⁵. Granulosa cells are characterized by small round and pale cells with characteristic nuclei of coffee beans. When these cells are well differentiated they have different patterns that are as follows: micro follicular, macro follicular, solid-tubular, trabecular, insular and hollow tubular⁵.

Although any patterns may be observed, but most commonly observed is the micro follicular pattern. These cells are characterized by Call- Exner bodies⁸. These are small rings made up of granulosa cells, which either surrounds the shrunken nuclei or eosinophilic fluid material⁵. In the case report, the girl is 20 years of age and she presents with a tumor identified by the histopathology report as the adult type. The adult form, which is a low-grade malignant sex cord-stromal tumor, occurs occasionally in young children. Therefore, for our 20-year-old patient to have this marks a rare case. Patients will usually present with abdominal pain, distention and irregular bleeding (adult type: postmenopausal bleeding) due to secretion of estradiol⁴.

Abnormal uterine bleeding and irregularities in the menstrual cycle are more commonly observed in adult women of reproductive age who are hormonally active and suffering from granulosa cell tumor. Another common finding is endometrial hyperplasia, which is again due to high levels of estrogen. Tumor torsion ovary may present at times, which causes acute abdominal pain. Tumor rupture is also seen in about 10% cases causing manifestations such as acute pain in abdomen, distention and hypotension because of hemoperitoneum⁵.

Unfavorable factors include advanced stage (most important), large size (>15cm), bilaterally present tumor and tumor rupture, which can then cause acute abdominal pain (6-10%). Morphological features including nuclear grooves, positivity of immunohistochemical Stain Inhibin, negative EMA and P53 support granulosa tumor, which may show necrosis. The imaging characteristics of both types are non-specific so difficult to distinguished easily from other types of ovarian cancers. Computed Tomography scans with a diffused pattern may also be mistaken in diagnosis of poorly differentiated carcinoma.

Circulating biomarkers are most commonly and accurately used for diagnosis of granulosa tumor such as; anti-Mullerian and Inhibin B. In such type of tumors FOXL2 gene is mutated at missense point 402->G(C134W), resulting in increased proliferation along with enhanced surviving ability of granulosa cells which in turn encourages the hormonal changes⁹. Histologically adult granulosa cell tumor identification is a difficult task. Therefore, FOXL2 mutation testing is very helpful in detecting problematic cases. It has shown that 97% of adult granulosa cell tumors showed mutation of FOXL2 gene⁴.

Staging of tumors is one of the most important features that help in establishing the prognosis of tumors⁹. As staging of tumors increase, the survival percentage drastically decreases. A 10-year survival rate of stage I tumor is 84-95%, 50-60% for stage II tumors and a further 17-33% for stage III and IV¹⁰. FIGO staging system is the preferred staging system in granulosa cell tumors and helps in identification of patients who are at risk or those who require therapy. Out of all its subtypes, FIGO

subtype 1C is found to be predictive of recurrence. When treated it can result in disease-free survival in early-stage patients of adult granulosa cell tumors (AGCTs)⁷.

AGCTs are less likely to be malignant compared to the juvenile type but they have a higher chance of recurrence hence, a follow up is highly required. It is also very important to know that follow up is essential because when relapse occurs it is more aggressive in nature with poor outcomes. When assessing via pelvic examination, the most common finding is a tumor mass. It can then be subsequently confirmed with imaging techniques⁴.

The primary treatment for granulosa cell tumors should be complete tumor resection. In this case, chemotherapy is planned for the patient, which is usually a combination of anticancer agents including; bleomycin, etoposide, cisplatin; etoposide/cisplatin and paclitaxel and carboplatin. Relapse rates are as high as 31% so regular follow-ups are very important whereas pelvis is the most common site of recurrence. If relapsed, there is no standard approach for management. Surgery may possibly provide long-term control for localized disease⁴.

If a more traditional approach is adopted then a unilateral salpingo-oophorectomy is suggested in patients of reproductive age having stage I tumor, whereas total abdominal hysterectomy and bilateral salpingo-oophorectomy is suggested in patients who have reached menopause or for those who have a higher grading of the tumor. It is hence emphasized that stage of the AGCT is a very important prognostic factor correlated with risk of prolapse⁴.

AGCT can also affect younger women so fertility preservation is another important problem in management AGCT. However, the importance of fertility surgery is unclear. Few studies report the association of fertility surgery with a higher rate of recurrence and decreased rate of survival⁷.

CONCLUSION

It was concluded that conservative surgery could play an effective role in management of young adults reported with granulosa cell tumor. Further investigated that majority of the patient with granulosa cell tumors of the ovary present in early-stage and have favorable prognosis. In A prospective multi-centric trial is needed to address the role of adjuvant therapies for optimal management of these rare neoplasms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT

Patient consent was taken before starting the procedure and for publishing.

AUTHORS' CONTRIBUTION

RH gave the idea; SC supervised the study; MA collected the data; RR wrote the manuscript and finalized it and SN conducted literature search.

REFERENCES

1. Bergamini A, Cormio G, Ferrandina G, Lorusso D, Giorda G, Scarfone G, *et al.* Conservative surgery in stage I adult type granulosa cells tumors of the ovary: Results from the MITO-9 study. *Gynecol Oncol.* 2019; 154(2):323-327.
2. Sakr S, Abdulfatah E, Thomas S, Al-Wahab Z, Beydoun R, Morris R, *et al.* Granulosa cell tumors: novel predictors of recurrence in early-stage patients. *Int J Gynecol Pathol.* 2017;36(3):240-252.
3. Ottolina J, Ferrandina G, Gadducci A, Scollo P, Lorusso D, Giorda G, *et al.* Is the endometrial evaluation routinely required in patients with adult granulosa cell tumors of the ovary? *Gynecol Oncol.* 2015;136(2): 230-234.
4. Koukourakis GV, Kouloulas VE, Koukourakis MJ, Zacharias GA, Papadimitriou C, Mystakidou K, *et al.* Granulosa cell tumor of the ovary: tumor review. *Integr Cancer Ther.* 2008;7(3):204-215.
5. Kottarathil VD, Antony MA, Nair IR, Pavithran K. Recent advances in granulosa cell tumor ovary: a review. *Indian J Surg Oncol.* 2013;4(1):37-47.
6. Al-Agha OM, Huwait HF, Chow C, Yang W, Senz J, Kalloger SE, *et al.* FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. *Am J Surg Pathol.* 2011;35(4):484-494.
7. Babarović E, Franin I, Klarić M, Ferrari AM, Karnjuš-Begonja R, Eminović S, *et al.* Adult granulosa cell tumors of the ovary: a retrospective study of 36 FIGO stage I cases with emphasis on prognostic pathohistological features. *Anal Cell Pathol.* 2018;9148124.
8. Fujita F, Eguchi S, Takatsuki M, Kobayashi K, Kanetaka K, Ito M, *et al.* A recurrent granulosa cell tumor of the ovary 25 years after the initial diagnosis: a case report. *Int J Surg Case Rep.* 2015;12:7-10.
9. Färkkilä A, Haltia UM, Tapper J, McConechy MK, Huntsman DG, Heikinheimo M. Pathogenesis and treatment of adult-type granulosa cell tumor of the ovary. *Ann Med.* 2017;49(5):435-447.
10. Khosla D, Dimri K, Pandey AK, Mahajan R, Trehan R. Ovarian granulosa cell tumor: clinical features, treatment, outcome, and prognostic factors. *N Am J Med Sci.* 2014;6(3):133-138.

CASE REPORT

A Rare Presentation of Primary Hydatid Cyst in an Adolescent – A Case Report

Asad Aziz, Javeria Junaid, Imtiaz Hashmi

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ABSTRACT

The aim of this case study was to report a very uncommon and rarely seen, but serious condition, involving spine. Hydatid cyst is caused by *Echinococcus granulosus* mainly. It is rarely found in spine, lumbar spine is least commonly involved in spine. A 14 years old boy with lower back pain radiating to both lower limbs observed in the hospital. Associated with progressive weakness and sensory deficit in both lower limbs investigations showed a space occupying lytic lesion extending from L3-S1 with cystic nature, showing nerve root compression at L3, L4 and L5. Managed with excision of cyst through posterior approach to lumbar spine and stabilized with posterior spinal instrumentation L2-L5 and S2Al screw. Hydatid cyst in lumbar spine should be considered as differential diagnosis of cystic lesions of spine. Treatment of hydatid cyst in spine is primarily removal of lesion. Postoperatively the patient improved, mobilized on first post-operative day and has been complication free in one-week follow up.

Keywords: Hydatid Cyst; Spine; *Echinococcus granulosus*; Lumbosacral Spine.

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INTRODUCTION

Hydatid means "watery cyst" in a Greek language. This disease is caused by the *Echinococcus granulosus* and *Echinococcus multilocularis*¹. It is not very common as humans are intermediary host. Usually found in lungs and liver commonly sites involved. Spine is least involved site comprising of 1% of all hydatid cyst cases²⁻⁵. It is commonly found in young patients those are in contact with dogs³.

Spine is involved secondary to spread from pulmonary, abdominal or pelvic infection, most commonly involving the thoracic spine, in decreasing order of frequency to lumbar, sacral and cervical spine^{4,6}. We presented a case of boy with hydatid cyst in lumbosacral spine, with progressive weakness and sensory deficit bilateral lower limbs that was a very rare site for involvement of hydatid cyst.

CASE PRESENTATION

A 14-years old, male patient no known comorbid presented to outpatient department with complaint of pain in lower back since 1 year. Pain was gradual in onset and progressed with time, does not give any history of trauma. Pain was radiating to bilateral lower limbs associated with sensory deficit and progressive weakness bilateral lower limbs. Since last few months mobility was restricted to home, because of pain and progressive weakness patient was hardly able to walk for more than thirty minutes. On examination healthy boy weighing 69kg, walks with waddling gait. Motor examination was unremarkable in bilateral lower limbs. There was sensory deficit in right foot at distribution of Lumbar 5-Sacral 1, ankle jerk was absent. MRI (Magnetic Resonance Imaging) and CT (Computerized Tomography) was performed (Figure 1a-f) which showed space occupying lytic lesion extending from L3-S1 with cystic nature, with nerve root compression at L3, L4 and L5.

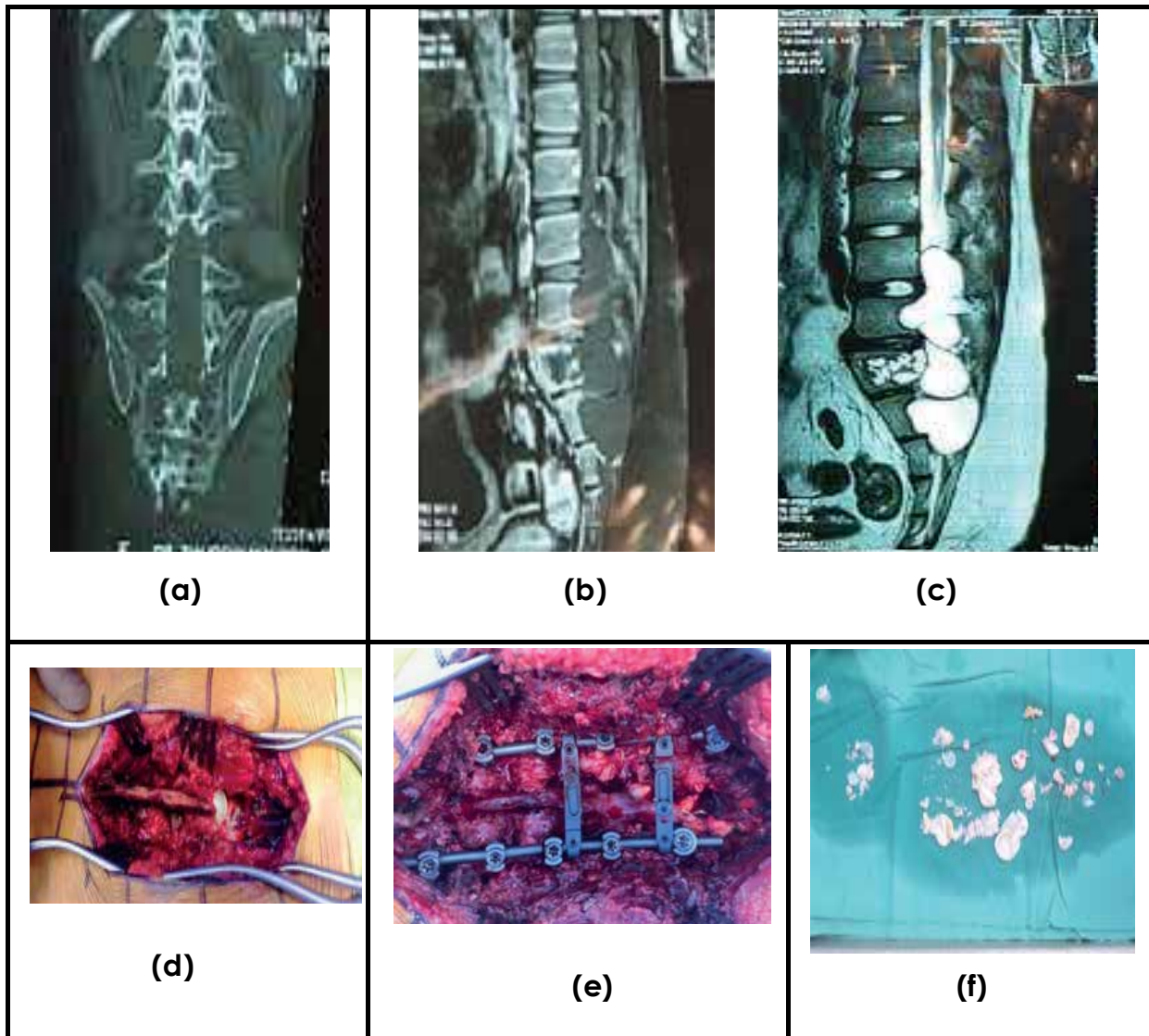


Figure 1: (a) Coronal cuts of CT scan showing involvement and extent of cyst in lumbosacral spine, also involving the right side sacrum (b) Sagittal cut of MRI T-1 image – showing dark signals around the involved area, as CSF and fluid appears dark. Sagittal cut of MRI (c) T-2 image – showing high uptake along posterior border of L3-S1 also involving vertebral body of S1 (d, e) Perioperative images of cyst and fusion surgery (f) Postoperative removed cyst.

Patient was planned for excision of cystic lesion and spinal instrumentation through posterior approach. After taking consent from family patient was given general anesthesia and was kept in prone position, skin incision was given following midline approach to lumbo-sacral spine. While doing soft tissue dissection he was found to have cystic lesion extending from spine superficially on right side with clear fluid and lytic sacral bone. Cyst was excised completely and sent for frozen section per-operatively and it came out to be hydatid cyst. Excision was done followed by posterior spinal instrumentation from

L2-L5 and S2A1 screw (Sacrum 2 Alar iliac) connected with rods and connector (Figure 2a, b). Later decompression was completed and curettage of all necrotic bone was performed and irrigation done with hypertonic saline. Patient remained stable on immediate post-operative day, with intact lower limbs neurology. Patient was ambulated full weight bearing on first post operation day without any brace, with relieved pre-operative symptoms; hospital course was uneventful and discharged home on fourth post-operative day without any walking aid.

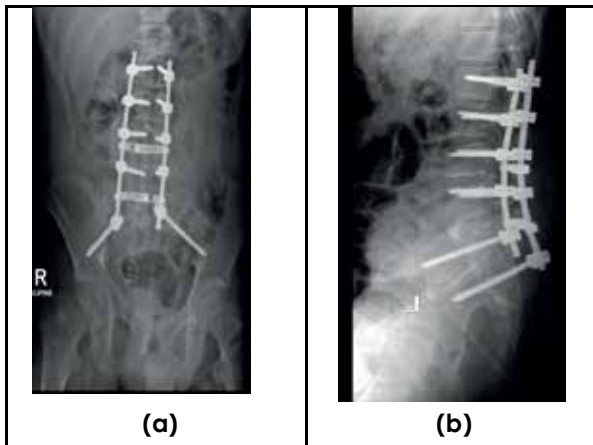


Figure 2a, b: Post operative images of posterior spinal instrumentation L2-L5 and S2A1 screws.

DISCUSSION

Hydatid disease is usually formed in liver (60-70%) and lungs (10-15%). Bones are involved rarely which is 0.5 to 2%^{7,8}. Neurological deficit have been reported to be found in patients with hydatid cyst of spine in 25-84% of cases⁹. It is more common in males' 11:1 female with an age range of 7 years to 56 years average age of 27 years. The parasite extends the paravertebral tissue or the epidural space through the cortex and pore front of the vertebral body. Cyst can progress from body of vertebrae to anterior space, on the sides to extradural space and paravertebral tissues, and to spinal canal putting pressure on the cord, causing radiculopathy symptoms and neurological deficit. Cyst is usually located in posterior element of spine or posterior aspect of cord¹⁰. It is more common in sheep raising areas of world, it affects human by ingesting the eggs excreted by dogs.

Hydatid cyst has two layers, inner germinal layer and outer fibrous layer. It has two types unilocular and multilocular, commonest among them is unilocular. Three layers of hydatid cyst are further classified histopathologically, peripheral adventitial layer consist of eosinophils, intermediate cuticular layer and inner germinal layer¹¹. Other conditions can be expected such as syrinx, hematoma, arachnoid cyst, epidermoid cyst, hemangioblastoma, myelomalacia and astrocytoma like cystic neoplasms.

Braithwaite and Lees⁷ classified the spinal disease in five types: 1) primary intramedullary hydatid cyst, 2) intradural extramedullary hydatid cyst, 3) extradural intraspinal hydatid cyst, 4) spinal hydatid cyst disease of the spine and 5) paravertebral hydatid cyst disease. In our case, it was type 4 spinal hydatid cyst diseases of spine.

Clinical manifestation of hydatid cyst usually shows no sign or symptoms other than symptoms related

to cord compression¹². Patient usually presents with chronic continuous lower back pain with radicular pain. Because of pressure over the cord or nerve roots in lower spine, patient may presents with neurological deficit, it can cause paraplegia in 25% to 84% of cases. Symptoms found in our patient were chronic lower back pain since 1 year, with radiculopathy in both lower limbs and now progressing to neurological deficit.

To confirm the diagnosis CT and MRI is necessary to see extant of disease. CT scan is done to see bone involvement and damage to vertebral structures. As hydatid cyst contents has same density as CSF MRI is considered as best imaging tool. The density of the cyst fluid is the same as the CSF in T1-and T2-weighted imaging¹³. Irregular vertebral body margins with non-enhancing flattened sausage shaped lesions, non-septated walls are all signs of vertebral hydatid disease. Cyst lying in extradural area are always multiple involving the bone¹⁴.

Lesion should be completely excised as a block and instrumentation must be performed for stabilization. Location of cyst and surgical area should be washed off with hypertonic saline. Goal of surgery is too remove the cyst without rupturing it, and it should be excised as much as possible without damaging any adjacent structure. However, it is not possible to do complete radical excision of lesion¹⁵. certain solution has not been proved yet, how potent they are, but most of the studies recommend use of (3%,10%,20% hypertonic saline, 0.5% betadine, 0.5% silver nitrate and 2% formalin¹⁰.

Surgical procedure varies from only excision of cyst to complete decompression depending on extant of disease; MRI (magnetic resonance imaging) is performed on regular follow up in post-operative period to ensure that recurrence is picked up, as re-operation is usually required. 30%-40% recurrence rate is found in previous studies¹⁶. Surgery is choice of treatment for spinal hydatid cyst as most of patients present with signs of cord compression, which requires immediate intervention. Surgeons usually act promptly in these cases.

Cyst has a tendency to be burst during the surgery, and its recurrence will exceed to 40% as reported before¹⁷. If there is, a single cyst or solid cyst wall recovery is possible post-surgical excision. It is often been misdiagnosed, a review from one study found that error among radiologists averages from 3% to 5%, in the same study it was noted that 26 % of cases diagnosis is missed clinically¹⁸. For the primary diagnosis of hydatid cyst and for follow up in outpatient department immunodiagnostic is useful. For serodiagnosis of hydatid cyst complement fixation test was the primary immunologic method. Most reliable test for diagnosis of hydatid cyst is Casoni test, which is positive in 90% of cases¹⁹.

CONCLUSION

Hydatid cyst is rare disease involving spine, but it is related to very severe morbidity. Patient with hydatid cyst of spine may end up in neurological deficit. Although it is a rare case but possibility should be suspected in patients with chronic back pain. Cystic lesion in spine diagnosed with help of MRI and CT scan should be considered as hydatid cyst under the differentials until proven.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT

Patient had been informed regarding the study and written consent was taken.

AUTHOR'S CONTRIBUTION

All authors contributed equally in this case study.

REFERENCES

1. Gun E, Efit D, Buyuktalanci DO, Cakalagaoglu F. Unusual locations of hydatid disease: a 10-year experience from a tertiary reference center in Western Turkey. *Ann Diagn Pathol.* 2017;29:37-40.
2. Agnihotri M, Goel N, Shenoy A, Rai S, Goel A. Hydatid disease of the spine: a rare case. *J Craniovertebr Junction Spine.* 2017;8(2):159-160.
3. Abera A, Teklebran T. Study on prevalence and cyst characterization of hydatidosis in cattle slaughtered at Wolayta Soddo Municipal Abbatior. *Int J Res Granthaalayah.* 2017;5(7):60-78.
4. Gezeran Y, Ökten AI, Çavuş G, Açıık V, Bilgin E. Spinal hydatid cyst disease. *World Neurosurg.* 2017;108:407-17.
5. Lakhdar F, Arkha Y, Rifi L, Derraz S, El Ouahabi A, El Khamlichi A. Spinal intradural extramedullary hydatidosis: report of three cases. *Neurosurg.* 2009;65(2):372-377.
6. Sarkar M, Pathania R, Jhobta A, Thakur BR, Chopra R. Cystic pulmonary hydatidosis. *Lung India : official organ of Indian Chest Society.* 2016;33(2):179-191.
7. Braithwaite PA, Lees RF. Vertebral hydatid disease: radiological assessment. *Radiol.* 1981;140(3):763-766.
8. Abedi M, Karimi A, Ghaffari S, Izanloo A, Mirkazemi M. Bone hydatid disease with long term follow-up: a case report. *Arch Clin Infect Dis.* 2018;13(6):e57823
9. Morshed AA. Hydatid disease of the spine. *Neurochirurgia.* 1977;20(06):211-215.
10. Rehman SU, Saeed N, Khan S, Vasenwala SM. A hydatid cyst of the lumbar spine: a rare cause of paraplegia. *Shafa Orthop J.* 2017;4(2)1-2.
11. Lotfinia I, Sayyahmelli S, Mahdkhah A, Shoja MM. Intradural extramedullary primary hydatid cyst of the spine: a case report and review of literature. *Eur Spine J.* 2013;22(3):329-336.
12. Pamir MN, Akalan N, Özgen T, Erbenji A. Spinal hydatid cysts. *Surg Neurol.* 1984;21(1):53-57.
13. Agnihotri M, Goel N, Shenoy A, Rai S, Goel A. Hydatid disease of the spine: a rare case. *J Craniovertebr Junction Spine.* 2017;8(3):291.
14. Fahl M, Haddad FS, Huballah M, Kana'an S, Husheimi I, Azizi T. Magnetic resonance imaging in intradural and extradural spinal echinococcosis. *Clin Imaging.* 1994;18(3):179-183.
15. Salduz A, Koyuncu LO, Dikici F, Talu U. Long-term result of treatment for paraspinal and extradural hydatid cyst: a case report. *Acta OrthopTraumatol Turc.* 2009;43(3):267-271.
16. Fares Y, Khazim R, El Zaatari MM, Haddad GF, Barnes PR. Spinal hydatid disease and its neurological complications. *Scand J Infect Dis.* 2003;35(6-7):394-396.
17. Caglar YS, Ozgural O, Zaimoglu M, Kilinc C, Eroglu U, Dogan I, *et al.* Spinal hydatid cyst disease: challenging surgery-an institutional experience. *J Korean Neurosurg Soc.* 2019;62(2):209-216.
18. Berlin L. Radiologic errors and malpractice: a blurry distinction. *Am J Roentgenol.* 2007;189(3):517-522.
19. Leder K, Weller PF. Clinical manifestations and diagnosis of cystic and alveolar echinococcosis. *UpToDate Online.* 2003; p.17. Available from: <https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-echinococcosis>



STUDENT CORNER**Biological Role of CDK 11 and 12 in Cell Cycle and its Function in Tumorigenesis**Danish Mohammad¹, Rida Tafveez¹, Zain Jawed Abubaker¹, Shamim Mushtaq²¹Student, Ziauddin Medical College, ²Department of Biochemistry, Ziauddin University Karachi, Pakistan.**ABSTRACT**

Uninhibited proliferation and abnormal cell cycle regulation are the hallmarks of cancer. The main role of cyclin dependent kinases is to regulate the cell cycle and cell proliferation. These protein kinases are frequently down regulated or up regulated in various cancers. Two CDK family members, CDK 11 and 12, have contradicting views about their roles in different cancers. For example, one study suggests that the CDK 11 isoforms, p58, inhibits growth of breast cancer whereas, the CDK 11 isoform, p110, is highly expressed in breast tumor. Studies regarding CDK 12 show variation of opinion towards different parts of the body, however there is a consensus that upregulation of cdk12 increases the risk of breast cancer. Hence, CDK 11 and CDK 12 need to be analyzed to confirm their mechanism and their role regarding therapeutics, prognostic value, and ethnicity in cancer. This article gives an outline on both CDKs of information known up to date from Medline, PubMed, Google Scholar and Web of Science search engines, which were explored and thirty relevant researches were finalized.

Keywords: Cyclin-Dependent Kinases; Cancer; Cell Cycle; CDK 11; CDK 12.

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INTRODUCTION

The cell cycle is an essential component of cell-biology that controls cell growth and duplication. Cell cycle control mechanisms by protein phosphorylation involving a highly regulated kinase family called cell cycle regulator or "cyclin dependent kinases (CDKs)". The mechanism of these processes is that cyclins bind to CDKs. As a result, the cyclin/CDK complex phosphorylates a target protein and is then degraded. Development and progression of cancer are usually connected by many changes in the activity of cell cycle regulators. In human, twenty distinct family members of CDKs have been described. CDK 2 plays an important role in replication during S phase¹⁻⁵. However, there does not seem to be much research on other CDKs. For example, two such CDKs are 11 and 12. Research indicates that both CDKs belong to the serine threonine family⁶. There are limited studies on CDK 11 that state that it has important function in cancer cell growth and proliferation⁷. Furthermore, various genetic and epigenetic actions are suspected to

cause increased expression of CDK 11 in certain cancers. Especially for breast cancer, CDK 11 is debated to be both over and under expressed⁸. As for CDK 12, it is considered a transcription related kinase that participates in DNA damage response, splicing, cellular differentiation, and pre mRNA processing⁹. Specifically in breast cancer, it is debated to be overexpressed¹⁰. Therefore, this article will focus on CDK 11 and 12 because of their role in the prognosis of different cancers.

DISCUSSION

The cell cycle is when growing cells replicate their own components and divide into two daughter cells¹¹. There are four stages in the cell cycle, which includes G1, S, G2 and M phase¹². G1 and G2 are resting phases for cell growth. These stages have checkpoints that make sure the cell is prepared to move forward to the next phase. S phase is a stage for DNA replication. M phase is a stage for cell division. These two phases are considered active phases. Dysregulation of different CDKs, such as

CDK11 and 12, can give us a finer understanding on how the cell cycle is affected and how cancer progresses.

CDK 11 has critical roles in different cancers. For example, expression of CDK 11 has major roles in osteosarcoma cell growth¹³. If Cdk11 is inhibited it can reduce the invasion of osteosarcoma cells. Another article states that expression of CDK 11 has a role in melanoma survival by interacting with proto oncogenes, BRAF and nRAS¹⁴. If CDK11 is inhibited it can cause death of melanoma cells. Therefore, CDK11 is involved in multiple cancers.

CDK11 has three different isoforms: p110, p58, and p46⁷. Each isoform has different roles. The p110 isoform is has a major role in pre mRNA splicing¹⁵. The p58 isoform has a major role in centrosome maturation and cytokinesis¹⁶. The p46 isoform has a major role in apoptosis by acting on Ran binding protein and activating caspases¹⁷. Because of these varying functions of different isoforms CDK 11 has

varying effects in different cancers (Table 1).

The major effects have been studied on isoforms p110 and p58. The p110 isoforms effects show that overexpression causes cancer cell growth. For example, in esophageal squamous cell carcinoma cancer cell growth is majorly associated with expression of cdk11 (p110)¹⁸. The mechanism as to how this works is unknown (Figure 1). Furthermore, the p110 isoform is highly expressed in breast tumor tissue⁸. However, the mechanism for this is also unknown. On the other hand, the p58 isoform inhibits the growth of breast cancer¹⁹. This is done by inhibiting mRNA levels of VEGF that reduces angiogenesis of tumor cells. The P58 isomer seems to have other roles besides cancer such as maturation of testis in mice²⁰. It also has a role in apoptosis of neuronal cells after being affected by Lipopolysaccharide²¹. Due to these varying studies, there is a need to have an overall understanding on what affect CDK 11 has on different cancer cell tissues (Figure 2a, b).

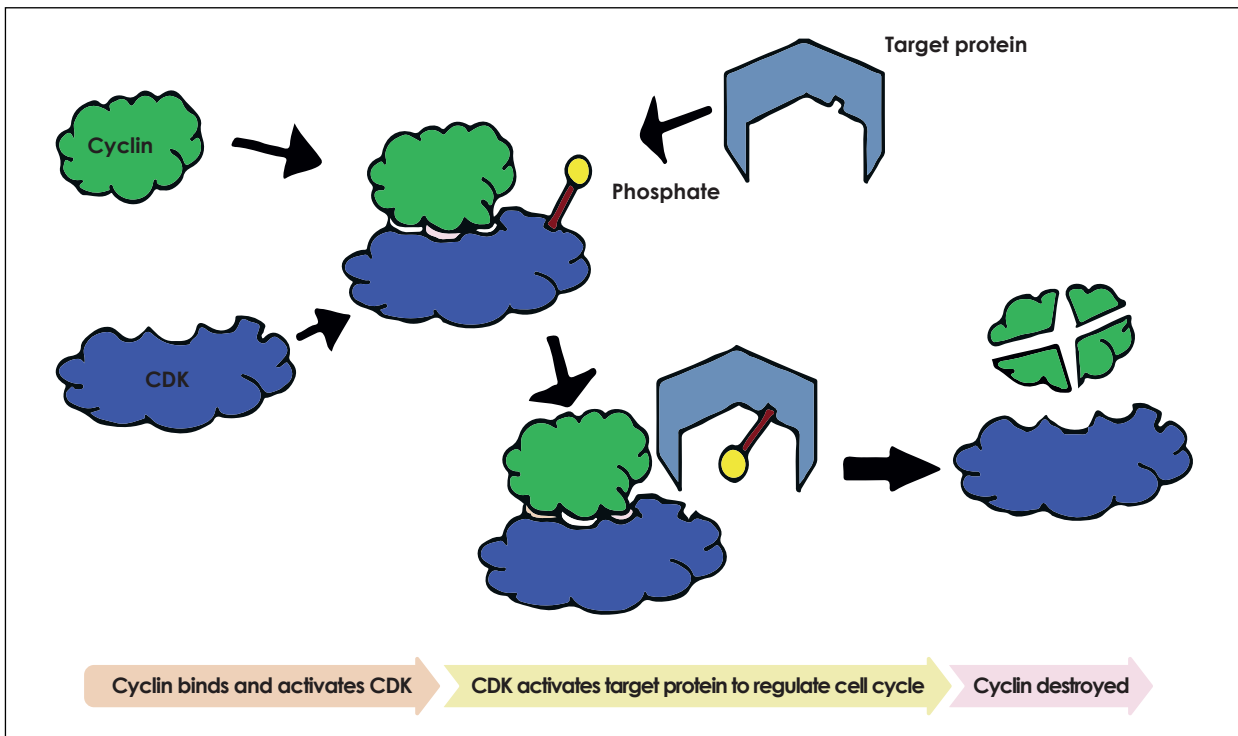


Figure 1: Mechanism of action of CDK with cyclins.

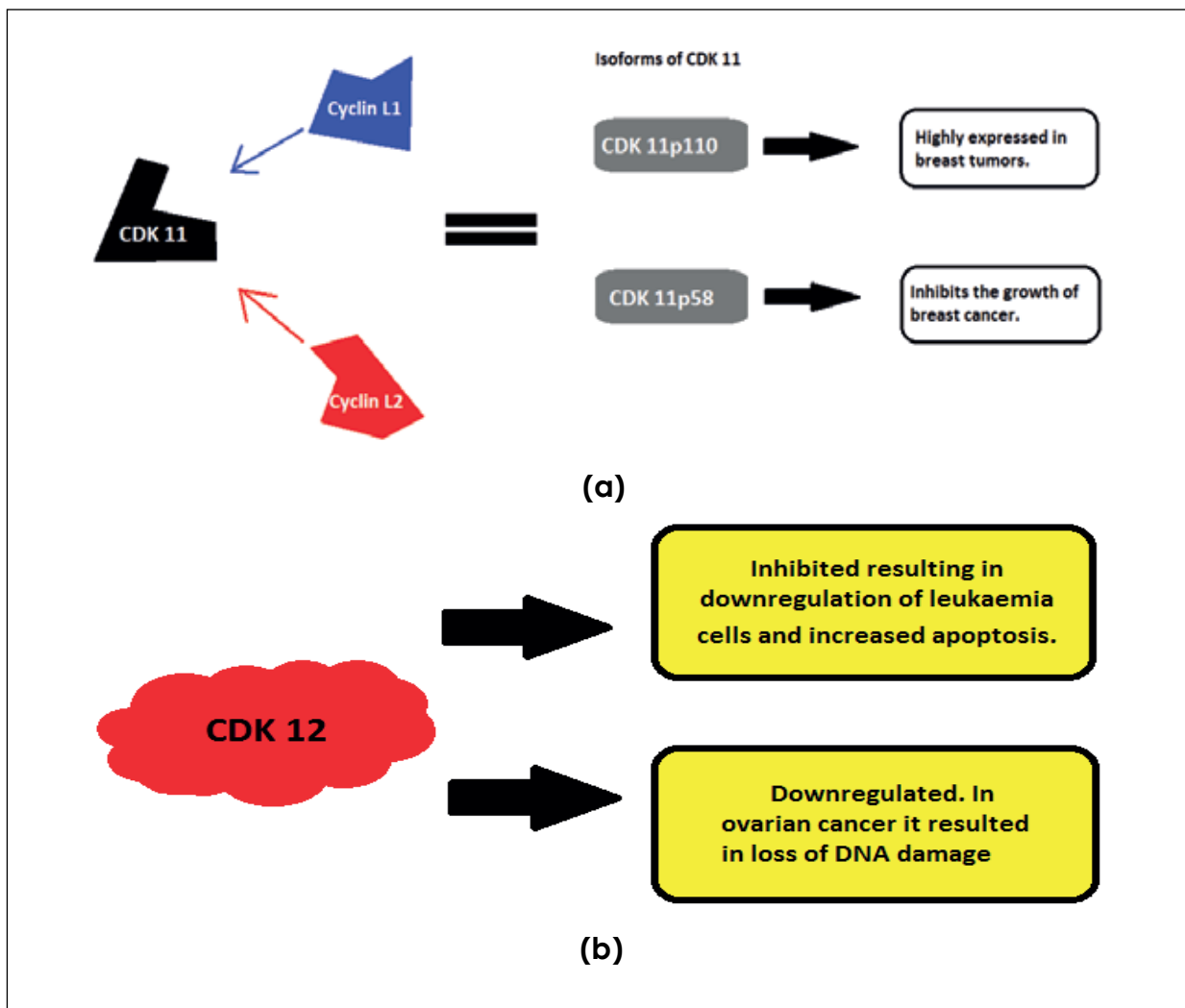


Figure 2: (a) Action of CDK 11 and (b) Action of CDK 12.

Table 1: Studies on CDK 11 and CDK 12 in various cancers.

Study Type	Patient Group	Principle Findings
Clinical Trial ¹⁸	Eight patients with non-small-cell lung carcinoma along with bone metastasis participated.	Out of all the genes responsible for Lung cancer signaling pathways, fibroblast growth factor receptor and CDK12 were the only ones found to be mutated in all eight patients.
Cohort Study ¹⁹	240 patients with ovarian cancer in the late stage.	In the cohort of patients, no genes, ATM, ATR, Chk2 polymorphisms have been found to significantly affect the overall survival and progression.
Clinical trial ²⁰	Patients with circulating tumor cells as well as paired leukocytes with metastatic castration resistant prostate cancer.	All patients with metastatic Castration-Resistant Prostate Cancer (mCRPC) also had resistance to Abiraterone acetate or Enzalutamide (primary or acquired). Furthermore genomic gains were observed to be >25% in many genes including CDK12.

Cohort study ²¹	556 patients of ovarian carcinoma, 760 patients of breast carcinoma as well as 401 patients of prostate carcinoma.	An increased prevalence of interstitial gains is associated with CDK12 inactivation. Tandem duplication results in regular gains in CDK12 mutated ovarian tumors.
Clinical trial ²²	2 patients diagnosed with breast adenoid cystic carcinoma with heterogeneous morphology associated with triple negative breast cancer area of high grade.	In the second patient, three additional mutations were limited to STAG2, KDM6A, and CDK12 to high-grade triple – negative breast cancer.
Cross sectional ²³	105 genomically annotated breast cancers.	Highly phosphorylated kinases associated with amplicon have been identified, CDK12, PAK1, PTK2, RIPK2, and TLK2.
Case Control ²⁴	Immediately after surgical resection, 18 parts of breast tumor samples with adjacent normal tissues were collected. No patients were treated before operation.	In each of the tested breast tumor tissue samples, CDK11p110 was highly expressed as to the normal tissues ($p < 0.01$). Various histological staining characteristics showed high levels of expression of CDK11p110, mainly in BT-474, MCF-7, and MDA-MB-468 cells.
Case control ²⁵	A total of 78 samples of epithelial ovarian cancer.	CDK11 levels in metastatic samples (average 2.08, $p < 0.01$), recurrent samples (average 2.269, $p < 0.01$) were significantly higher than in primary samples (average 1.207)
Case Control ²⁶	For tissue microarrays, 250 FFPE blocks of breast cancer tissues and ANCT have been collected. Thus, 2 tissue cores for breast cancer and 2 ANCT cores from the FFPE blocks of the same patient.	CDK11 levels in metastatic samples (average 2.08, $p < 0.01$), recurrent samples (average 2.269, $p < 0.01$) were significantly higher than in primary samples (average 1.207)

CDK 12 is responsible for responding to DNA damage, splicing, pre-mRNA processing, cellular development, and cellular differentiation²⁷. CDK 12 has contrary views on its function. In one article, inhibiting it resulted in downregulation of leukemia cells and increased apoptosis²⁸. However, in a study on serous ovarian cancer, downregulation of CDK 12 resulted in loss of DNA damage repair²⁹. Down regulation had caused expression of tandem repeats in a patient. This evidence emphasizes that it has varying opinion on different areas of the body. In terms of breast cancer, the consensus seems to be that it increases the chances of breast cancer. According to Mertens et al, amplification of cdk12 is one of the causes to somatic mutations in breast cancer²⁹. The pathology to CDK 12 and breast cancer is unclear. Tien et al hinted that CDK12 has a role in alternative splicing of mRNA

strands, which is considered a factor to invasive cells in breast cancer¹⁰. However, it is important to reassure these results because some articles state that cdk12 has a role in tumor suppression²⁷.

CDKs give a better understanding towards the cell cycle and cancer. If studies figure out where each CDK is over or under expressed during cancer then this information can give clues to provide better treatment such as CDK inhibitors. Even though studies have been done on cdk11 and 12, several of them contradict each other. More studies must be done to confirm certain findings (Figure 3). Moreover, certain studies must be done to see whether certain CDKs being expressed have an ethnic relevance or not. In conclusion, there is information about CDKs, but there are many gaps to be filled.

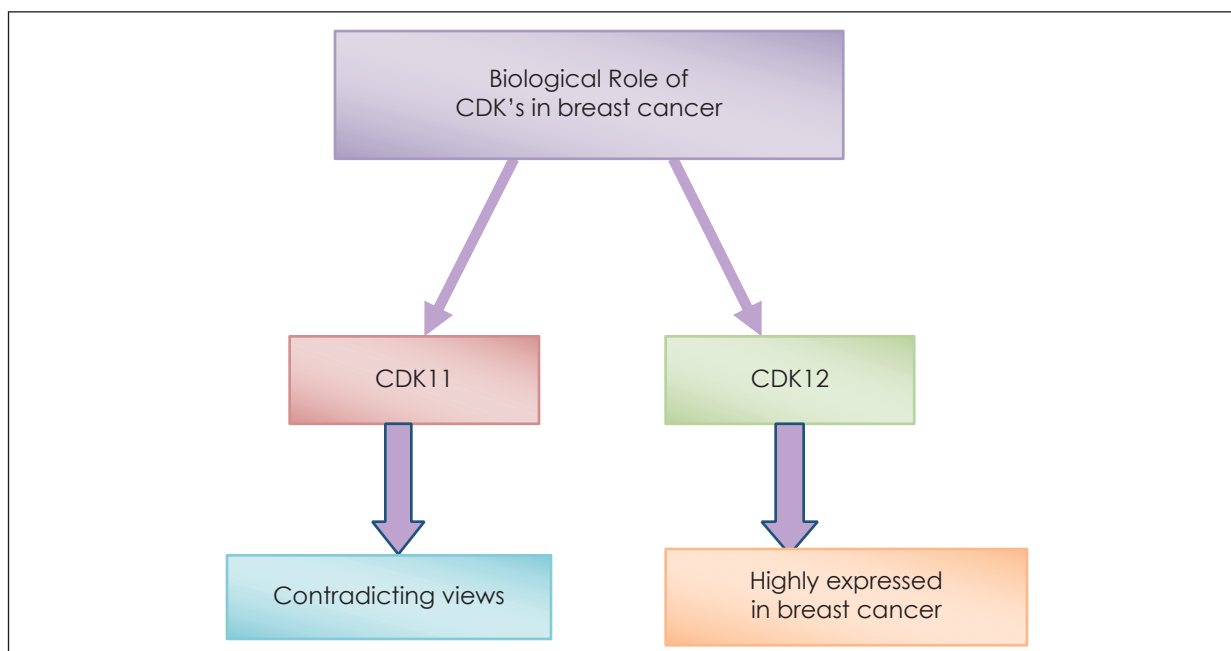


Figure 3: Summarized biological roles of CDK 11 and 12.

CONCLUSION

Studies on CDK 11 and 12 explain that their expression can indicate prognosis for different cancers. These CDKs seem to vary in expression during different cancers. Specifically, for breast cancer, CDK 11 has contradicting views on expression, whereas CDK 12 research seems to lean towards overexpression in breast cancer. Even though this seems to be the trend, findings need to be confirmed on a much larger scale. There could be a chance that ethnicity and region may play a larger role in amounts of expression. Once understood scientist can further act on this information by creating CDK inhibitors to fight cancers. There are already studies on CDK inhibitors, but there has not been much progress. Therefore, cdk11, and 12 must be studied to analyze greater importance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally in this write-up.

REFERENCES

1. Malumbres M. Cyclin-dependent kinases. *Genome Biol.* 2014;15(6):122-132.

2. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. *Trends Biochem Sci.* 2005;30(11):630-641.
3. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discov.* 2015;14:130-146.
4. Finn RS, Aleshin A, Slamon DJ. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. *BCR.* 2016;18(1):17.
5. Lui GYL, Grandori C, Kemp CJ. CDK12: an emerging therapeutic target for cancer. *J Clin Pathol.* 2018;71(11):957-962.
6. Zhou Y, Shen JK, Hornicek FJ, Kan Q, Duan Z. The emerging roles and therapeutic potential of cyclin-dependent kinase 11 (CDK11) in human cancer. *Oncotarget.* 2016;7(26):40846-40859.
7. Zhou Y, Han C, Li D, Yu Z, Li F, Li F, *et al.* Cyclin-dependent kinase 11(p110) (CDK11(p110)) is crucial for human breast cancer cell proliferation and growth. *Scientific Rep.* 2015;5:10433.
8. PaculováH, Kohoutek J. The emerging roles of CDK12 in tumorigenesis. *Cell Div.* 2017;12(1):7-17.
9. Tien JF, Mazloomian A, Cheng SG, Hughes CS, Chow CCT, Canapi LT, *et al.* CDK12 regulates alternative last exon mRNA splicing and promotes breast cancer cell invasion. *Nucleic Acids Res.* 2017;45(11):6698-6716.
10. Hopkins M, Tyson JJ, Novák B, Solomon MJ. Cell-cycle transitions: a common role for stoichiometric inhibitors. *Mol Biol Cell.* 2017;28(23): 3437-3446.
11. Au - Welschinger R, Au - Bendall LJ. Temporal tracking of cell cycle progression using flow cytometry without the need for synchronization. *JoVE.* 2015(102): e52840.

12. Bendris N, Lemmers B, Blanchard JM. Cell cycle, cytoskeleton dynamics and beyond: the many functions of cyclins and CDK inhibitors. *Cell Cycle*. 2015;14(12):1786-1798.
13. Malumbres M. Cyclin-dependent kinases. *Genome Biol*. 2014;15(6):122-130.
14. Kalra S, Joshi G, Munshi A, Kumar R. Structural insights of cyclin dependent kinases: implications in design of selective inhibitors. *Eur J Med Chem*. 2017;142:424-458.
15. Schmitz ML, Kracht M. Cyclin-dependent kinases as coregulators of inflammatory gene expression. *Eur J Med Chem*. 2016;37(2):101-113.
16. Chi Y, Huang S, Peng H, Liu M, Zhao J, Shao Z, *et al*. Critical role of CDK11(p58) in human breast cancer growth and angiogenesis. *BMC Cancer*. 2015;15:701-711.
17. Zhang K, Zhang M, Zhu J, Hong W. Screening of gene mutations associated with bone metastasis in nonsmall cell lung cancer. *J Cancer Res Ther*. 2016;12(7):186-190.
18. Guffanti F, Fruscio R, Rulli E, Damia G. The impact of DNA damage response gene polymorphisms on therapeutic outcomes in late stage ovarian cancer. *Sci Rep*. 2016;6:38142.
19. Gupta S, Li J, Kemeny G, Bitting RL, Beaver J, Somarelli JA, *et al*. Whole Genomic Copy Number Alterations in Circulating Tumor Cells from Men with Abiraterone or Enzalutamide-Resistant Metastatic Castration-Resistant Prostate Cancer. *Clin Cancer Res*. 2017;23(5):1346-1357.
20. Ekumi KM, Paculova H, Lenasi T, Pospichalova V, Böskén CA, Rybarikova J, *et al*. Ovarian carcinoma CDK12 mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex. *Nucleic Acids Res*. 2015;43(5):2575-2589.
21. Fusco N, Geyer FC, De Filippo MR, Martelotto LG, Ng CKY, Piscuoglio S, *et al*. Genetic events in the progression of adenoid cystic carcinoma of the breast to high-grade triple-negative breast cancer. *Mod Pathol*. 2016;29:1292-1305.
22. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, *et al*. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature*. 2016;534:55-62.
23. Zhou Y, Han C, Li D, Yu Z, Li F, Li F, *et al*. Cyclin-dependent kinase 11p110 (CDK11p110) is crucial for human breast cancer cell proliferation and growth. *Sci Rep*. 2015;5:10433.
24. Liu X, Gao Y, Shen J, Yang W, Choy E, Mankin H, *et al*. Cyclin-Dependent Kinase 11 (CDK11) Is Required for Ovarian Cancer Cell Growth In Vitro and In Vivo, and Its Inhibition Causes Apoptosis and Sensitizes Cells to Paclitaxel. *Mol Cancer Ther*. 2016;15(7):1691-1701.
25. Chi Y, Huang S, Wang L, Zhou R, Wang L, Xiao X, *et al*. CDK11p58inhibits ERa-positive breast cancer invasion by targeting integrin β 3 via the repression of ERa signaling. *BMC Cancer*. 2014;14(1):577-787.
26. Paculova H, Kohoutek J. The emerging roles of CDK12 in tumorigenesis. 2017;12:7-17.
27. Zhang T, Kwiatkowski N, Olson CM, Dixon-Clarke SE, Abraham BJ, Greifenberg AK, *et al*. Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors. *Nat Chem Biol*. 2016;12(10):876-884.
28. Popova T, Manie E, Boeva V, Battistella A, Goundiam O, Smith NK, *et al*. Ovarian cancers harboring inactivating mutations in CDK12 display a distinct genomic instability pattern characterized by large tandem duplications. *Cancer Res*. 2016;76(7):1882-1891.
29. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, *et al*. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature*. 2016;534(7605):55-62.



MEDICAL EDUCATION

Perception about Case Based Learning among Medical Students of a Public Sector Medical University in Karachi

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ABSTRACT

Background: There has been a shift in medical education from didactic teaching to small group teaching which stresses on the importance of student active participation in problem-solving and critical thinking. We aimed to evaluate perception about Case Based Learning (CBL) as a learning strategy among medical students.

Methods: A cross-sectional study was conducted in Jinnah Sindh Medical University, Karachi. A structured questionnaire was distributed to 300 male and female students of 1st and 3rd semester, MBBS. Data was entered and analyzed by using Statistical Package of Social Sciences (SPSS) version 25. The Independent Samples t-test was used and $p < 0.05$ was considered statistical significant.

Results: We reported an increase in the perception regarding CBL from Cambridge students and a reduced scoring of CBL in the non-Cambridge group ($p > 0.05$). There was a significant difference in the perception score about CBL obtained from O-Level students (34.1 ± 4.4) and Matric systems (35.8 ± 2.9) ($p = 0.02$) in the first semester, however, it was not significant in the 3rd semester ($p > 0.05$). A significant difference between the A-Level (33.3 ± 5.4) and intermediate (35.8 ± 2.8) college systems was observed regarding the opinion about CBL ($p = 0.009$) in the first semester as compared to third semester but statistically insignificant.

Conclusion: Students tend to have better perception about CBL at the entry level. The educational background of students influences the perception. However, learning environment and quality of facilitation may influence their perception and opinion regarding CBL. It is therefore critical to consider the quality of facilitations and learning environment along with CBL.

Keywords: Education; Medical Students; Perception; Undergraduate.

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INTRODUCTION

Medical education has progressed rapidly in the past few decades with the improvement in scientific knowledge revolutionizing the medical curricula, from didactic teaching to small group teaching¹. The small group learning technique is motivated by the importance of student's energetic involvement in learning, building up their own ideas, rationales, and developing novel notions, grounded on their existing

understanding of the topic, testing their hypotheses and their application and integration of those ideas into future circumstances¹.

Case-based learning (CBL) is defined as a directed probing strategy where the teacher utilizes pre-defined queries to guide the students back to the main learning objectives. CBL also offers a more defined and structured discussion using small group methodology. The foremost objective of the imple-

enter of the CBL is to support the students in micro-analyzing the problem statement, helping them develop new strategies or the solutions¹⁻². In a Case-based learning format, the students study the given topic first and then solve the problem in the class. The CBL approach works best when applied to a small group, where it has shown to have a substantial positive effect on the overall learning and academic outcome of students³.

Srinivasan et al. claimed that in contrast to problem-based learning (PBL), case-based learning (CBL) demanded preparation in advanced by the students and offers more structured learning to the students⁴. This strategy helps students to develop critical thinking, develop their ability to correlate so that they can integrate their knowledge and share among themselves and facilitators to get clear understanding of the topic^{1,4}.

In Pakistan, students in medical schools are still following the traditional teaching methods and only a few institutions have started to incorporate CBL in their curricula as well upon the insistence of Pakistan Medical and Dental Council (PMDC) to adopt CBL for undergraduate curriculum⁵⁻⁶. After the recommendation of the Medical Council of India, a medical college in North India adopted Case based learning for students to develop logical scientific reasoning and ability to gather and analyze information^{7,8}. CBL also allows students to develop a collaborative team based approach to their education. Learners and facilitators favor CBL as it helps in development of clinical skills but it is necessary to have a stronger and deeper fundamental knowledge when following CBL as a learning modality along with trained faculty to lead discussion⁹.

Case based learning (CBL) is a student-centered, facilitator-led approach of learning which have been reported as more effective than traditional¹⁰. Jinnah Sindh Medical University (JSMU) adopted a case based learning strategy recently. As Jinnah Sindh Medical University is a public sector university, most of the students belong to the Matric/Intermediate school system where case based teaching system has not yet been introduced. Whereas, relatively very few students belong to the Cambridge schooling system, where students are already exposed to problem or case-based learning techniques. The present study aimed to assess the perception of 1st and 3rd semester students of MBBS on the application of Case Based Learning system as a part of their MBBS curriculum.

METHODS

An observational cross-sectional study was conducted at the Department of Community Medicine, Jinnah Sindh Medical University, Karachi, Pakistan. After obtaining the ethical approval from the institutional review board committee, 350 students from 1st semester and 3rd semester MBBS were enrolled in the study.

Year back students and those who did not give consent to participate in the research study were excluded from the study.

After taking written informed consent, a structured questionnaire was distributed among the students of 1st semester and 3rd semester students of MBBS. The questionnaire was distributed to all the available students. It was distributed and collected by the principal investigator at the end of the lecture. The content validity of the questionnaire was established by getting feedback from four experienced persons in medical education through the internet. A pilot study was done on a group of ten students before finalizing the questionnaire. The reliability of the questionnaire was determined by using Cronbach's alpha for identifying the internal consistency of the responses. The mean scores for the perception and opinion of participants about the CBL system were recorded.

Data was entered and analyzed by using Statistical Package of Social Sciences (SPSS version 25). Mean±SD was used for the numerical variables. Categorical data was represented in frequency or percentages. The Independent Samples t-test was used to compare the perception of the respondents by gender and type of pre-medical schooling system. The statistical significance was taken at $p < 0.05$. The internal reliability of instrument items was determined through Cronbach's alpha. A Cronbach's alpha value of 0.68 and 0.73 was obtained for perception and opinion about benefits of CBL respectively¹⁰.

RESULTS

A total of 300 students participated from 1st and 3rd Semester, 150 each. The mean age of the study participants from 1st semester and 3rd semester was 18.3 ± 0.9 years and 19.3 ± 3.2 years respectively. Among the 1st semester students, 12 (8%) students completed their higher secondary education in a Cambridge schooling system while 138 (92%) students belonged to the Board of Intermediate Education (BIEK) system. Among the 3rd semester students, 20 (13.3%) belonged to the A-levels, Cambridge, while 128 (85.3%) belonged to BIEK.

The responses of the students regarding their perception and opinions about CBL are shown in Table 1. Almost all 149 (99%) students of first semester and 100 (66.7%) students of 3rd semester agreed that CBL triggers their prior knowledge, 148 (98%) student of first semester and 78 (52%) students of third semester agreed that CBL improves their ability for understanding the case. Responding to the question that CBL helps in developing diagnostic skills, 142 (95%) of first semester and 86 (57.3%) agreed with the statement. Responding to the question that CBL has no benefit, 143 (95%) of first semester and 59 (39.3%) of third semester students disagreed that they do not find any benefit of using CBL strategy for learning (Table 1).

Table 1: Perception and opinions of students (1st and 3rd semester) about case based learning (CBL) (n=150).

Perception about CBL								
Perceptions	Disagree n (%)		Neither Agree nor Disagree n (%)		Agree n (%)			
	1 st	3 rd	1 st	3 rd	1 st	3 rd	1 st	3 rd
There is no benefit of CBL	143 (95.3%)	59 (39.3%)	5 (3.3%)	14 (9.3%)	2 (1.3%)	8 (5.3%)		
CBL triggers prior knowledge	---	8 (5.3%)	1 (0.7%)	9 (6%)	149 (99.3%)	100 (66.7%)		
CBL improve my ability for understanding the case	1 (0.7%)	3 (2%)	1 (0.7%)	15 (10%)	148 (98.7%)	78 (52%)		
CBL helps in developing diagnostic skills	2 (1.3%)	7 (4.7%)	6 (4%)	10 (6.7%)	142 (94.7%)	86 (57.3%)		
I enjoy CBL	1 (0.7%)	13 (8.7%)	8 (5.3%)	26 (17.3%)	141 (94%)	67 (44.7%)		
CBL methods is useful strategy for life-long learning	3 (2%)	15 (10%)	15 (10%)	31 (21.7%)	132 (88%)	65 (43.3%)		
CBL helps me in developing lateral thinking	1 (0.7%)	4 (2.7%)	18 (12%)	32 (21.3%)	131 (87.3%)	81 (54%)		
CBL increases my self - confidence in clinical reasoning	2 (1.3%)	10 (6.7%)	20 (13.3%)	37 (24.7%)	128 (86.3%)	69 (46%)		
Opinion about CBL								
Options	Less than half n (%)		About half of the Time n (%)		Most of the Time n (%)		Always n (%)	
	1 st	3 rd	1 st	3 rd	1 st	3 rd	1 st	3 rd
The discussion in CBL helps me to understanding of the topic	1 (0.7%)	2 (1.3%)	6 (4%)	33 (22%)	100 (67%)	101 (67%)	43 (28.7%)	14 (9.3%)
The discussion session facilitate interaction between teachers and students	1 (0.7%)	22 (14.6%)	13 (8.7%)	30 (20%)	44 (29%)	71 (47.3%)	92 (61.3%)	27 (18%)
I collect new information after every CBL session	2 (1.3%)	25 (16.6%)	13 (8.7%)	41 (27.3%)	55 (37%)	54 (36%)	79 (53%)	30 (20%)
The cases presented are interesting	0	19 (12.7%)	12 (8%)	50 (33.3%)	72 (48%)	63 (42%)	66 (44%)	18 (12%)

The cases presented involve several disciplines	4 (2.7%)	18 (12%)	19 (13%)	57 (38%)	79 (53%)	62 (41%)	48 (32%)	13 (8.7%)
Through CBL, we have more efficient use of our time	3 (2%)	13 (8.7%)	13 (8.7%)	56 (37.3%)	51 (34%)	62 (41.3%)	83 (55.3%)	19 (13%)
CBL provides more opportunity for participation	2 (1.3%)	17 (11.3%)	9 (6%)	29 (19.3%)	46 (31%)	62 (41.3%)	93 (62%)	42 (28%)

Table 1 showed the responses of the students' opinion (effect of perception) about the benefits of CBL; 43 (28.7%) of first semester students and 14 (9.3%) responded that the discussion in CBL always help them to understand the topic. When asked does discussion sessions facilitate interaction between teachers and students, 92 (61.3%) of first semester and 27 (18%) students affirmed the efficacy of CBL in enhancing the facilitator and student interaction. There were 79 (53%) of first semester and 30 (20%) of third semester medical students who agreed that CBL sessions help them collect new information every time.

The mean score of students' perception of CBL was 35.7 ± 3.2 and maximum possible score was 40. Similarly, the mean score of the students for the

opinion about benefits of CBL was 23.7 ± 2.9 and maximum was 28. The 1st year students from secondary school education (O-Level) had a mean score of 34.1 ± 4.5 for the perception about CBL as compared to students from the Matric school system score of 35.9 ± 2.9 ($p=0.02$) (Table 2). No such significance was observed in 3rd year students. No significant difference was observed in the opinion of benefits of CBL between the students from O-Level or Matric schooling system. However, the mean score of 1st semester students from A-Level group was 33.3 ± 5.4 which was significantly lower as compared to the score of students from Intermediate Board which was 35.9 ± 2.9 ($p=0.009$). However, for the 3rd semester students no significant difference between the groups was observed ($p>0.05$) (Table 2).

Table 2: Assessment of 1st and 3rd semester CBL Score with various factors.

Assessment of 1 st Semester CBL Score				
Variables	Score of Students Perception about CBL (n= 40)		Score of Students' opinion about benefits of CBL (n=28)	
	Mean \pm SD	p-value	Mean \pm SD	p-value
Overall Mean Score	35.7 ± 3.2		23.7 ± 2.9	
Male	35.7 ± 2.9	0.88	23.6 ± 2.9	0.96
Female	35.6 ± 3.3		23.7 ± 2.9	
Secondary School O-Level (n=20)	34.1 ± 4.5	0.02	22.9 ± 3.0	0.19
Matric (n=129)	35.9 ± 2.9		23.8 ± 2.8	
Higher School A-Level (n=12)	33.3 ± 5.4	0.009	22.6 ± 3.4	0.18
Intermediate (n=138)	35.9 ± 2.9		23.7 ± 2.8	
Assessment of 3 rd semester CBL Score				
Overall Mean Score	35.7 ± 3.2		23.7 ± 2.9	
Male	19 ± 3.4	0.86	17.1 ± 3.6	0.096
Female	19.1 ± 3.7		18.2 ± 3.4	
Secondary School O-Level (n=20)	20.3 ± 4.3	0.06	17.5 ± 3.9	0.54
Matric (n=129)	19 ± 3.4		17.9 ± 3.4	
Higher School A-Level (n=12)	19.1 ± 4.1	0.98	18.2 ± 3.4	0.83
Intermediate (n=138)	19 ± 3.6		18 ± 3.5	

Independent sample t-test shows no significant difference ($p > 0.05$) between the perception and opinions of CBL in two groups i.e. Cambridge and non-Cambridge. Independent sample t-test on the data shows significant increase ($*p < 0.05$) in the positive perception of CBL in 3rd semester. Similarly, when independent sample t-test was applied, a significant increase ($*p < 0.05$) in a positive opinion regarding CBL was observed among the 3rd semester students which were exposed to the CBL system as compared to 1st year students who had no exposure of CBL system as of yet.

DISCUSSION

Case based learning (CBL) is student-centered, interactive and instructor-led learning approach. To implement CBL students and teachers are required to be very much motivated. It is a good method of teaching, exciting, motivating, practical and effective strategy^{11,12}. The present study evaluated the difference of perception and opinions of Case Based Learning (CBL) as an effective learning strategy of 1st year MBBS students of Jinnah Sindh Medical University, in context of gender and students coming from O/A levels and Matric/Intermediate schools.

Study by Joshi¹³ also reports that case based learning is motivating and interesting similar to our study showing about half of the students confirming CBL as interesting and motivating. Flynn and Klein¹⁴ also report that students enjoy more while working in groups rather than be alone, supporting our results in which more than half of the students said that they enjoy small group sessions as in CBL¹⁴. The results were supported by another study by Jesus et al, which reports that case based learning session helps in improving interaction and increased participation in discussions¹⁵. The results confirmed that CBL is interesting and helped learners in improving their educational performances and motivated them to actively participate¹⁶. This study showed that Case based learning promotes active learning, which is also supported by other studies^{17,18}. There also exist some studies which have shown no preference for CBL by students who thought CBL had no benefits^{5,19}. While another study on CBL perception found no significant differences in perception regarding CBL²⁰.

Interestingly, CBL facilitates improved communication skills, better problem solving skills and increased motivation consistent with our findings²¹. Scott²² observed similar findings where case based learning resulted in acquiring improved problem solving skills. We consistent with other studies²³ have observed student satisfaction and positive effect on their learning. The study did not find any significant difference between the male and female students regarding their perception of CBL and their opinion about benefits of CBL. While assessing

students of secondary education about perception of CBL, the Matric system students had a higher score regarding the perception about CBL, but there was no significant difference between the O-level and Matric students regarding their opinion about benefits of CBL. There was also no difference between the A-level and Intermediate system students regarding their perception about CBL as well as about their opinion about benefits of CBL.

It was interesting to observe a decline in overall scoring of perception by 3rd year students. The decline in the overall scoring may be due to the following factors: The sample group from 3rd semester was of the same as that of the first semester. This difference is observed due to several factors including absenteeism. In addition, the non-availability of the same group is due to the preference of students for a particular session or a particular facilitator as the data was collected immediately after an on-going session. Moreover, the faculty involved in taking sessions is not usually the same therefore; the students may reflect high scoring for a particular facilitator and session in mind. While the same facilitator for a different subject may influence the scoring on perception and opinion.

We may not ignore the influence of educational background on the overall scoring of CBL. It was surprising to observe a positive perception and opinion regarding CBL by non-Cambridge students, since these students are not previously exposed to such learning strategy. A similar study by Baher-Horenstein²⁴ has recognized the effectiveness of CBL in developing ownership of learning and increases active participation. Hashim et al.⁵ have shown that students preferred case based learning. Vora et al.⁶ observed a positive effect by case based learning in developing critical thinking and self-learning approach. In accordance with Gade et al²⁵.

CONCLUSION

The results of this study showed that the CBL is motivating, increases interaction among themselves and with teachers, increased participation and efficient use of time and helps in developing critical thinking. Further studies are needed to address the issues with more extensive data. More comparative group studies should be conducted in future.

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CONFLICT OF INTEREST

There is no conflict of interest reported in the study.

AUTHORS' CONTRIBUTION

The author designed, directed and coordinated the study, created study design, analyzed the data, studied and drafted the article.

REFERENCES

- Mir MM, Jeelani M, Alshahrani MS. A practical approach for successful small group teaching in medical schools with student centered curricula. *J Adv Med Educ Prof.* 2019;7(3):149-153.
- Lee MH, Park MS. The effect of case-based learning (CBL) on critical thinking disposition, communication ability, problem solving ability and self-directed learning ability of nursing students in pathophysiology course. *J Korean Biol Nurs Sci.* 2016;18(3):176-184.
- Suhasini P, Joshi KP, Swaroopachary RS, Yamini D, Sarma DV. An effective approach in learning clinical biochemistry-Case Based Learning. *J Educ Technol Health Sci.* 2017;4(2):62-64.
- Srinivasan M, Wilkies M, Stevenson F, Nguyen T, Slavin S. Comparing problem-based learning with case-based learning: effects of a major curricular shift at two institutions. *Acad Med.* 2007; 82:74-82.
- Hashim R, Azam N, Shafi M, Majeed S, Ali S. Perceptions of undergraduate medical students regarding case based learning and tutorial format. *J Pak Med Assoc.* 2015;65(10):1050-1055.
- Vora MB, Shah CJ. Case-based learning in pharmacology: Moving from teaching to learning. *Int J Appl Basic Med Res.* 2015; (1):521-523.
- Bhardwaj P, Bhardwaj N, Mahdi F, Srivastava JP, Gupta U. Integrated teaching program using case-based learning. *Int J Appl Basic Med Res.* 2015; (5): S24-S28.
- Medical Council of India [Internet]. Salient features of regulations on graduate medical education 1997; 2018 [updated 2018 May 30, cited 2020 June 5]. Available from: <http://www.mciindia.org/RulesandRegulations/GraduateMedicalEducationRegulations1997>
- Nadershahi N, Bender DJ, Back L, Lyon C, Blaseio A. An Overview of case-based and problem-based learning methodologies for dental education. *J Dent Educ.* 2013;(77):1300-1305.
- Ramnanan CJ, Pound LD. Advances in medical education and practice: student perceptions of the flipped classroom. *Advan Med Educ Pract.* 2017;8:317-320.
- Dalal EA, Kaja R. Evaluating Case-Based Learning versus traditional method of teaching-learning for undergraduate medical student: a comparative study. *J Res Med Educ Ethics.* 2019;9(1):43-50.
- Nasir J, Goldie J, Little A, Banerjee D, Reeves S. Case-based interprofessional learning for undergraduate healthcare professionals in the clinical setting. *J Interprof Care.* 2017; 31(1):125-128.
- Joshi KB, Anup N, Nilawar, Thorati AP. Effect of case based learning in understanding clinical biochemistry. *Intl J Bio Med Adv Res.* 2014;5(10):516-518.
- Flynn AE, Klein JD. The influence of discussion groups in a case based learning environment. *ETRD.* 2001; 49(3):71-86.
- Jesus A, Cruz A, Gomes MJ. Case based learner-centered approach to Pharmacotherapy. *Proc EDULEARN11 Confer.* 2011;74-79.
- Nair SP, Shah T, Seth S, Pandit N, Shah GV. Case based learning: A method for better understanding of biochemistry in medical students. *J Clin Diagn Res.* 2013;7(8):1576-1578.
- Surapaneni KM. The effect of integrated teaching with case based learning (CBL) in the Biochemistry of undergraduate medical curriculum. *J Clin Diagn Res.* 2010; 5:3058-3063.
- Meyers C, Jones TB. Case studies in: promoting active learning: Strategies for the college classroom. San Francisco, CA: Jossey-Bass. 1993;103-119.
- Mayo JA. Using case-based instruction to bridge the gap between theory and practice in psychology of adjustment. *J Constr Psychol.* 2004; 17:137-146.
- Dupuis RE, Persky AM. Use of case based learning in a clinical Pharmacokinetics course. *Am J Pharm Educ.* 2008; 72(2): 29-36.
- Yoo MS, Park HR. Effects of case-based learning on communication skills, problem-solving ability, and learning motivation in nursing students. *Nurs Health Sci.* 2015; 17(2):166-172.
- Scott TM. A case-based anatomy course. *Med Educ.* 1994; 28(1):68-73.
- Curran VR, Sharpe D, Forristall J, Flynn K. Student satisfaction and perceptions of small group process in case -based inter-professional learning. *Med Teach.* 2008;30(4):431-433.
- Behar-Horenstein LS, Catalanotto FA, Nascimento MM. anticipated and actual implementation of case-based learning by dental faculty members during and after training. *J Dent Educ.* 2015;79(9): 1049-1060.
- Gade S, Chari S. Case-based learning in endocrine physiology: an approach toward self-directed learning and the development of soft skills in medical students. *Advan Physiol Educ.* 2013; 37(4):356-360.

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