European Journal of Medicine

Has been issued since 2013.

E-ISSN 2310-3434
2023. 11(1). Issued once a year

Journal is indexed by: **CAS Source Index** (USA), **CiteFactor** (Canada), **CrossRef** (UK), **EBSCOhost Electronic Jornals Service** (USA), **Electronic scientific library** (Russia), **Open Academic Journals Index** (USA), **ResearchBib** (Japan), **Sherpa Romeo** (Spain).

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Postal Address: 1717 N Street NW, Suite 1, Washington, District of Columbia, USA 20036

Website: https://ejm.cherkasgu.press
E-mail: office@cherkasgu.press

Founder and Editor: Cherkas Global University

Release date 16.06.23
Format 21 × 29,7/4.

Headset Georgia.

Order № 26.

European Journal of Medicine

2023

Is. 1

C O N T E N T S

Articles

Published in the USA
European Journal of Medicine
Has been issued since 2013.
E-ISSN: 2310-3434
2023. 11(1): 3-18

DOI: 10.13187/ejm.2023.1.3
https://ejm.cherkasgu.press

Articles

Diagnostic Cytopathology of Human Buccal Mucosa Neoplasm

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Abstract

The objective of the present study is to investigate the frequency of pleomorphic cytological atypias in the exfoliated cytosmears of human buccal mucosa neoplasms (HBMN), pattern of cervical lymph node (CLN) metastasis and to establish its role as a diagnostic criterion for early detection of oral cancer.

In a hospital-based case-control study, a total of 126 subjects (63 HBMN cases and 63 healthy individuals) were included. Two scalpel-scraped exfoliated cytosmears were collected from the affected site of the buccal mucosa and were fixed in aceto-alcohol (1:3) immediately. Cytosmears were stained with Papanicolaou's stain and Giemsa's stain. *Cat Cam 1.30* (*1.3 Mega Pixel*) microscope camera was used for phptomicrography. Software package Palaeontological Statistics (PAST) ®, Version 2.17 was used for statistical analysis.

Cytological pleomorphism was well observed exhibiting a number atypias in the exfoliated cytosmears of HBMN. The frequencies of typically atypical cells like KSC, KTC, KSC-A, KFC, KRC, PKSC, MNC and NMSCs are observed to be directly correlated with the degree of pathogenicity at different stages of oral carcinogenesis. Diagnostically, the Sensitivity was calculated to be 93.1 % and Specificity was 100 %, Positive Predictive Value (PPV) was 100 %, Negative Predictive Value (NPV) was 55.6 % and the accuracy was 93.7 %.

Presence of any such cytological atypias in the exfoliated cytosmears of HBMN indicates the state of malignancy and thus, the finding is practically helpful in determining the stage of the HBMN. However, the role of tobacco and alcohol in connection to unusual CLN metastasis with particular reference to the HBMN needs further research.

Keywords: exfoliated cytosmear, Buccal mucosa neoplasm, carcinoma, cytological atypia.

1. Introduction

Squamous cell carcinoma of human buccal mucosa (SCCHBM) is a more common form of oral cavity cancer in South-east Asia than in North America and Western Europe. SCCHBM is relatively uncommon in the developed countries, as compared to the Indian subcontinent (Parkin et al., 2001). In India, it is the most common cancer in men and the third most common cancer in women which accounts for up to one-third of all tobacco-related cancers (Agrawal et al., 2016).

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The higher rate of buccal mucosa carcinoma (BMC) in Asia is likely related to the widespread practice of betel nut chewing. Betel nut, composed mainly of the cut-pieces of the areca nut mixed with processed tobacco, is either placed along the side of buccal mucosa or chewed slowly to induce a feeling of euphoria. Buccal nucosa carcinoma related to betel nut chewing tends to develop at an earlier age, with most cases occurring between the ages of 40-70. In addition to the chewing of betel nut, the high incidence of SCCHBM in India is also attributable to the chewing and smoking of tobacco and drinking of alcohol (Lin et al., 2005).

The buccal mucosa includes all of the mucosa of the inner surface of the cheek from the mouth angle (lips) to the attachment of the mucosa of the upper and lower alveolar ridges and the pterygo-mandibular raphe, which is a continuation of the retromolar trigone. About 30-80 % loco-regional recurrence in patients with buccal mucosa carcinoma is the main cause of treatment failure (Lapeyre et al., 1995; Strome et al., 1999). There is a strong influence of overall disease stage, along with tumor size, nodal status, final histopathological report and habits of tobacco and betel quid chewing on prognosis, emphasizes the importance of early diagnosis and prevention of BMC and aggressive treatment for patients with advanced stage cancer (Singhania et al., 2015).

Despite various technological advancement and implementation of sophisticated methods, exfoliative cytopathology has primarily been accepted as an important tool for early detection of oral cancer. Due to its simplicity, reliability and economically affordability (with respect to time and money), there is a growing interest on oral exfoliative cytopathology world-wide. Mohanta et al. (2009) have reported that cytological pleomorphism is a common and well observed feature in oral squamous cell carcinoma (OSCC). However, site specific cytological pleomorphisms in oral cavity are not reported so far. Therefore, an attempt has been undertaken to investigate the frequency of pleomorphic cytological atypias in the exfoliated cytosmears of human buccal mucosa neoplasms (HBMN), pattern of cervical lymph node (CLN) metastasis and to establish its role as a diagnostic criterion for early detection of oral cancer.

2ıo Methodology

The subjects

In a hospital-based study, out of 136 oral cases, 63 (46.32 %) HBMN cases (37 males and 26 females) registered at the Out-patient Department of Acharya Harihar Regional Cancer Centre (AHRCC), Cuttack, Odisha, India during May 2007 to May 2009 were included. All the patients were referral cases, belonging to different regions of Odisha State. They had undergone neither chemotherapy nor radiotherapy earlier. Detailed case-history including age, sex, oral site, gross clinical pathogenicity, the nature, types of addiction and occupation of each individual was recorded prior to the collection of samples. Among them, 56(88.9 %) were addicted to different forms of tobacco and alcohol for more than 15 years and the rest 7(11.1 %) were non-addicted. Age-group, site and sex matched non-addicted 63 healthy individuals were also included in this study as Control group. Thus, a total of 126 subjects were taken into account for this study.

Collection of Samples, Fixation and Staining

A written consent of the respective subject was obtained prior to the collection of sample. Two scalpel-scraped exfoliated cytosmears were collected from the affected site of the buccal mucosa on the pre cleaned-coded glass-slides. Collected cytosmears were fixed in 1:3 aceto-alcohols (1 part of glacial acetic acid and 3 parts of ethyl alcohol) immediately. A set of smears was stained with Papanicolaou's stain and the other set was counterstained with Giemsa's stain for cytopathological analysis. Stained slides were observed under Hunds-H500 light microscope fitted with a computer assisted *Cat Cam 1.30* (*1.3 Mega Pixel*) microscope camera (Catalyst Biotech®, Maharashtra, India). Photomicrographs were taken out as the supporting evidences.

Statistical Analysis

Out of 1000 observed cells, the cytological atypias were scored. Software package Palaeontological Statistics (PAST) ®, Version 2.17 was used for statistical analysis. Z-test was carried out for the test of significance at 1 % ($p < 0.01$) level of confidence.

Ethical considerations

This study was approved (Reference No EC/UU-38832/2007) by the Subject Research Committee (SRC) of Utkal University, Bhubaneshwar, Odisha, India and necessary permission from the Director, AHRCC, Cuttack, Odisha, India was also obtained for the same purpose.

3. Results

The cases: clinical aspects

Out of 63 collected samples, 18 (28.6 %) cases were with premalignant lesion and 45 (71.4 %) were malignant. Four (10.8 %) male and 5(19.2 %) female were suffering from leukoplakia. Seven (18.9 %) male and 2(5.5 %) female were with erythroplakia. Out of 45(71.4 %) malignant cases, 26 (70.3%) were male and 19 (73.1 %) were female (Figure 1).

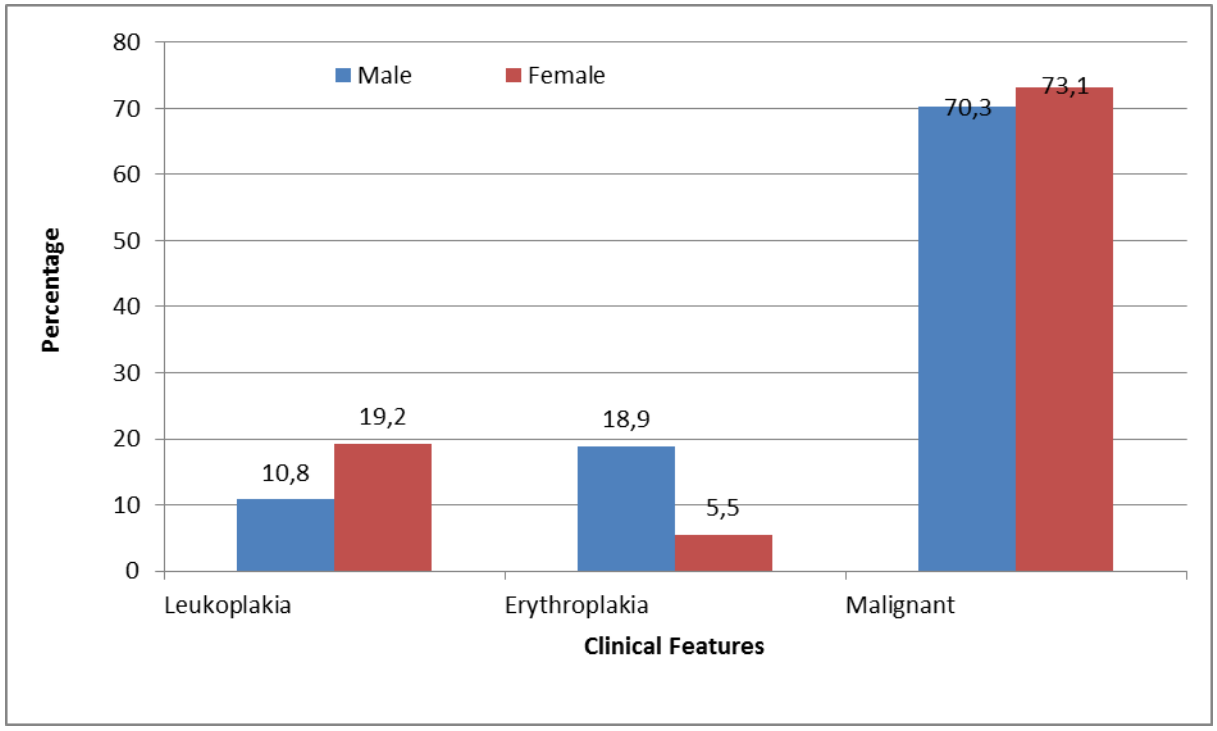

Fig. 1. Clinically diagnosed patients with buccal mucosa neoplasms.

Left buccal mucosa (LBM) was found to be the most common sites (90.5 %) whereas right buccal mucosa (RBM) was observed to be the least (9.5 %) affected site in this study (Figure 2).

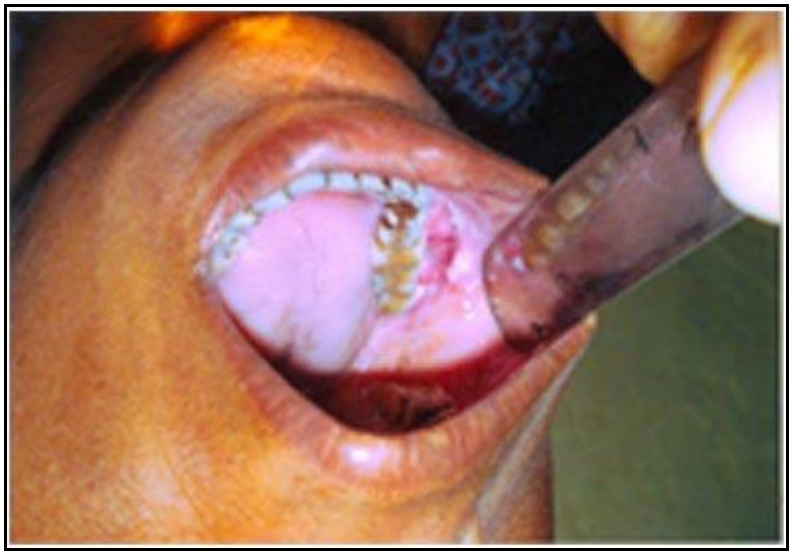

Fig. 2. Buccal mucosa neoplasm at the left cheek

Cervical lymph nodes (CLN) were observed in 35(55.6 %) cases and absent in 28(44.4 %) cases. Among addicted groups, 21(33.3 %) highest cases were recorded to be chewers, followed by

mixed group (habituated with chewing and smoking of tobacco and drinking of various forms of alcohol-30.2 %) and the least case was recorded in the smokers group (1.6 %).Occupationally, 73.2 % cases were belong to labourer group, 20.6 % were service holder and the rest 6.2% were auto-drivers. The age of the patients was ranged from 32 year to 77 year with an average of 53.32 ± 12.15 year. A brief general attributes of the subjects is summarized in Table 1.

Table 1. General characteristics of 63 buccal mucosa carcinoma patients

Variables	**Number of Patients**		**Total (%)**
	Male (%)	**Female (%)**	
Sex	37 (58.7)	26 (41.3)	63(100)
Specific Site			
LBM	35 (94.6)	22 (84.6)	57 (90.5)
RBM	02 (5.4)	04 (15.4)	05 (9.5)
Age			
30-49	14 (37.9)	04 (15.4)	18 (28.6)
50-69	16 (43.2)	19 (73.1)	35 (55.6)
70-89	07 (18.9)	03 (11.5)	10 (15.8)
Clinical Pathogenicity			
Leukoplakia	04 (10.8)	05 (19.2)	09 (14.3)
Erythroplakia	07 (18.9)	02 (7.7)	09 (14.3)
Malignant	26 (70.3)	19 (73.1)	45 (71.4)
CLN Metastasis			
Positive	18 (48.6)	17 (65.4)	35 (55.6)
Negative	19 (51.4)	09 (34.6)	28 (44.4)
Addiction			
Tobacco Chewers	13 (35.2)	08 (30.8)	21 (33.3)
Tobacco Smokers	01 (2.7)	00 (0.0)	01 (1.6)
Alcoholics	04 (10.8)	11 (42.3)	15 (23.8)
Mixed	14 (37.8)	05 (19.2)	19 (30.2)
Non-addicted	05 (13.5)	02 (7.7)	07 (11.1)

Variables	Number of Patients		Total (%)
	Male (%)	Female (%)	
Occupation			
Labourer	25 (67.6)	21 (80.8)	46 (73.2)
Driver	04 (10.8)	00 (0.0)	04 (6.2)
Service holder	08 (21.6)	05 (9.2)	13 (20.6)
Brushing of teeth			
Once/day	32 (86.5)	26 (100)	58 (92.1)
Twice/day	05 (13.5)	00 (0.0)	05 (7.9)
Tooth brush	18 (48.6)	14 (53.8)	32 (50.8)
Plant stick	19 (51.4)	12 (46.2)	31 (49.2)

Cytopathology

Cytological pleomorphism was well observed exhibiting a number atypias in the exfoliated cytosmears of human buccal mucosa neoplasm (HBMN). Generally, the normal epidermal cells of the BM were found to be more or less polyhedral with well-defined cell boundary, non-keratinized cytoplasm and centrally located rounded or oval nucleus. Normal oral mucosal cells (NOSC) are observed to be sky-blue colour in Papanicolaou's stain and magenta colour in Giemsa's stain (Figure 3). Nuclear-Cytoplasmic ratio (N:C) in NOSCs was found to be 1:34.5. In due course of carcinogenesis, the normal cells of the buccal mucosa were metamorphosed into various pleomorphic cytological atypias, such as Micronucleated cell (MNC), Plump keratinized squamous cell (PKSC), Keratinized spindle cell (KSC), Keratinized strap (Anitschkow) cell (KSC-A), Keratinized fiber cell (KFC), Keratinized round cell (KRC) and Non-keratinized malignant squamous cell (NMSC) with drastic and dramatic modification. KFC and KRC were observed to be in two different forms- large and small. Thus, large keratinized fiber cell (LKFC), small keratinized fiber cell (SKFC), large keratinized round cell (LKRC) and small keratinized round cell (SKRC) have their own identity so far as cytological pleomorphism is concerned (Figure 4). MNC and PKSC were observed to be well differentiated squamous cell (WDSC); KSC, KTC, KSCA, KFC and KRC were moderately differentiated squamous cells (MDSC) and NMSC was absolutely poorly differentiated squamous cell (PDSC). All these atypical pleomorphic cells exhibited intense cellular and nuclear pleomorphism, loss of cellular-cohesion, nuclear hyperchromatism, individual cell keratinization, atypical mitoses and formation of keratin pearls. Nuclear hyperchromasia, increased nuclear to cytoplasmic ratio (N:C), anisonucleosis, nuclear pleomorphism, irregularities of nuclear membrane, nuclear crowding, nuclear moulding, clumping and irregular distribution of chromatin are the changes observed during malignancy. Universal occurrence of MNCs in all exfoliated cytosmears with gradual increase in its frequency from normal to malignant cases proves itself to be an onco-indicator as well as a potential biomarker of oral carcinogenesis (Mohanta et al., 2017).

PKSCs were chiefly found in premalignant lesions (leukoplakia and erythroplakia) and rarely found if present, in benign and malignant cases. The moderately differentiated KSC, KTC, KSC-A, KFC and KRC are generally observed in pre-malignant and malignant tumors. However, these were also frequently observed in the cytosmears of buccal mucosa premalignant lesions. Presence of these moderately differentiated in premalignant cases clearly indicates that the lesions were unexpectedly in an advanced stage and trigger aggressiveness. It is noteworthy that, both KSC and MNC were found to be the modal cytological atypias in the HBMNs. It has been observed that except PKSC and MNC, other detected pleomorphic cytological atypias may or may not be found in

all cases, but presence of either one or more type of these atypias in a buccal mucosa cytosmear indicates the state of malignancy.

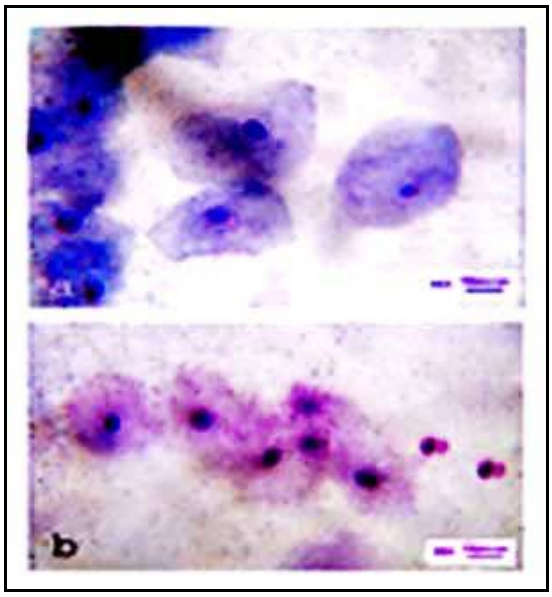

Fig. 3. Normal oral squamous cells(a. Papanicolaous' stain, b. Giemsa Stain)

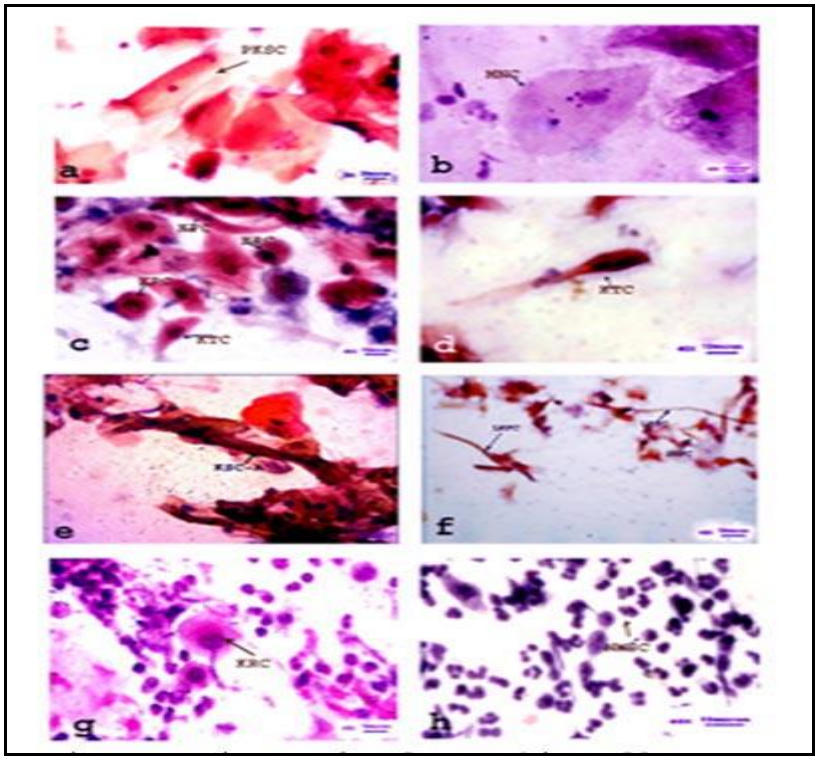

Fig. 4. Diagnostic pleomorphic cells (a. PKSCs, b.MNC, c. KSCs, d. KTC, e. KSCA, f. KFCs, g. KRCs, h. NMSCs)

On the basis of Broder's cytological differentiation, 35 (55.5 %) cases were well differentiated squamous cells (WDSC), 15 (23.9 %) cases were moderately differentiated squamous cells (MDSC) and the rest 13 (20.6%) were of poorly differentiated squamous cells (PDSC) type among buccal mucosa neoplasm cases. However, on the basis of present cytopathological differentiation (pattern of keratinization, chromatisation and nuclear pleomorphism), our finding indicates that 3 (4.8 %) cases were WDSC, 45 (71.4 %) cases were MDSC and 15 (23.8 %) were PDSC type (Table 2).

Therefore, the detected cytological atypias have both prognostic and diagnostic importance during difficult diagnosis of HBMN cases.

Table 2. Comparative account of cytopathological differentiation of 63 buccal mucosa cases

Sl.No.	Cytopathological differntiation	Broder's System	Present System
1	WDSC	35 (55.5%)	3 (4.8%)
2	MDSC	15 (23.9%)	45 (71.4%)
3	PDSC	13 (20.6%)	15 (23.8%)

Statistical Analysis

In normal exfoliated cytosmear of buccal mucosa, along with normal oral squamous cells (NOSCs) two types of atypias such as MNC and PKSC were observed. MNCs were observed to be non-keratinized while, PKSCs were mostly hypo-keratinized. The percentage sof cytological atypias were recorded to be 1.62, 1.61 and 1.74 among males and 1.725, 1.35 and 1.45 among females in the age group 30-49, 50-69 and 70-89 years respectively. Thus, the mean percentages of 1.64 in male and 1.72 in females were calculated in the Control group.

In pre-cancerous group, the percentages of cytological atypias were recorded to be the lowest (11.8) in male in the age-group of 30-49 years and highest (20.7) in female in the age group of 70-89 years respectively (Table 3). It has also been observed that the frequencies of cytological atypias were in increasing order from lower to higher age group. The mean percentage of atypias was thus found to be 16.74 in males and 16.175 in females. The z-values, in precancerous cases were recorded to be 19.627 in males and 8.214 in females, which signify the test of significance ($p \leq 0.01$) itself.

Table 3. Age groups and sex-wise frequencies of cytological atypias from buccal mucosa of normal and cancer patients

Group	Age group in years	Total no of samples screened		No. of atypical cells scored		Percentage of atypical cells		Mean percentage of atypical cells		z- value	
		M	F	M	F	M	F	M	F	M	F
Control	30-49	14	04	228	69	1.62	1.725	1.64	1.72	-	-
	50-69	16	19	258	336	1.61	1.35				
	70-89	07	03	122	43	1.74	1.43				
	30-89	**37**	**26**	**608**	**448**	**1.64**	**1.72**	**1.64**	**1.72**	-	-
Pre-cancerous	30-49	02	02	236	282	11.8	14.1	16.74	16.175	19.627*	8.214*
	50-69	07	05	1204	805	17.2	16.1				
	70-89	02	01	402	207	20.1	20.7				
	30-89	**11**	**08**	**1842**	**1294**	**16.74**	**16.175**	**16.74**	**16.175**	**19.627**	**8.214**
Cancerous	30-49	12	02	4569	508	37.575	25.4	39.769	42.267	31.855*	34.018*
	50-69	09	14	3928	5871	43.644	41.935				
	70-89	05	02	1843	1301	36.86	65.05				
	30-89	**26**	**18**	**10340**	**7680**	**39.769**	**42.267**	**39.769**	**42.267**	**31.855**	**34.018**

*z-values are highly significant ($p \leq 0.01$)

Similarly, in buccal mucosa carcinoma group, the frequencies of cytological atypias were observed to be more than that of pre-cancerous group. Although, the highest percentage (65.05) of cytological atypias was recorded among females; a dramatic fall in percentage (39.769) of cytological atypias was observed among males in the age group of 70-89 years in comparison to those of preceding age groups (Table 3). The real cause, though, is not clear; inclusion of two non-addicted subjects (one male and one female) in the 79-89 years of age group may be attributed to this unusual effect. The mean percentages of atypias were recorded to be 39.769 in males and 42.267 in females. The z-values were calculated to be 31.855 in males and 34.018 in females, which are found to be highly significant ($p \leq 0.01$).

Metastasis and TNM Staging

Out of 63 HBMN cases, cervical lymph nodes (CLN) were found in 35 (55.6 %) cases (18 male and 17 female) and absent in 28 (44.4 %) cases (19 male and 9 female) irrespective of their single or multiple (mixed) addiction habit. CLN were observed either in single, double or multiple in numbers with either ipsilateral or contra-lateral in position. Pattern of metastasis differs from individual to individual is really unpredictable. American Joint Committee for Cancer (AJCC) Staging and End Results Reporting-2010 was followed for Tumor-Node-Metastasis (TNM) staging of buccal mucosa neoplasms. Accordingly, only 3 (4.76 %) cases were recorded to be in Stage I, 22 (34.92 %) cases were in Stage II and 38 (60.32 %) cases were in Stage IV. None of the cases were in Stage III (Table 4). It indicates that, the HBMN aggressively progresses from premalignant lesions towards malignancy during buccal mucosa carcinogenesis.

Table 4. TNM Staging of 63 buccal mucosa carcinoma patients

Stage	No. of Patients	Percentage (%)
I	03	4.76%
II	22	34.92%
III	Nil	0%
IV	38	60.32%
Total	63	100%

Sensitivity, Specificity and Accuracy of the Diagnostic test

Considering the malignancy potential, 54 (32 male and22 female) cases were found to be true positive (TP) and 5 (3 male and 2 female) cases were of true negative (TN). No cases were recorded to be false positive (FP), whereas, false negative (FN) cases were recorded to be (2male and 2 female) in number. Therefore, the Sensitivity was calculated to be 93.1 % (male – 94.1 %, female – 91.7 %) and Specificity was 100 % in both male and female cases. However, the Positive Predictive Value (PPV) was calculated to be 100 % in both sexes, whereas Negative Predictive Value (NPV) was 55.6 % (male – 60 %, female – 50 %). Ultimately, the accuracy of our diagnostic test was calculated to be 93.7 % (Tables 5-6, Figure 5).

Table 5. Status of malignancy potential among the buccal mucosa carcinoma patients

Final Reports	Male	Female	Total
TP	32	22	54
FP	0	0	00

Final Reports	Male	Female	Total
FN	02	02	04
TN	03	02	05
Total	37	26	63

Table 6. Sex-wise diagnostic tests for cytopathological outcomes

Attributes	Calculating formula	Sex	Outcome (Decimal)	Outcome (Percentage)
Sensitivity	TP/ (TP+FN)	Male	0.9411	94.1
		Female	0.9166	91.7
Specificity	TN/ (FP+TN)	Male	1.0	100
		Female	1.0	100
Positive Predictive Value (PPV)	TP/ (TP+FP)	Male	1.0	100
		Female	1.0	100
Negative Predictive Value (NPV)	TN/ (FN+TN)	Male	0.6	60.0
		Female	0.5	50.0
Accuracy	(TN + TP)/ (TN+TP+FN+FP)	Male	0.9189	91.9
		Female	0.9230	92.3

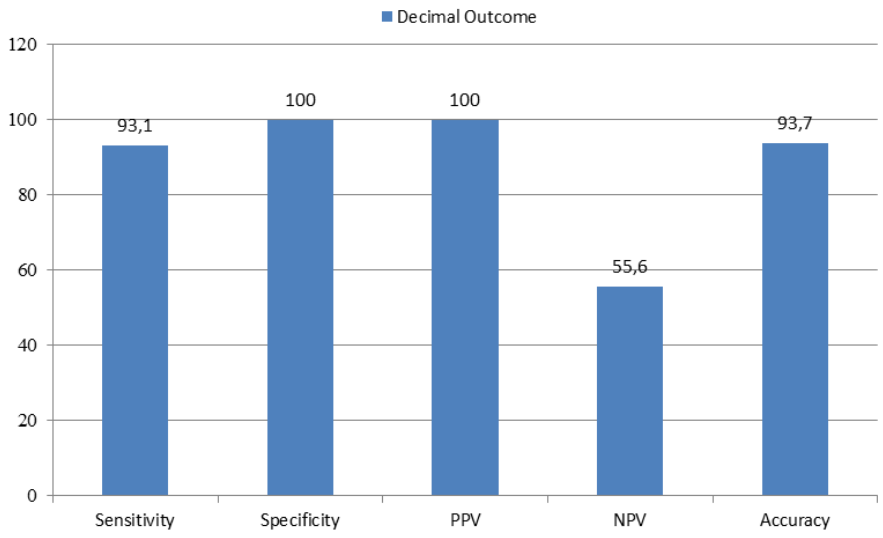

Fig. 5. Overall outcome of the diagnostic test

4. Discussion

According to the International Classification of Diseases-10th Edition (ICD-10) by World Health Organizations (WHO), buccal (cheek) mucosa is a prominent intra-oral cancer-prone site (C06). National Cancer Registry Program (NCRP) of India has pointed out that SCCHBN is the most common cancer of the oral cavity in India (Anonymous, 2010). The high incidence of buccal mucosa carcinoma in India is attributable to the usage of tobacco in its various forms and smoking. The reported 5 year survival rates for buccal mucosa cancers in India ranges from 80 % for Stage I disease to 5-15 % for locally advanced disease (Pradhan, 1989; Iyer et al., 2004). Despite current progress in various treatment modalities, over the past 50 years survival rates have not improved drastically.

Oral Squamous Cell Carcinoma (OSCC) is a long latency multistep disorder presents different clinical aspects which are related with the location of the tumor, evolution time, precancerous lesions and risk factors (Boring et al., 1994). During oral carcinogenesis, leukoplakia, erythroplakia or erythro-leukoplakia are the most frequent clinical aspects, which may present superficially eroded areas. The lesion can progress and develop as an exophytic, irregular lobulated lesion or adopt an endophytic growth pattern characterized by a depressed ulcer with greyish-white edges, elevated, everted and indurate borders and an infiltrated base. In most cases, lesions are asymptomatic; pain appears only when muscles or nerves are invaded at advanced stages of the disease (Silverman et al., 1998; Neville, Day, 2002). Generally, OSCC arises from within a field of pre-cancerized epithelium either from a pre-existing potentially malignant lesion, or de novo. The use of tobacco and betel quid, heavy drinking of alcoholic beverages and a diet low in fresh fruits and vegetables are the major risk factors for OSCC (Feller, Lemmer, 2012).

Macroscopically, tumours may be exophytic, ulcerating, infiltrating or nodular or any combination. Most tumours are squamous cell carcinomas. Other histopathological types are: glandular carcinomas, lymphosarcomas, melanomas. Precancerous lesions are observed in one third of patients. Nodal involvement is noted at the time of the initial diagnosis in about 40 % of patients (Gerbaulet, Pernot, 1985; Urist et al., 1987). High mitotic activity, infiltrative borders, lymphatics and vascular invasion indicate aggressive behaviour of buccal mucosa carcinoma.

OSCC is characterized by both cytopathological and histopathological manifestations. Cellular alteration due to increased molecular entropy is the root cause of neoplastic transformation. All carcinogenesis evolves from initial cell injury to the formation of a malignant neoplasm (Hanahan, Weinberg, 2011). Histologically, the lesion passes from reactive epithelial changes (such as hyperkeratosis, hyperplasia and acanthosis) or pre-neoplastic changes (including mild, moderate and severe dysplasia) prior to the establishment of an invasive carcinoma (Neville et al., 2009; Rivera, 2012). In a case-control study, Minhas et al. (2021) have reported that cytological changes were observed not only in neoplastic epithelial cells but the nonneoplastic epithelial cells are also affected, resulting in cytopathological atypical changes in patients receiving concomitant chemoradiotherapy (CCRT) as a treatment for oral squamous cell carcinoma (OSCC), Nuclear atypia features were higher on the 17th day and end of treatment; whereas, epithelial atypia was mainly observed on the 17th day of CCRT (40 %). Atypia was not observed in any control group. In the present study, atypias were also observed in control group, insignificantly.

OSCC may be graded according to their degree of differentiation, mitotic activity and other factors. Grade I carcinomas are the most differentiated and Grade III, the least differentiated. The higher the grade the worse is the prognosis. Well differentiated carcinoma form broad bands and nests. Microscopic observation reveals that the tumor cells have abundant eosinophilic cytoplasm, intercellular bridges and prominent keratinization either as single cell keratinisation or keratin whorls. Grade II squamous cell carcinoma resembles squamous epithelial cells, but there is less evidence on squamous differentiation and less keratinisation than those in Grade I carcinoma. Grade III has infrequent keratinisation, cellular pleomorphism, increased mitosis, bizarre tumor giant cells and even mitotic spindling are prominent. Otherwise, poorly differentiated squamous cell carcinoma have sheets of uniform cells either small, large or spindle cell type. In this exfoliative cytopathological study, it has been observed that MNC and PKSC were found chiefly in precancerous conditions (leukoplakia and erythroplakia stage). These cells are well differentiated and resemble with the normal buccal mucosal cells. Moderately differentiated pleomorphic atypical cells, such as KSC, KTC, KFC, KRC and KSC-A were keratinized and mostly observed in benign and malignant lesions. However, an exceptionally peculiar NMSC was observed to be non-keratinized and neither found in any precancerous conditions nor in premalignant neoplasms, at all; but was

restricted to the malignant neoplasms only (Mohanta, Mohanty, 2016). Spindle cell squamous cell carcinoma (SpSCC) is a rare phenomenon in head and neck region. Ferrisse et al. (2021) have reported that post-radiotherapy occurrence of recurrent SpSCC at the same site of lingual origin after an immunohistochemical analysis. In our study, KSCs are also frequently observed in HBMN cases. KSCs are moderately differentiated and are usually related to poor prognosis. Therefore, these pleomorphic cells have diagnostic importance in early detection of HBMN cases.

Metastasis

Lymph node involvement correlates with tumor size, depth of invasion and primary tumor site. Increased risk of nodal metastases are directly co-related with tumor grade and are more prone to the posterior than anterior site of the oral cavity. Lung, bone and liver are the most common site for distant metastases from OSCC. Mishra et al. (1999) reported the relation between treatment failure and tumor thickness in a series of 176 patients with early buccal mucosa cancer. Tumor thickness of more than 5 mm into the underlying tissues, are more likely to metastasize to lymphnodes with a poorer prognosis (Shah and Gil, 2009; Massano et al., 2006).

Borges et al. (1989) reported that low incidence of neck node metastasis even in presence of large tumors. According to them, carcinoma of buccal mucosa is very aggressive and biologically a different disease. During this investigation, we have observed that clinically 7 erythroplakia cases (5 male and 2 female) had CLN and on the contrary, 5 malignant cases (2 male and 3 female) were of without CLN. Cytopathological analysis indicated that in addition to MNC and PKSC, multiple number of pleomorphic cells (KSC, KTC, KFC and KSC-A) were present in such 7 erythroplakia cases. It clearly indicates that some times, the malignant lesions mimic to be non-malignant/ benign one and vice-versa. It also indicates that presence of the moderately differentiated cells in premalignant cases turn the carcinogenic event more aggressive and more advanced to be a malignant one in a short-span of long latency multistep oral carcinogenesis.

Distant metastases are uncommon in buccal mucosa carcinoma. An exhaustive pubmed search suggests that carcinoma buccal mucosa rarely metastasising to brain, pericardial cavity, liver, lung, thyroid, bone and bone marrow (Pichi et al., 2009; Manon et al., 2008). Suhag et al. (2011) have recently report a case of locally advanced BMSCC which metastasised to left adrenal on presentation, picked up by PET-CT and later confirmed by CT-guided FNAC. The ability to metastasize is directly associated with the differential grade of tumor cells, similar to that of the neoplasm tissue architecture and normal epithelium (Sapp et al., 2004). Occurrence of cutaneous metastasis (CM) is very rare in squamous cell carcinomas of the head and neck CM generally develops near the primary site of recurrence, then spreads outwards. Prakash and Upadhyay (2022) have reported a case who had already been treated for carcinoma of the right side buccal mucosa but presented with local site recurrence as primary and subsequently developed distant skin metastases to the lower neck and upper trunk during the treatment of the primary recurrence site.

Buccal mucosa SCC (BMSCC) is reported to be highly aggressive malignant tumor. It grows rapidly, and has a high recurrence rate (Bobdey et al., 2018; Karthikeyan et al., 2018). About 90 % of recurrences occur within the first 1.5 years after treatment. Local recurrence is more common than regional recurrence, and has been reported between 23 to 32 % (Diaz et al., 2003; Yoo et al., 2009). Locoregional control and survival rates may be greater with surgical excision plus post-operative radiation than with treatment with either modality alone (Lin et al., 2008). SCC of the buccal mucosa seems to be a cancer of poor prognosis characterized by a high local failure rate. Possible explanations include inadequate treatment and intrinsic tendency to an aggressive nature requiring multimodality treatment (Lin et al., 2006). Fang et al. (2013) have reported that buccal mucosa SCC is an aggressive malignant tumor, with its degree of differentiation being the most important factor affecting prognosis and survival. Lymphovascular invasion was associated with poor survival at the late stage especially those with primary at the buccal mucosa and the tongue (Liu et al., 2017). Therefore, more aggressive post-operative therapy is suggested for patients with buccal mucosa carcinoma excised with a close margin of ≤3 mm (Chiou et al., 2010). Commissural skin involvement and in situ invasion in buccal mucosa carcinoma poses unique challenges in choosing treatment modalities (Shanmugam et al., 2020).

BMSCC is the most common oral cancer in men and the third most common oral cancer in women in India; and accounts for up to one-third of all tobacco-related cancers (Misra et al., 2008). This higher rate of BMSCC in India is likely related to the widespread practice of betel nut chewing, in addition to tobacco and alcohol. Subapriya et al. (2007) found that combination of

tobacco use or smoking with alcohol consumption is a very potent risk factor for the development of OSCC in a population in South India. Frequency and duration of alcohol and tobacco consumption have also been found to play a role in oral cancer development (Krishna et al., 2014; Mohanta et al., 2013). Other suspected but not confirmed etiologic factors include human papilloma virus, poor oral hygiene and chronic irritation (Krishna Rao et al., 2013).

Hande and Chaudhary (2010) carried out buccal mucosa cytomorphometric analysis of tobacco chewers and concluded that cytomorphometric changes could be the earliest indicators of cellular alterations. They showed a progressive decrease in cytoplasmic area (CA), increase in nuclear area (NA) and increase in the ratio of nuclear area to cytoplasmic area (NA: CA) in smears taken from all tobacco users. This indicates that there could be a relationship between tobacco usage and quantitative alterations of cells. The concept of cellular or nuclear alteration on exposure to different forms of tobacco can be best explained by reviewing the nature of the cellular response to the end products of tobacco usage. The decrease of cellular diameter and increase of nuclear size are significant morphologic changes characteristic of actively proliferating cells. In this study, NA: CA ratios were observed to be increased state in all aforesaid pleomorphic atypias and found to be culminated as 1:1 in NMSC of both addicted and non-addicted HBMN cases (Mohanta, Mohanty, 2016).

In the past, the controversies surrounded over the use of oral exfoliative cytology (OEC) in the management of oral malignancy, because of a large number of false-negative results and subjective interpretation of abnormal oral mucosa cells. The low sensitivity of oral cytology is related to various factors including inadequate sampling, procedural errors and subjective interpretation of the findings. Kumar et al. (2011) have reported that oral exfoliative cytology is a reliable adjuvant to biopsy with the 69 % sensitivity and 100 % specificity in oral leukoplakia and 75 % sensitivity and 100 % specificity in squamous cell carcinoma. Based on a comparative study, Fontes et al. (2013) have reported that the diagnostic concordance between histopathological (gold standard) and cytopathological examinations was 83.1 % for OSCC and 85.7 % for non-neoplastic lesions. Specifically, the sensitivity was 83.1 %, the specificity was 100 %, the positive predictive value was 100 %, the negative predictive value was 49.0 %, and the accuracy was 85.5 %. In a current study, Hafez and Fahim (2014) reported to have the diagnostic reliability in the form of sensitivity was 93.5 %; specificity 96.2 %; predictive value (PPV) 97.7 %; negative predictive value (NPV) 89.3 % and overall diagnostic accuracy was 94.4 %. A statistically significant relation was found between cytological and pathological diagnosis ($p < 0.001$). Kappa was 0.882 indicating a good agreement between cytological and histopathological results. Namala et al. (2016) have studied on micronuclei frequency and reported that there was 60 % correlation between the cytological grade and histological grade and the difference between them was found to be insignificant. They have also concluded that cytological grading can be used as an alternative to histological grading in grading of OSCC. Recently, Mohanta and Mohanty (2017) in a cytopathological diagnostic test reported that the Sensitivity was calculated to be 83.5 %, Specificity was 100 %, positive predictive value (PPV) was 100 %, negative predictive value (NPV) was 30 % and the accuracy was found to be 84.6 % Therefore, the nuclear pleomorphism-based cytopathological grading system makes itself an ideal screening test for early detection of human oral cancer. The findings of the present study also highly corroborates with the earlier studies.

A quantitative technique increases the diagnostic ability of exfoliative cytology. It is precise, objective and reproducible. This technique is not only useful for preliminary diagnosis of many oral mucosal diseases but also it can be used as an onsite mass screening tool for a definitive diagnosis. The present finding can be helpful to the Oncopathologists in screening and early detection of potentially malignant oral disorders and oral cancer to a greater extent. However, self-consciousness and public awareness of these lesions are critically important to manage the disease. The knowledge, attitude and behaviour of the oral clinicians and dental practitioners regarding these lesions are important for successful diagnosis. Contrary to that, Bataineh et al. (2015) have pointed out that inaccurate diagnosis or a delay in diagnosis of these life crumbling lesions might have profound implications for both the patients and practitioners.

5. Conclusion

Among the intra-oral neoplasms, the human buccal mucosa neoplasm (HBMN) poses a difficult diagnostic dilemma for the clinician as well as the oncopathologists (Misra et al., 2008). Clinically indistinguishable HBMN may mimic to be a benign one, but cytopathologically the

patient might be in an advanced stage. Chewing and smoking of various form of tobacco and drinking of alcohol contribute a lot to the genesis of cytological pleomorphism in HBMN. Typically atypical cells like KSC, KTC, KSC-A, KFC, KRC, PKSC, MNC and NMSCs although found less in number, modal occurrence of the moderately differentiated KSC and MNC in all the HBMNs may be directly correlated with the degree of pathogenicity. Probably, the carcinogens present in the tobacco and alcohol play a major role in the drastic cellular alternation followed by carcinogenesis in the buccal mucosa of oral cavity. Diagnostically, it also indicates that the Sensitivity was calculated to be 93.1 % and Specificity was 100 %, Positive Predictive Value (PPV) was 100 %, Negative Predictive Value (NPV) was 55.6 % and the accuracy was 93.7 %. To conclude, the stage of advancement of SCCHBM at the time of diagnosis is the most important prognostic factor. Presence of any such cytological atypias in the exfoliated cytosmears of BMN is practically helpful in determining the stage of the HBMN. However, the role of tobacco and alcohol in connection to unusual CLN metastasis with particular reference to the HBMN needs further research.

6. Acknowledgements

The authors are thankful to Prof. Gadadhar Parida, M.D, formerly Professor and Head, Department of Oncopathology, Acharya Harihar Regional Cancer Centre (AHRCC), Cuttack, Odisha, India for his guidance and supervision during cytopathological analysis. We are also indebted to the Head P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneshwar, Odisha, India and to the Director, AHRCC, Cuttack, Odisha, India for permitting us to collect samples from oral cancer patients and also for providing library and laboratory facilities. One of us (AM) is grateful to the University Grants Commission (UGC), New Delhi, India for awarding UGC Meritorious Research Fellowship to carry out the research work.

7. Conflict of interests

The authors declare that they have no conflict of interest.

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Published in the USA
European Journal of Medicine
Has been issued since 2013.
E-ISSN: 2310-3434
2023. 11(1): 19-24

DOI: 10.13187/ejm.2023.1.19
https://ejm.cherkasgu.press

Factors Associated with COVID-19 Vaccine Acceptance and Hesitancy Among Health Care Workers in ATBUTH Bauchi

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Abstract

COVID-19 Vaccine acceptance among the general public and healthcare workers appears to play a key role in the successful control of the pandemic. It is critical to understand and investigate how much hesitancy toward COVID-19 vaccines might occur and factors responsible for these public concerns as this will greatly assist public health workers in their efforts to maximize vaccine uptake and thus, control the pandemic. This study investigated factors responsible for covid 19 vaccine hesitancy among health care workers in ATBUTH Bauchi. A cross-sectional study was carried out using questionnaires administered to health care workers of ATBUTH. A total of 339 were administered. Random sampling was employed in the selection of participants. Simple Percentages and Means were used to analyze the data. It was observed that out of the 339 administered questionnaires, 321 were returned. 70 % of respondents were aged 30-40 years, 20 % 20-30 years and 10 % 40-50 years respectively. 198 (62 %) health care workers were vaccinated, out of which 43 % were fully vaccinated, 57 % had received at least 1 dose. The most common reason for vaccination was travel restriction (74 %). Most common reason for not being vaccinated was fear of side effects (68 %). Highest qualification of respondents that were vaccinated was masters (5 %).

Fear of side effects and limited knowledge about the vaccine appear to be the main reasons for vaccine hesitancy among health care workers in ATBUTH.

Keywords: COVID 19, Vaccine, vaccine hesitancy, health care workers, cross- sectional, endemic, pandemic, epidermic, messenger-RNA, ATBUTH.

1. Introduction

COVID-19 vaccines were developed within a period of one year in late 2020 and early 2021 with the aim of ending the COVID-19 pandemic and vaccine acceptance among the general public and healthcare workers appears to play a key role in the successful control of the pandemic (Parsons et al., 2022). The World Health Organization has approved a total of 8 vaccines as at 12th November 2021 (Soares et al., 2021). These include Moderna mRNA 1237 and Pfizer/BioNTech BNT162b2 which are both mRNA vaccines, Janssen (Johnson, Johnson), Oxford/AstraZeneca and Covishield (Oxford/AstraZeneca formulation) which contain non-replicating viral vectors and the

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last 3 are Covaxin, CoronaVac and BBIBP CorV (Vero Cells) which contain inactivated virus (Soares et al., 2021).

Front-line fighters, primarily health professionals, are at a high risk for the disease (UNCTAD, 2020). Their susceptibility to diseases has many implications for health care systems. Their morbidity and mortality can cause severe crises in health care personnel shortages (Toor et al., 2022).

Healthcare workers are at higher risk of COVID-19 infection with ease of infection transmissibility to co-workers, patients and their relatives (Watanabe et al., 2022).

Several studies report COVID-19 vaccination hesitancy in the general public with Africa as one of the continents with low rates of COVID 19 vaccine acceptance (Workforce, 2021), thus, mitigating the global efforts to control the covid 19 pandemic (Ruiqiang et al., 2021). However, little is known about the nature and extent of COVID-19 vaccination hesitancy in healthcare workers worldwide (Workforce, 2021).

According to Nigeria Center for Disease Control (NCDC) as at 10th November 2021, only 2.8 % of Nigerians have received at least one dose and 1.8 % are fully vaccinated (Tanko et al., 2020). However, little is known about intention of healthcare professionals to accept COVID-19 vaccination and the factors affecting it are not known. The findings from these professionals would help policy makers in the health sector to improve vaccine acceptance, which would contribute to the control of COVID-19 pandemic (Allen, Butler, 2017; Al-mulla et al., 2021; Toor et al., 2022; Woo, Dimova, 2022).

The research therefore determined the factors associated with COVID-19 vaccine acceptance and hesitancy among health care workers in ATBUTH Bauchi.

2. Methodology

Study area

Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) is located in Bauchi town, Bauchi State, North Eastern Nigeria and it is located within my immediate community. It is well-equipped with more than 10 clinical and non-clinical departments with the aim of providing standard health care to both indigent and non-indigent patients.

Study design: A cross-sectional study was carried out using a structured questionnaire administered to health care workers.

Study population: Consenting Staff of ATBUTH Bauchi.

Sample size: The sample size determined for this study was determined by a single population proportion formula, with the assumption of 50 % acceptability of vaccination against COVID-19, a 95 % confidence interval, 5 % margin of error, and addition of 10 % non-response rate. This calculator uses the following formula for the sample size n:

$n = N^*X / (X + N - 1)$,

where,

$X = Z_{\alpha/2}^2 \ ^*p^*(1-p) / MOE^2$,

and $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g. for a confidence level of 95 %, α is 0.05 and the critical value is 1.96), MOE is the margin of error, p is the sample proportion, and N is the population size. Note that a Finite Population Correction has been applied to the sample size formula.

Therefore, the sample size for this study was 319.

Inclusion criteria: All consenting staff of ATBUTH.

Exclusion criteria: Non-consenting staff of ATBUTH.

Data collection: Data was collected using a self-administered questionnaire.

Data analysis: Data was analysed using Microsoft Office tools.

3. Results

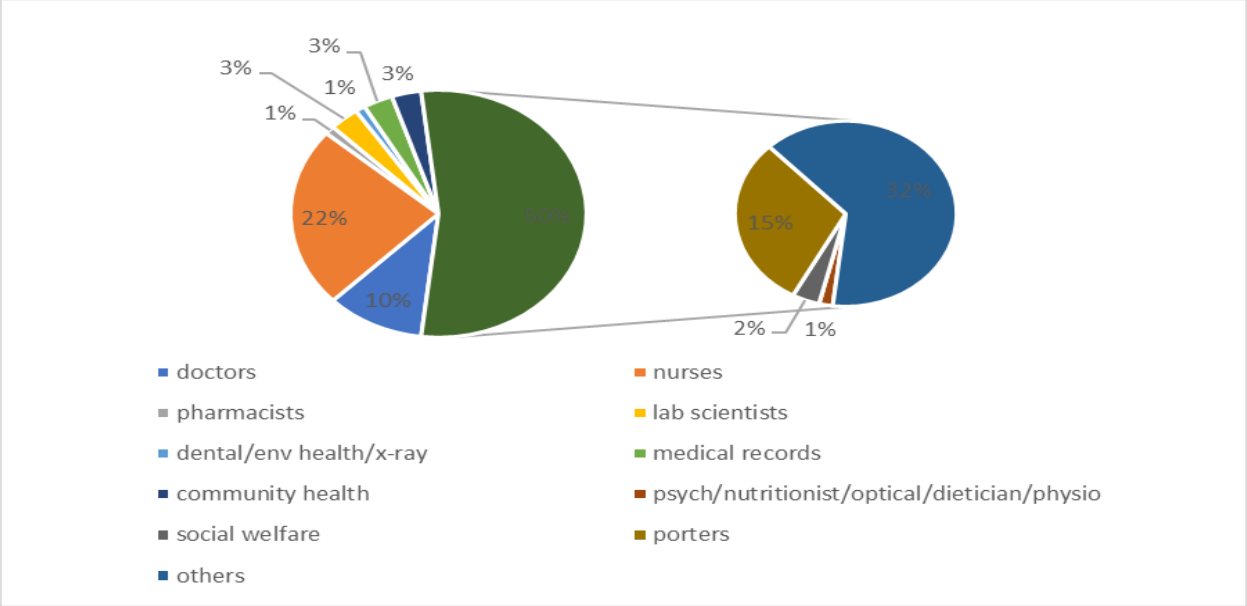

Fig. 1. Distribution of ATBUTH Staff by Cadre

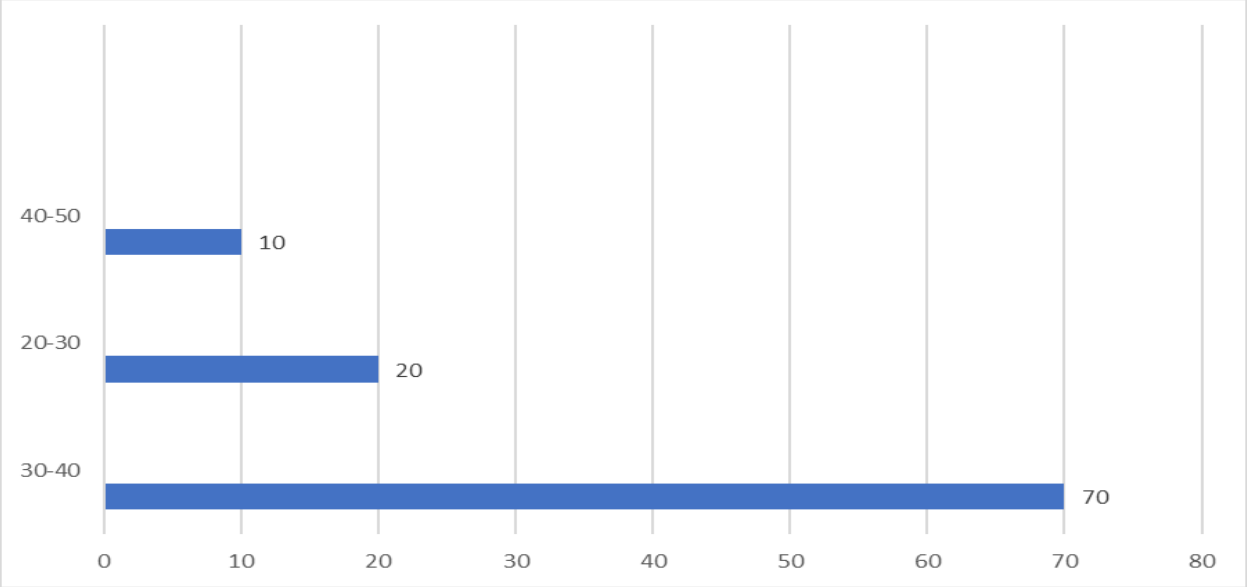

Fig. 2. Percentage distribution of respondents in years

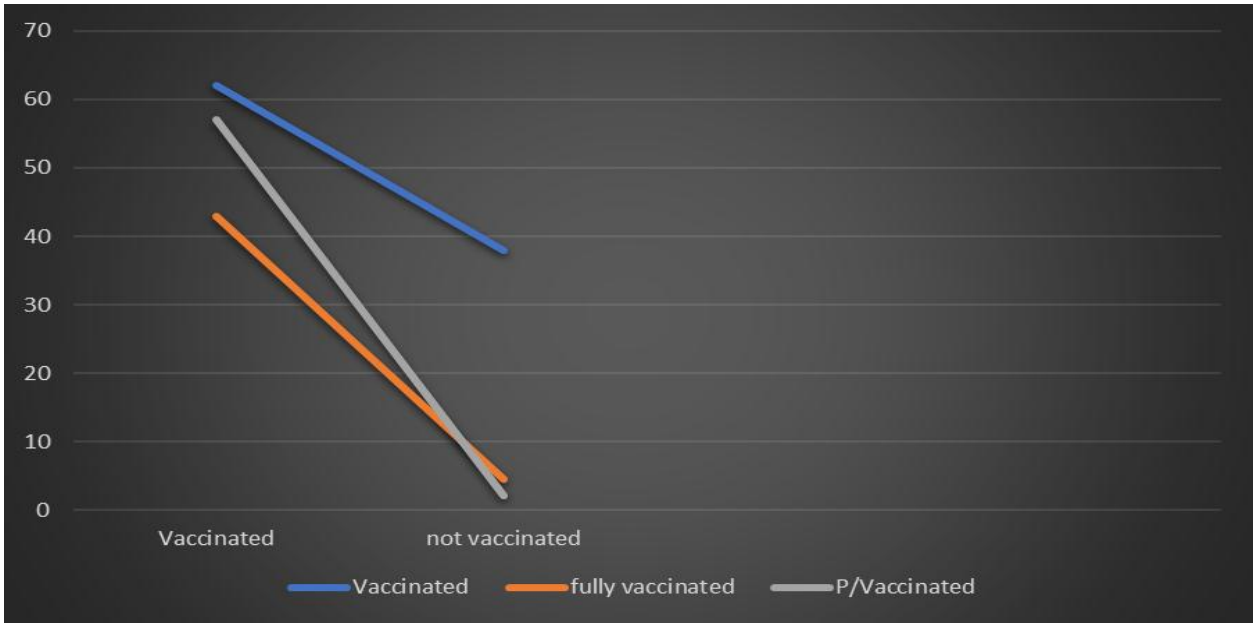

Fig. 3. COVID 19 vaccination status of ATBUTH staff

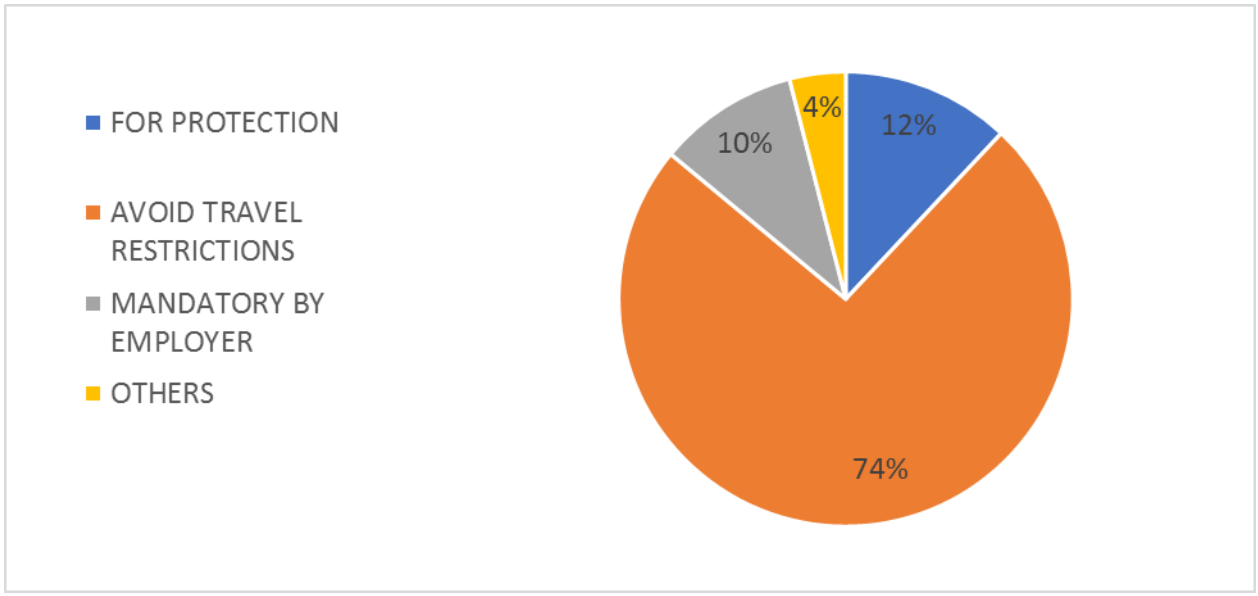

Fig. 4. Reasons for being vaccinated against COVID 19

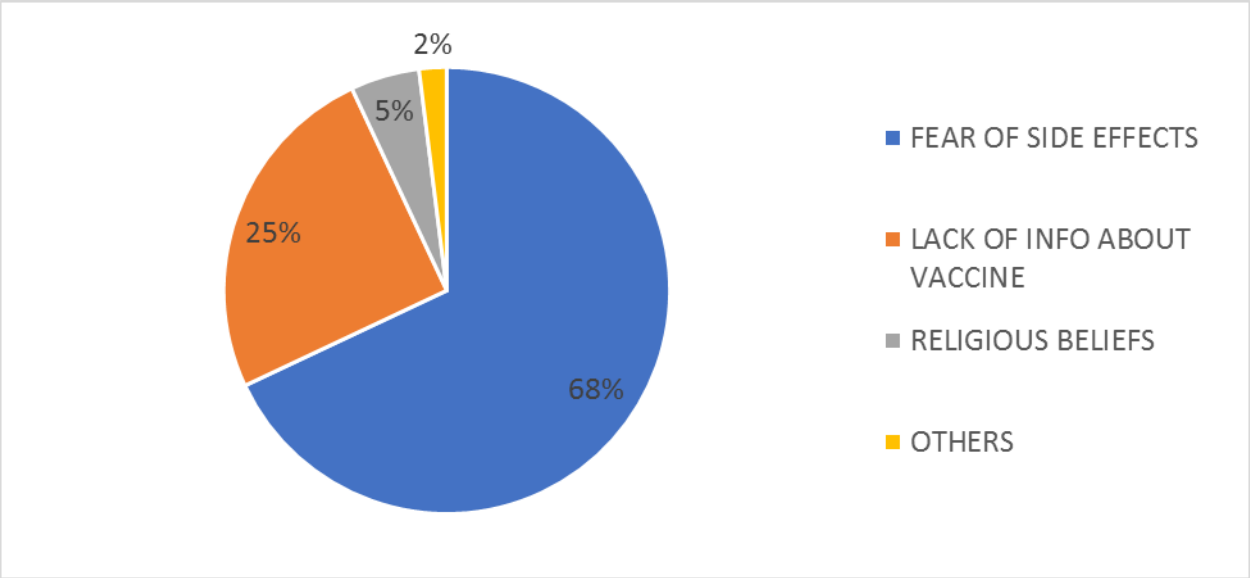

Fig. 5. Reasons for not being vaccinated against COVID 19

4. Discussion

Concerns about vaccine safety, efficacy, and potential side effects are top reasons for COVID-19 vaccination hesitancy in healthcare workers, this is similar to findings from a study done by Li et al, 2021. In the current study it was observed that 62 % were vaccinated, while 48 % were vaccine hesitant; it showed a lower vaccine hesitancy compared to 52.3 % reported in Ethiopia, 12.9 % reported in Qatar which was buttressed by Biswas et al, 2021. Reasons for being vaccinated ranged from for protection, avoid travel restrictions, mandatory by employer, others which were not so different from reports by Carcelen et al., 2021. Though the statistics vary, the common causes for acceptance and hesitancy remain similar in most studied health facilities.

5. Conclusion

Vaccine hesitancy is high among health care workers. Measures need to be put in place to adequately sensitise health care workers on the importance of vaccination.

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