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# AN OVERVIEW OF THE NOTTINGHAM RESEARCH PROGRAMME ON PRIMARY BREAST CANCER IN OLDER WOMEN

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### ABSTRACT

The incidence of breast cancer increases with age. Despite this, most research in this field is aimed at the younger patient. Preliminary studies have shown that older women with breast cancer have distinct biological features compared to their younger counterparts. In addition, the focus of treatment of older women may not simply be curative but may be a trade-off between risks of treatment and impact on independence and quality of life. The Nottingham research programme is a unique programme dedicated to primary breast cancer in older women. There are two arms of the research programme 1) determining the biological differences of breast cancer in this cohort 2) exploring the use of geriatric assessment to understand the complex needs and factors contributing to treatment decision making in this group of patients. The overall aim of the research programme is to optimise both the biological and geriatric assessment of older women with primary breast cancer, to provide personalised data at diagnosis, on likely survival and quality of life outcomes following breast cancer treatment. This article will outline why this research programme is important, what it has achieved so far and future aspirations.

**Key Words:** Primary breast cancer, older women, biology, biomarkers, geriatric assessment, personalised medicine

## INTRODUCTION

### Importance of Breast cancer in older women

Breast cancer is the most common cancer in women worldwide (1) and age is the biggest risk factor for development of breast cancer; risk increasing proportionally with age (2).

In 2018, the number of new breast cancers diagnosed worldwide was approximately 2 million (3) with around 25% of cases in patients  $\geq 70$  years (4). Due to ageing of the world's population, it is expected that the number of older women living with breast cancer will double by 2040 to nearly 1 million cases in patients  $\geq 70$  worldwide per year (1, 5).

The ageing of the world's population is the result of the continued decline in fertility rates and increased life expectancy (6). This demographic change has resulted in increasing proportions of older persons. In 2019, approximately 9% of the global population were  $\geq 65$  years of age (7) and this is expected to double by 2050 (8). The crisis of our ageing population has been well documented (9, 10); the burden on health service resources in terms of financial and service provision will be unprecedented. Breast cancer in older women will have a significant impact on this.

### Differences between breast cancer in older compared to younger women

Existing data suggests that the biology of breast cancer in older women is different compared to their younger counterparts (11) and this may impact treatment decision making, alongside conventional factors, patient preference and discussion with the multidisciplinary healthcare team. For example, ER-positivity of breast cancer increases with increasing age (12), thereby making primary endocrine therapy (PET) a potential primary treatment option in older

women. A number of other biomarkers have been identified outside of those routinely measured, which could potentially have predictive and prognostic significance in older women with primary breast cancer (13). Furthermore, it is recognized that there are other factors which influence treatment decision making in older compared to younger women, such as impact of treatment on quality of life and preservation of independence (14, 15). Whilst younger women may consider curative intent the end goal of breast cancer treatment, older women may not be willing to tolerate the same level of invasive treatment if it means a reduction in their usual functioning, especially giving the slow growing nature of breast cancer in this age group.

### **Nottingham Breast Cancer Research Programme**

The Nottingham team based in the United Kingdom has a dedicated research programme focused on primary operable breast cancer in older women. The centre describes a large consecutive series of older women with primary breast cancer, for whom long-term follow-up data is available (11, 16). There is also a younger cohort of primary breast cancer patients, available for comparative analysis (17). There are currently two arms of the research programme in older women, one focussing on profiling the unique biology of primary breast cancer in this series and the second arm, focussing on the application of geriatric assessment (GA).

#### ***Biology theme***

The cohort consists of 1,758 women with early operable breast cancer aged  $\geq 70$  years. Long-term follow-up data (up to 37 years) and survival outcomes for the whole cohort has been collected and described (18) for all patients, where available. From the whole series, 813 patients underwent primary surgery. It has been possible to construct 575 tissue microarrays (TMAs) using the surgical excision (SE) samples and a panel of 25 biomarkers has been assessed in these SE TMAs (11). It has been possible to construct 693 TMAs from CNB samples from the overall cohort. A panel of 18 biomarkers has been measured in the CNB TMAs in patients who had ER-positive breast cancer (19).

#### Summary of significant findings to date

Cluster analysis in both the SE TMAs and CNB TMAs identified a novel biological cluster of disease, which is distinct from standard clusters seen in younger women (11, 19). The unique cluster, termed 'low ER luminal' had high expression of luminal cytokeratins, mucin (MUC)1 and HER3 compared to the other conventional clusters and had different BCSS compared to conventional clusters. Similar findings suggesting a differing biology (with differing clinical outcomes) according to age, have also been found in terms of histological type (20), as well as in HER2-positive (21) and TNBC (22) and in response to treatment (23). Both the work in the SE and CNB TMAs have identified potential biomarkers which may be of use in predicting response to therapy and overall survival in older women with primary breast cancer.

#### ***Geriatric assessment theme***

A prospective pilot study implementing a cancer-specific GA in older patients with primary breast cancer is in progress and is currently being conducted in four centres internationally. Patient recruitment commenced in 2009 and invites women aged  $\geq 70$  years with early-stage operable primary breast cancer to participate. The aim is to examine the value of using a validated cancer-specific comprehensive geriatric assessment (CGA) (24) to assess older patients undergoing surgery versus non-operative treatment. The CGA is performed within 6-weeks and 6-months post-diagnosis, supplemented by using EORTC QLQ C-30 and BR23 as formal measures of QOL (25, 26).

#### Summary of significant findings to date

A pilot study performed in Nottingham specifically examined feasibility of implementation of GA in 47 older women ( $\geq 70$  years) with early operable primary breast cancer (27). Decision of primary treatment followed consultation with the clinical team and was not guided by GA. GA determined that increasing age, greater comorbidity, greater number of daily medications and slower timed up and go (a measure of physical function) were significantly related to non-surgical treatment. Quality of life remained stable at 6 months in all patients regardless of treatment. Average time to complete the GA was 32 minutes (range 15 – 65 minutes) and was conducted by a variety of trained research team members, who were not necessarily clinicians; this pilot study confirmed the feasibility of GA in a research setting.

The most recent findings of the study data from three UK centres, was presented at the International Society of Geriatric Oncology (SIOG) 2019 annual conference and reported results from 88 women who underwent surgery (28). Results

were conflicting in that there was an improvement in some questions related to functional status and a decline in others, with no clear pattern and no relationship with intensity of surgery.

The study is currently ongoing and involves several centres across the UK and Hong Kong, with the aim of recruiting over 1000 patients.

### Summary – future of the research programme

In the future we see the development of a tool to analyse an extensive panel of biomarkers for each individual older women with primary breast cancer, based on their core needle biopsy specimen. This would help to generate a predicted outcome for each potential treatment option they are considering.

Furthermore, development of a GA specific for primary breast cancer patients, which can be utilised alongside the above measures of biology either in the surgical clinic or before referral at the level of general practice in the community, will provide a detailed understanding of individual issues which must be considered in discussions between healthcare professionals and patient and their families. Clear guidance needs to be developed as to how to use information derived from GA further and how to standardise potential interventions.

Effective utilisation of the unique biology and geriatric needs of older women with primary breast cancer will result in optimal treatment and quality of life outcomes for these patients and will provide a truly personalised approach.

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## LIGATION OF INTERSPHINCTERIC FISTULA TRACT AS AN ALTERNATIVE TREATMENT FOR PERIANAL FISTULA AT TERTIARY CARE HOSPITAL

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### ABSTRACT

This study was aimed to evaluate clinical outcome of patients with perianal fistula operated with the ligation of intersphincteric fistula tract (LIFT) technique. The study was conducted at Department of Surgery, Liaquat University of Medical & Health Sciences, Jamshoro from July 2018 and August 2019. A total of 22 patients diagnosed with perianal fistula operated with the Ligation of Intersphincteric Fistula Tract (LIFT) technique were selected using non-probability consecutive sampling technique. Physical examination and magnetic resonance imaging (MRI) and/or endosonography (ES) were performed to make a confirmed diagnosis of fistula. They were classified according to Parks classification. All patients were subjected to intersphincteric fistulous tract ligation using the technique defined by Rojanasakul modified. There were 16 males and 6 females. Mean age was 42 years (range 23-68 years of age). Out of 22 patients, 17 patients had a history of perianal abscess drainage prior to surgery and an average preoperative symptom presentation was 2.2 years (range 0-10 years). Therapeutic success with first surgery was achieved in 77% and with a second surgery in 90%. None of this patients modified their preoperative Wexner. It was concluded that LIFT technique appears to be a convenient, reproducible, and effective surgical alternative. This provides an appropriate closing rate and without continence modification, Therefore indicated for complex cryptoglandular fistulas.

**Key Words:** Ligation of intersphincteric fistula tract (LIFT); perianal fistula; magnetic resonance imaging; endosonography; fistulotomy.

### INTRODUCTION

Anorectal fistula represents the chronic phase of anorectal abscess and is defined as the persistence of a fibrous path covered by granulatory tissue between an internal primary hole and one or multiple external secondary holes in the skin of the perianal region, clinically resulting in fouling, wet anus, cyclic perianal pain and chronic drainage of associated purulent material, abscess formation and intermittent spontaneous drainage.<sup>1</sup> Its origin is cryptoglandular in about 90% of cases and in the remaining it is due to other conditions such as Crohn's disease, local radiation, cancer and trauma. The cryptoglandular theory is based on the constant exposure of fecal load in the internal fistulous orifice associated with the tightness of the path, ultimately causing the septic focus to be perpetuated.<sup>2</sup>

The surgical treatment of the fistula is basically aimed at achieving healing of the fistulous tract without altering function of the anal sphincter and avoiding the recurrence of the path as much as possible.<sup>3</sup> The literature shows a variety of surgical techniques for resolution of the complex fistulas, although some achieve

efficient control of the disease, with an average recurrence of 5%. However incontinence resulting from procedures range between 10% and 57%.<sup>4</sup>

Currently, the best surgical alternative in complex anorectal fistula is being sought, unfortunately the evidence is low, given the series reported are heterogeneous, with variable and short post-operative follow-up.<sup>5</sup> Classical techniques such as fistulotomy and the seton or cutting line technique have a recurrence rate of up to 9%, causing variable deterioration of anorectal function with the figures already described. Fecal incontinence is one of the main problems of fistula treatment, since in both techniques the internal anal sphincter is sectioned.<sup>6</sup>

When considering aspects related to preservation of sphincter continence and rate of recurrence new techniques have been brought in order to solve the problem. Such as biological sealants have emerged (ie. Fibrin Glue Injection), use of caps of different materials, advanced therapies related to the use of Stem Cells and novel surgical techniques including LIFT.<sup>7</sup>

The LIFT fundamentally considers ligation and excision of the intersphincteric path, managing to block the entry of fecal material into it, eliminating the chronic cryptoglandular septic sinus.<sup>8</sup> The technique contemplates the preservation of both anal sphincters, an aspect that almost completely reduces the risk of incontinence.<sup>9</sup> The first report of this technique was a Thai experience developed by Rojanasakul et al.,<sup>10</sup> In 2007, as a modification to the technique described by Matos et al. where the description of this technique was made in 18 patients with a rate of success as 94%, without reporting alterations in continence. Since then, other authors have published their experiences with success rates in relation to cure between 47% and 95%, not forgetting the real benefit regarding the maintenance of fecal continence.<sup>8,9</sup>

One of the most important bias identified in systematic reviews was the variability detected in the technique with respect to the classic LIFT. In addition, hybrid LIFT techniques have been incorporated with the combined use of biomaterials, which makes evaluating the potential of the technique even more complex. The objective of this study was to present our results in terms of clinical efficacy and incontinence with the LIFT technique and to enhance its role as a treatment alternative for anorectal fistula.

## **METHODOLOGY**

This study was conducted at department of Surgery, Liaquat University of Medical & Health Sciences, Jamshoro, from July 2018 and August 2019. Twenty-two patients diagnosed with perianal fistula operated with the LIFT technique were selected using non-probability consecutive sampling technique. Patients included in this study were informed of the procedure to be performed and the possible complications of the procedure. Detailed written informed consent for the operation was obtained from the hospital treatment chart. This study was approved by local Ethics Committee.

A physical examination and magnetic resonance imaging (MRI) and/or endosonography (ES) were performed to confirm diagnosis of fistula. They were classified according to Parks classification<sup>11</sup>. Complex fistulas identified as medium and high trans-sphincter were exclusively included, depending on the proportion of external anal sphincter involved (> 30%) and anterior fistulas in women. Patients with fistulas whose origin was not cryptoglandular were excluded. Colonoscopy was performed on all patients to rule out Crohn's disease. All patients were subject to LIFT using the technique defined by Rojanasakul modified<sup>10</sup>.

### **Surgical procedure**

The procedure was carried out under regional anesthesia. Curved incision in the intersphincteric space over the path of the fistula was made and dissected until it was released. Ligation and cutting of the fistula was done, curettage of the distal path and enlargement of the external orifice (OFE) was performed. The modified technique included curettage of the proximal path with gauze and invagination of the ligation ends with resorbable material. The internal orifice (OFI) was not closed (modification proposed in other series) due to the theoretical risk of a new abscess. Demographic and clinical data were recorded in a prospective database, all patients had a preoperative clinical evaluation and an average postoperative follow-up of 48 weeks. Clinical

success was defined as the absence of symptoms and signs of fistula within 3 months of surgery. Incontinence was measured and classified by the Wexner scale<sup>12</sup>.

### Statistical methods

Data was recorded and analysed by using Statistical Package for Social Sciences (SPSS version 22.0). Mean of the continuous variables is presented along with range and frequency distribution was presented for categorical variables.

### RESULTS

Out of 22 patients underwent surgery for perianal fistula using the LIFT technique, 16 were males and 6 were females, as shown in Figure 1. The mean age was 42 years ranging between 23 and 68 years. 18 patients were ASA I and 4 ASA II while 17 patients had a history of perianal abscess drainage prior to surgery and mean preoperative symptom presentation was 2.2 years (range 0-10 years). In 16 patients, it was decided to install a non-cutting seton prior to final surgery. This was done in order to manage the infectious part of the fistula and to obtain a more fibrous path. Mean time between seton and LIFT was 60 days.

The LIFT was performed on 6 patients without prior settling. In the preoperative study, 20 patients underwent enhanced contrast pelvic MRI and 1 endosonography as described in Table 1. Twenty patients had a Wexner score of 0 points and 2 of 1 point in the preoperative assessment. Mean duration of surgical procedure was 39.7 minutes. All patients received a prophylactic antibiotic with 1 gram IV ceftriaxone and 500 mg IV metronidazole. None of the patients had intraoperative complications and all were discharged 24 hours post-operatively. At discharge, all patients were given oral antibiotics for 7 days (ciprofloxacin 500 mg every 12 hours and metronidazole 500 mg every 8 hours), pain relievers and nonsteroidal anti-inflammatory drugs for 3-5 days.

An average follow-up of 48 weeks was performed with a range of 24 to 96 weeks. Complete closure was achieved in 77% (n=17 patients). In postoperative follow-up, fistula recurrence was observed in 5 patients (23%) with a simpler fistula. All of them underwent fistulotomy along the way. After this second intervention, 90% clinical success was obtained in the follow-up. None of the 5 cases had reported any incontinence. No patient out of 22 has modified their Wexner at follow-up as shown in Table 2.

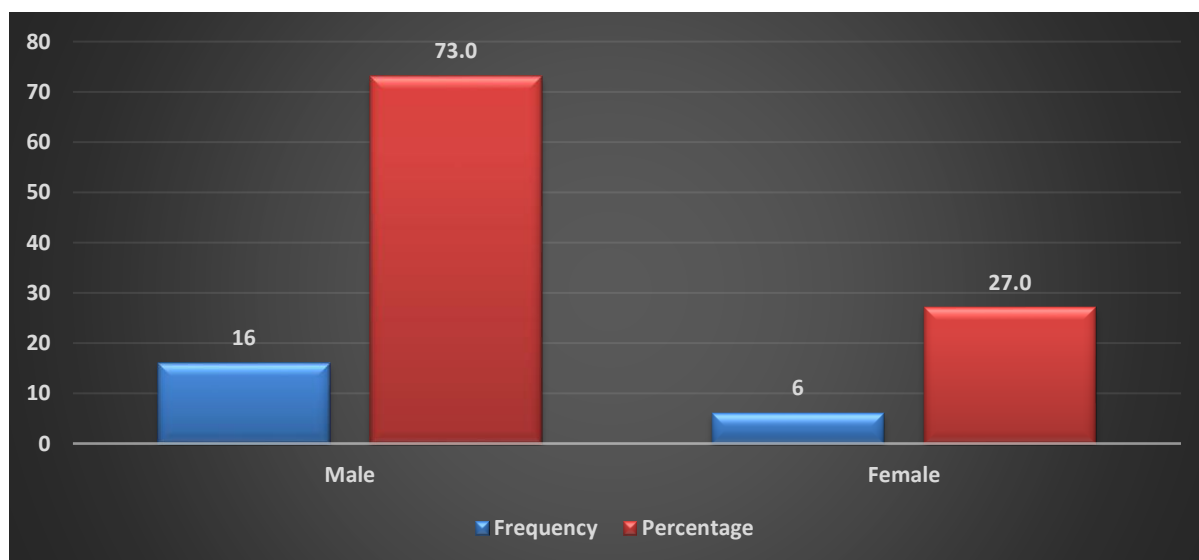


Figure 1. Distribution of the Gender of the patients included in the study

**Tables 1. Characteristics of the study patients (n=22)**

CHARACTERISTICS	NO. (% OR RANGE)
Mean age	42 years (23-68 years)
Mean symptoms time	2.2 years (0-10 years)
Previous abscess drainage	17
Fishing line 60 days prior	16
MRI-endosonography	20-2

**Table 2. Summary of results (n=22)**

Operated	22 patients
Mean operating time	39.7 minutes
ATB prophylaxis	22
Mean days of hospitalization	one
Mean postoperative EVA - High	0-1
Early complications	0
Tracing	48 weeks (24-96 weeks)
Full closure	In 2 stages *77% (17/22 patients)90% (20/22 patients)
Recurrence (all were treated with a simple fistulotomy)	23% (5 patients with OFE secretion, down staging)
Recurrence and previous line	4/5 had no line **
Wexner Modification	0

\* After the first LIFT, primary closure was obtained in 77% of the cases, 90% of closure was achieved after a second intervention in the relevant cases. \*\* Recurrence occurred in 4 out of 5 patients, in whom a seton was not positioned in the first fistulotomy.

## DISCUSSION

For a long time, the anal rectus mucosa advancement flap was considered the gold standard for the surgical treatment of complex fistulas with retention of sphincter function, showing success rates between 27-100%, but with incontinence rates of up to 35%<sup>8,9</sup>. Precisely in order to improve these results, the LIFT procedure has recently emerged as an attractive alternative for the surgical treatment of complex anorectal fistulas. Its principle is to treat the fistula and its origin without compromising the apparatus of the sphincter. It presents a final closing rate of 47% to 94% in different reported series, with no continence commitment in any of these series<sup>13</sup>. Other advantages of the procedure are low postoperative pain, quick reintegration into the workplace and lower economic costs compared to other techniques requiring the use of biological materials. Reviewing the literature, there is only one national publication on the subject, from the Bahawal Victoria Hospital Bahawalpur Pakistan. This work shows an experience in 30 patients subjected to ligation of Intersphincteric fistula tract. Twenty-five (83%) patients were cured and only five (17%) patients had fistula recurrence and were thus considered as not cured. None of the patient was presented with incontinence<sup>14</sup>.

The present study shows similar results of those who experienced the LIFT treatment, recorded in the literature. While the number of patients was small, the proportion of patients who had an indication to fistulotomy or other non-surgical procedures for a complicated and alternative treatment was similar to that. It should be noted that during the study period, 240 fistulas were operated at the main study center (Department of Surgery at Liaquat Univeristy hospital, LUMHS Jamshoro), of which only 22 (9%) were operated in the LIFT; this indicates that the option of performing the technique is very limited and is not currently one of preferred techniques of colorectal surgeons.

In relation to the patients in the present case, as in the series of patients with perianal fistula of cryptoglandular origin, these occur at intermediate ages, without significant comorbidities, and an outpatient procedure can

be provided comfortably, with regional anesthesia, without greater analgesic requirements and with rapid functional and occupational recovery. No patient without recurrence had to consult urgently when monitoring patients during the postoperative period and all were able to control their surgery-related discomfort with the indications provided at discharge<sup>15</sup>.

Analyzing the findings, considering the closure of the fistula as the main goal, this series indicates a closure of 77% only with the LIFT treatment and if we find closure in two stages after a more basic (intersphincteric) recurrence, the closure rate is close to 90% (20 out of 22 patients), with 100% of them without sphincter continence modification, which is the main objective.

When analyzing the recurrence cases (ie 5 patients, which makes only 23% of the total), 4 of them had a recurrence that was intersphincteric, as the literature indicates as a "downstaging" of a complex process in which the recurrence corresponds to the most frequent, proximal portion of the fistula, and that a simple fistulotomy can show good results and protection in the sphincter can be solved in a second period.

It is interesting that most primary success patients have previously installed a line, indicating that previous drainage and pathway conduction may affect the success of fistula healing, but the literature is not categorical in this regard, given the limited number of cases, and this variable cannot be assigned a meaning. Neither can we assume that the modifications in the procedure affect the success rate, but we postulate that the installation of a line in advance, the curettage with gauze of the fistulous tract, the closure of the internal orifice and the invaginating muscle plasticity of the ends of the ligation may impact better healing of the closure by decreasing the risk of recurrence.

## CONCLUSION

In conclusion, the LIFT technique appears to be a convenient, reproducible, and effective surgical alternative. This provides an appropriate closing rate and without continence modification, so we suggest it as a good option for cryptoglandular fistulas.

**Ethical Consideration:** The study was approved by Ethics Committee of Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan

**Conflict of Interest:** There is no conflict of interest.

**Funding:** This study was not funded by any agency

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## ANALYSIS OF FACTORS INHIBITING WOMEN FROM AVAILING ANTENATAL CARE FACILITY AT PRIVATE SECTOR OUTPATIENT DEPARTMENT

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### ABSTRACT

This study aimed to evaluate the factors influencing women to avail antenatal care among women attending outpatient department (OPD), of private sector hospitals in Hyderabad. This was an interview-based survey conducted during a period of six months (from 1<sup>st</sup> July 2019 to 31<sup>st</sup> December 2019) at OPD of private sector Hospital in Hyderabad. All the pregnant women and their female companions attending the antenatal OPD were included. Those women who regretted being part of the study were excluded. Participants of the study were interviewed regarding the knowledge and factors affecting utilization of antenatal care among women attending OPD as defined in a pre-set questionnaire. All the data was recorded in self-made proforma and analyzed by using SPSS version 20. A total of 500 pregnant women were interviewed regarding awareness of antenatal care. The study participants were grouped according to their age, 3% were 15-20 years of age, 30.9% were 21-30 years of age, 34.5% were 31-40 years of age and 31.5% were more than 41 years of age. Out of those who did not avail antenatal care, 8.5% were not allowed due to family restrictions, 47.3% reported ignorance, 9.7% lived far away from the facility, 24.2% could not afford it and 10.3% had other reasons of not availing antenatal care. The study concluded that the main reason for not utilizing antenatal care was ignorance by the women as well as their family members; affordability was also an important factors in a considerable number of participants.

**Key Words:** Antenatal care (ANC), pregnancy, factors, primigravida, grandmultigravida

## INTRODUCTION

Antenatal care is a program specifically designed for pregnant women provided from the period of conception till the onset of labor. It falls under the auspice of preventive medicine which deals with the early detection of general medical disorders, nutritional deficiencies, and immunological disorders. The program also includes the provision of health education and social medicine and early detection of pregnancy-related disorders which in turn provides timely management of existing co-morbidities and minimizes potential causes of maternal and neonatal mortality and morbidity (1). Organized antenatal care historically dates back to the beginning of the 20th century in Europe and North America and now it's a global facility with a well-developed system in more advanced countries(2).

Recent research reported a correlation of inappropriate antenatal care with poor pregnancy outcomes. Initially, following the World Health Organization (WHO) recommendations, four antenatal visits per pregnancy in normal conditions starting at the 12th week of gestational age (3) were advised however

according to recently revised suggestions a minimum level of care to be eight visits all over the pregnancy to reduce maternal morbidity and mortality (4). Maternal mortality is reported to be very high with the rate of 830 women passing away during or after pregnancy or childbirth-associated complications per day worldwide. In 2015, approximately 303,000 women died during pregnancy or childbirth. Such a high rate of maternal mortality around the world indicated disproportions in the provision of health care services where nearly all maternal deaths (99%) occurred in developing countries. More than 50% of these mortalities were reported from sub-Saharan Africa and approximately one-third from South Asia (5,6,7). Globally, while 85% of pregnant women access antenatal care with skilled health care providers at least once, only six out of ten (ie 58 %) be given at least four antenatal visits. Though the areas with high maternal mortality the rate of antenatal visits were even low. Regular contact with a doctor, nurse, or midwife during pregnancy allows women to get services crucial to their health as well as their baby. It is suspected that only half of pregnant women around the globe receive WHO-recommended four antenatal care visits during their pregnancy period (8).

Previous studies conducted in different countries on demographic and socio-cultural factors influencing the use of maternal health care services have shown that factors such as maternal age, number of living children, education, place of residence, occupation, religion, and ethnicity household level (women's autonomy, husband's attitude, husband's support, and family income) and health service level appeared to be significantly associated with the use of antenatal care (4,9,10,11,12,13,14). However, there is limited literature available looking at the influencing factors from Sindh. Therefore this study was designed to explore factors influencing utilization of antenatal care among women attending the outpatient department of a private sector hospital in Hyderabad.

#### **METHODOLOGY**

This study was an interview-based survey conducted at OPD of the private sector in Hyderabad. The study duration was 6 months from 1<sup>st</sup> July 2019 to 31<sup>st</sup> December 2019. All the pregnant women and their female companions attending the antenatal OPD were included in the study. Women who were not willing to participate in the study were excluded. After taking informed consent women were interviewed regarding information about the factors affecting utilization of antenatal care among women attending OPD, demographic information regarding age, education, occupation, socioeconomic status, and residence were obtained from the participants All the data was recorded in pre-defined proforma and data was analyzed by using SPSS version 20.

#### **RESULTS**

A total of 500 women were interviewed, according to their age, 3% were 15-20 years of age, 30.9% were 21-30 years of age, 34.5% were 31-40 years of age and 31.5% were more than 41 years of age. Out of 500 respondents, 15.8% were primigravida, 49.7% were multigravida and 34.5% were found to be grand-multigravida (Table 1).

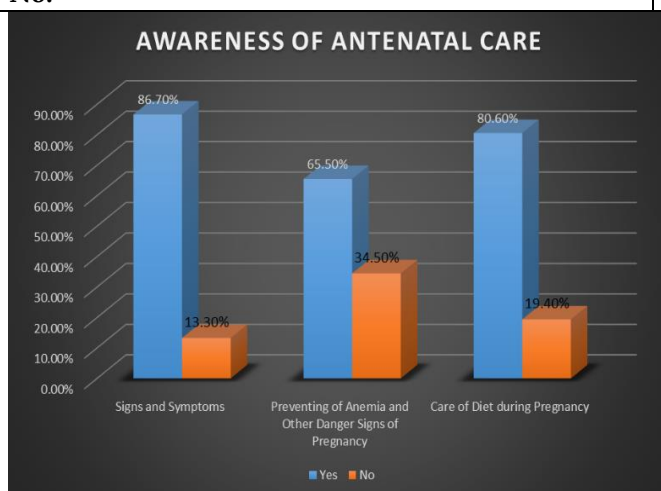
Majority (ie 44.2%) were reported to be from urban areas while the rural residents were 33.9% and 21.8% of patients resided in semi-urban. Most of the participants were 40% lower financial class and 40% middle class. While only 20% belonged to the upper-middle class. In our study, 52.1% of women were illiterate while the rest 47.9% were literate and 63% had Literate husbands while 37% were married to illiterate. There were 74.5% of women who were reported to be aware of antenatal care and 25.5% were unaware. 86.7% reported to be experiencing signs and symptoms of pregnancy whereas 13.3% did not report any symptoms. 65.5% of women knew the benefits of antenatal care regarding prevention of anemia and other dangerous signs of pregnancy while 34.5% did not know about it. 80.6% of women took care of their diet in pregnancy whereas 19.4% did not take any effort (Figure 1).

66.7% of women were acknowledged the antenatal care benefits and 33.3% were ignorant. In 50.3% of cases, husbands were the decision-makers, 21.8% of women made the decision themselves, Father/Mother-in-law was responsible for deciding 16.4% of cases, and 11.5% of women had other means(Figure 2). Out of those who did not avail antenatal care, 8.5% could not get permission from relatives to go to the antenatal care facility, 47.3% cited ignorance about the

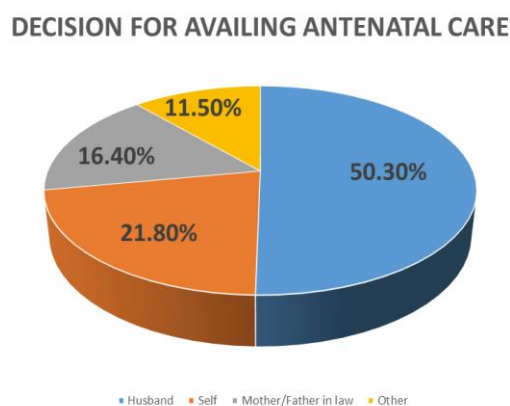
importance of antenatal care, 9.7% said that antenatal care facility was far away, 24.2% could not afford it and 10.3% had other reasons of not availing antenatal care (Figure 3).

**Table. 1. Summary of the demographic characteristics of the study population**

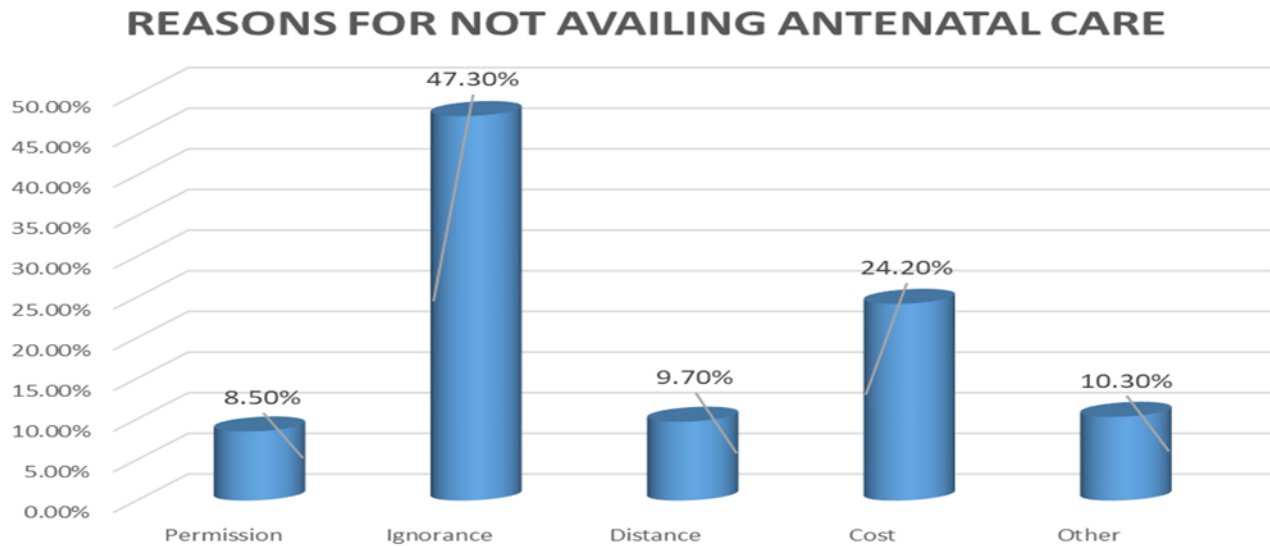
Demographic data	Frequency	Percentage
<b>Age</b>		
15-20 yrs	15	3%
21-30 yrs	154	30.8%
31-40 yrs	170	34%
>41 yrs	161	31.2%
<b>Parity</b>		
Primigravida	77	15.4%
Multigravida	245	49.9%
Grand-Multigravida	178	35.6%
<b>Residence</b>		
Urban	220	44%
Semi-urban	105	21%
Rural	56	35%
<b>Socio-economic Status</b>		
Upper	100	20%
Middle	200	40%
Low	200	40%
<b>Literacy rate:</b>		
Literate:	211	42.2%
Illiterate :	289	57.8%
<b>Awareness of antenatal care:</b>	374	
Yes	126	74.80%
No		25.20%
<b>Awareness of benefits of antenatal care:</b>	333	
Yes:	167	66.6%
No:		33.4%



**Figure 1. Summary of the participants regarding knowledge of signs and symptoms, complications, and diet care during pregnancy**



**Figure 2. Summary of the data regarding antenatal care decision of the participants**



**Figure 3. Summary of the factors inhibiting women from availing antenatal care facility**

## DISCUSSION

Four pillars for safe motherhood concept suggested by WHO includes family planning, antenatal care, clean & safe delivery & emergency obstetric care(1,6). Maternal death is an important issue of developing countries and it can be prevented by identifying & treating its common causes in their antenatal period. By giving adequate antenatal and obstetric care most perinatal deaths can be prevented.

Our study evaluated the factors which can potentially affect the utilization of antenatal care among women attending a private hospital in Hyderabad. In our study 3% of women were 15-20 years of age, 30.9% were of 21-30 years, 34.5% were 31-40 years and 31.5% were more than 40 years of age. Age is an important factor because it serves as a proxy for women's accumulated knowledge of health care utilization, where older women were more likely to have maternal care as compared to their younger counterparts. However, there are conflicting findings available in literature where some suggested younger age as a determinant of antenatal care while others suggested that advancing age was reported to be associated with more utilization of antenatal care (15,16,17,18,19).

Education plays a significant role in the utilization of antenatal care. In our study 52.1% women and 37% husbands were illiterate, here it is worth mentioning that even in the illiterate category, all those included had no years of schooling and could just read and write simple words, it may be the quality of education which increases the likelihood of using antenatal care services, educated couples are more aware of health issues, complications, and benefits of using health services. It is suggested that education empowers the female in terms of seeking health services, these mothers may have better chances to get health information and show more concern towards healthcare (4,20,21). It has been previously observed that mothers with less education were likely to have less information about antenatal care and added difficulties in its access. A study conducted in Central Ethiopia concluded that women with little schooling were more than two times more likely to avail of antenatal care (OR=2.645) as compared to those who had no education and similar results were observed in different studies done in North Ethiopia, Nigeria, and China (,20,21,22).

The Pakistan integrated household survey (PIHS) 96-97 shows that only 30% (urban 54%, rural 22%) of women who had given birth in the last three years had antenatal consultations, in our study the ratio of



women utilizing antenatal care was 74.5% and 25.5% were not utilizing antenatal care in south Asia the ratio of utilization is 52% and 68% in rest of the world(23).

The result of our study showed that the most common reason for not taking antenatal care was ignorance, while permission from family members, distance from health care centers, unaffordability were found to influence the use of antenatal care. According to a previously reported study carried out in Nurpur shah, the main factor was permission (60%) from family. Another study in Sindh showed that the main reason for not utilizing antenatal care was long distance(24,25). As lack of money was also a reason for not utilizing antenatal care in 24.20% in our study population, poverty, financial crises were also important factors inhibiting women from seeking the health care facilities, similar findings were observed in previously reported studies(20,21,22). Far-reaching hospitals were reported to be the important factor inhibiting women from seeking antenatal care, studies conducted in remote areas of Africa also suggested that far away antenatal clinics were a major problem of third world countries because lack of transport and long-distance walk is troublesome for pregnant women (26,27) .

As the main reason for not having antenatal care in our study was women being ignorant, therefore there is a dire need to continue educating expectant mothers and women in general public on regular basis. Government should make an effort to set up health centers in rural areas to improve utilization and to eliminate the problem of transportation.

### CONCLUSION

We concluded that the main reason for not utilizing antenatal care was ignorance by the women as well as their family members; this ignorance may be due to lack of knowledge or illiteracy. While other factors such as permission from family members or husbands, lack of money, a long distance from the health care centers were also involved to avoid antenatal care.

**Ethical Consideration:** The study was approved by Local Ethics Committee.

**Conflict of Interest:** There is no conflict of interest.

**Funding:** This study was not funded by any agency

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## PREVALENCE OF MUSCULOSKELETAL IMPAIRMENT IN TRAFFIC POLICE OF DISTRICT SWABI, PAKISTAN

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### ABSTRACT

The number of vehicles has been increasing out of proportion for the capacity of road infrastructure in particular in developing countries. The duty of a traffic police officer includes the implementation of daily traffic regulations. This involves long-standing hours and also particular repeated body movements. There is limited literature available on the musculoskeletal disorders develop among traffic police. Therefore this study was designed to include 219 traffic police officers from 23 traffic booths of district Swabi, Pakistan. Data was collected using a structured questionnaire and Cornell Musculoskeletal Discomfort Questionnaires (CMDQ). Informed consent was taken before filling the questionnaire. Age distribution of the participants was grouped as: 20 to 30 years (n=51, 23.4%), 31 to 40 years (n=141, 64.7 %), and over 40 years (n=26, 11.9%). Out of 219, 23% were diabetics, hypertensive participants were 21.9% (n=48), while 54% (n=120) had no history of chronic illness. The majority of traffic cops in district Swabi reported mild discomfort in their shoulders, arms, forearms, thighs, lower legs, knees, and feet, but moderate discomfort in their neck and back. The results of the study indicate that the traffic cops in the district of Swabi suffer back and neck pain, causing them to have moderate difficulty doing their duties.

**Key Words:** Traffic police, CMDQ, musculoskeletal impairments, WRMSDs

### INTRODUCTION

Musculoskeletal dysfunction has been identified as an occupational health issue in the working population resulting in reduced working capacity and productivity. Given the pattern and hours of duty, traffic police officers (TPP) are the most vulnerable category, as they are required to manage a busy street for extended periods in difficult conditions(1-2) Traffic cops are required to stand for the whole of their shifts, putting them at risk for significant musculoskeletal ailments. Additionally, being in a static posture exposes them to ergonomic risks. Because of the rising number of automobiles, many cities have experienced traffic congestion, making TPP's job even more difficult and complex. Traffic cops face occupational hazards related to their duties and obligations while managing a complicated traffic system. (3) They are also exposed to physical variables like noise, vibration, and radiation, which pose an additional health risk. While working on a busy and congested road, noise-induced hearing loss is a major health problem among TPP. They are susceptible to heat sensitivity and light-related illnesses as a result of their exposure to heat and light. Carbon oxyhydroxide,

sulfur oxyhydroxide, nitrogen oxyhydroxide, lead oxyhydroxide, and benzene oxyhydroxide oxyhydroxide oxyhydroxide oxyhydroxide oxyhydroxide TPP are also subjected to psychological stress as a result of the hurried nature of their employment, lengthy duty hours, traffic congestion, and monotonous nature of their labor. Taking these factors into account, TPPs are at higher risk of developing cardiovascular problems as well(4-7). A whole spectrum of inflammatory and degenerative cascades start causing pain and functional impairment particularly involving the neck, shoulders, elbows, wrists, and hands, according to the definition of work-related musculoskeletal disorder (WRMSD)(8) The prevalence of WRMSD has been reported to be high among TPP and is linked to work hours, years worked, awkward and static postures, repetitive movements, uncomfortable postures, vibration, manual handling, and other factors. This puts a strain on the joints and causes various musculoskeletal disorders, which is the leading cause of workplace absenteeism(9). WRMSD is characterized by aching, pain, or discomfort in the neck, shoulder, elbow, wrist, upper back, lower back, hips/thigh, knee, and ankle/feet(10-11). Low back pain (LBP) is the most common health problem among WRMSD all over the world (12). According to the World Health Organization (WHO) report from 2013, low back pain was the leading cause of time off work and visits to the doctor. Around 70% to 80% of the world's population will have at least one episode of low back pain during their lifetime(13). Low back discomfort is the most common ailment among workers due to a lack of information about good posture. It's also a primary cause of sick leave, productivity loss, and long-term occupational disability(14). Given the ever growing traffic congestion in Pakistan, TPPs are also at higher risk of musculoskeletal disorders. However there is limited literature available. Thus this study was conducted to look at the pattern of musculoskeletal disorders reported by TPPs in the district Swabi.

## **METHODOLOGY**

After receiving ethical approval, this cross-sectional study was conducted. Data was acquired from a total of 219 traffic police of district Swabi, KPK, using non-probability convenient sampling. The sample size was computed using a 95% confidence level. Work-related musculoskeletal pain, male, road traffic police, 20-60 years old were the inclusion criteria. Infection, tumor, trauma, recent fracture, and female were all ruled out. A questionnaire including past medical history chart and Cornell Musculoskeletal Discomfort Questionnaires (CMDQ) were used to collect data after getting informed consent. Participants completed the questionnaire whether or not they were in pain. The pain was separated into the following body areas: shoulder, arm, forearm, wrist, neck, lower back, leg, knees, and foot. Statistical Package for Social Sciences (version 23,0) was used for data analysis.

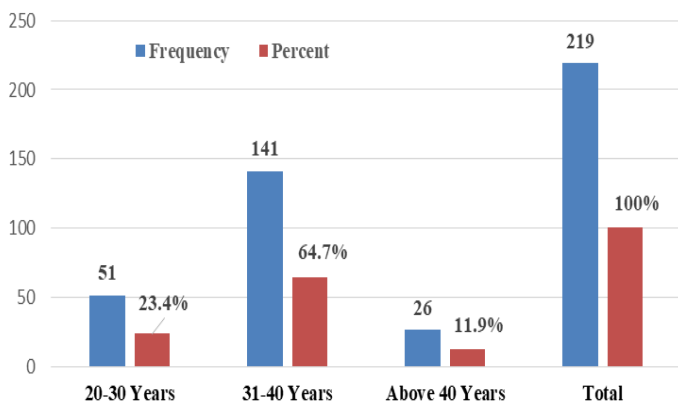
## **RESULTS**

A total of 219 males were included in the study. Ages of 20 to 30 years were represented by 51 (23.4%), 31 to 40 years by 141 (64.7 %), and over 40 years by only 26. (11.9) (Figure 1). The majority of the participants were between the ages of 31 and 40 years. Past medical history mainly focused on three categories including diabetics, hypertension, and none of these chronic medical conditions (Figure 2). Diabetic participants made up 23% (n=51), hypertensive participants made up 21.9 percent (n=48), and those with no prior history of disease made up 54.8 percent (n=120). Mild discomfort was reported by 28 (12.8%), moderate discomfort by 118 (54.1%), and severe discomfort was reported by 72 (33%). Table 1 presents summary of the pain distribution among participants.

**Table 1. Summary of pain distribution reported by traffic Police Officers**

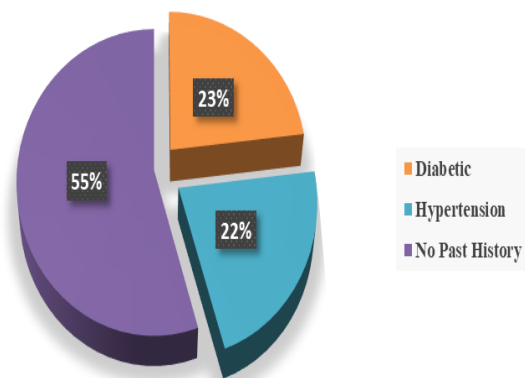
	N	Mean	Standard deviation
Neck	219	2.2018	2.2018
Right Shoulder	219	1.2156	1.2156
Left Shoulder	219	1.2523	1.2523
Upper Back	219	1.4587	1.4587
Right Upper Arm	219	1.0000	1.0000
Left Upper Arm	219	1.0000	1.0000
Lower Back	219	2.0917	2.0917
Right Forearm	219	1.2569	1.2569
Left Forearm	219	1.1284	1.1284
Right Wrist	219	1.0000	1.0000
Left Wrist	219	1.0000	1.0000
Hip/ Buttocks	219	1.0000	1.0000
Right Thigh	219	1.0000	1.0000
Left Thigh	219	1.0000	1.0000
Right Knee	219	1.1147	1.1147
Left Knee	219	1.0872	1.0872
Right Lower Leg	219	1.0367	1.0367
Left Lower Leg	219	1.0000	1.0000
Right Foot	219	1.2248	1.2248
Left Foot	219	1.1468	1.1468

**Age in Years**



**Figure 1. Age group distribution of the participants**

**Frequency of Past Medical History**



**Figure 2. Distribution of the chronic health reported by Traffic Police officers**



## DISCUSSION

Musculoskeletal disorders are a frequent health concern that affects people from all walks of life(15). These illnesses have resulted in enormous human misery, as well as diminished working ability and production(16). Research conducted in 1995 A.D. in one of China's main cities estimated that 1.2 million men and women were suffering from work-related musculoskeletal complaints. Lower back, neck, shoulder, and upper back were the most commonly affected areas, with prevalence rates of 28.0 percent, 24.0 percent, 18.6 percent, and 15.5 percent, respectively. About half of workers with MSD reported pain or discomfort in less than a month.(17) Physical stress was identified by Burton et al. in 1996 as one of the occupational risk factors for poor LBP among police officers controlling traffic, which often resulted in higher sickness and absenteeism(18).

As a result, stress can cause a variety of physical symptoms, such as muscle strain and back pain. In 2014 a study was conducted including 353 participants, the rate of pain was 44.2% in the shoulder, 41.4 percent in the waist, 31.2 percent in the neck, 26.1 percent in the legs/foot, 16.7 percent in the hands/wrist/fingers, and 14.7 percent in the arms/elbows, indicating that the global burden of pain was 44.2 percent in the shoulder, 41.4 percent in the waist, 31.2 percent in the neck, 26.1 percent in the legs/foot Furthermore, the shoulder had a 4.87 times higher risk in police lieutenants compared to those under the rank of corporal, and a 1.78 times higher risk in people with chronic diseases compared to those without chronic diseases, according to the comparative risk of the relevant part that was analyzed(19). In a recent study, 384 traffic cops in Pakistan were interviewed. 69 percent felt discomfort in their upper extremities, while 54 percent had pain in their lower extremities felt pain radiating to other parts of the body. In this study, the participants' pain was caused by their long working hours(20). The results of this study show that the prevalence of WMSDs varies significantly between participants with varying years of job experience. Participants with >30 years of job experience had the highest prevalence of neck, back and foot pain discomfort followed by those with 20–30 years of work experience. This finding suggests that having more years of work experience is linked to a higher prevalence of WMSDs, which is consistent with findings from a previous study(21), which found that having more years of work experience was a predisposing factor to the development of WMSDs. Furthermore, service length is linked to musculoskeletal problems(22). According to official Swedish statistics, MSK disorder accounts for roughly 74 percent of occupational diseases (23). The most painful areas in this study were the neck and back. According to one study, MSK pain is very common among Ibadan drivers, and the most common MSK pain region is the low back,(24) which is similar to a study in Mumbai, India.(25)

The current study suggests that the district Swabi, KPK traffic police are affected moderate discomfort of pain in neck and back but they are just mild discomfort of the shoulder, arm, forearm, thigh, knee, lower leg, and foot. Which concluded that the traffic police of District Swabi are feeling discomfort because of neck and back pain.

## CONCLUSION

The majority of traffic cops in district Swabi reported mild discomfort in their shoulders, arms, forearms, thighs, lower legs, knees, and feet, but moderate discomfort in their necks and backs, according to the survey. This indicates that the traffic cops in the district of Swabi are suffering from back and neck pain, causing them to have moderate difficulty doing their duties..

**Ethical Consideration:** This study was approved by research Ethics Committee

**Conflict of Interest:** There is no conflict of interest.

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## RICKETTSIAL AGENTS DETECTION FROM BLOOD OF TICK-INFESTED ANIMALS IN LOWER SINDH- COMPARISON OF CONVENTIONAL AND MOLECULAR APPROACH

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### ABSTRACT

Ticks are important vectors of human and animal pathogens. They are considered as main vectors for transmission of rickettsial agents affecting animal and human health. The study was designed to investigate district wise pattern and detection of rickettsial agents by using molecular and conventional techniques in blood samples of infected cattles, buffalos, sheep and goats. A survey study was carried out in lower Sindh (Tharparkar, Badin, Hyderabad, Karachi, Tando Muhammad khan, Thatta and Mirpurkhas). Blood samples were collected randomly from infected Cattles, buffalos, sheep and goats and transported to the Molecular Parasitology laboratory, Sindh Agriculture University, Tandojam, followed by examinations under stereomicroscope and Polymerase Chain Reaction (PCR). The study showed that overall infection of Rickettsial agents among infected animals was recorded following Microscopy/ Blood smear test in cattles, buffalos, sheep and goats was 41.79, 49.09, 46 and 41.66% respectively, whereas overall infection through PCR in cattle, buffalo, sheep and goat was 39.55, 43.55, 46 and 55.55% respectively. Whereas animal-wise data through PCR indicates that in case of Goats (55.55%) were more susceptible to rickettsial infection as compared to sheep (46%), buffaloes (43.55%) and cattle (39.55%). The highest rate of rickettsial agents was found in district Tharparkar and lowest rate was found in district Karachi. Microscopy/Blood smear method indicates that Buffaloes were more susceptible for infection. Whereas PCR indicates Goats were more susceptible for infection.

**Key Words:** Ticks (Vectors), Rickettsial agents (Pathogens), Molecular and Conventional techniques, Lower Sindh

### INTRODUCTION

Ticks are potential vectors and reservoirs of many infectious agents such as. *Pasteurella multocida*, *brucella abortus* and *salmonella typhimurium* in both humans as well as animals (1). Ticks have enormous capability to adapt to changing geoclimatic conditions and can therefore expand their distribution range (2). They are known as main vectors for transmission of many pathogens such as viral, bacterial, rickettsial and parasitic infestations (3). Till date 899 species of ticks are known which belong to three families, namely Ixodidae, Argasidae and Nuttalliellidae (represented by a mono- typic species restricted to South Africa (4). After sucking blood, the outer surface of a tick grows to 200-600 times as compared to its unfed body weight (5). Prevalence of tick-borne pathogens (TBPs) and their occurrence in bovines have been found all over the

Pakistan (6). *Hyalomma anatolicum* transmitted some of tick-borne pathogens (TBP) which have zoonotic importance (e.g. Crimean Congo haemorrhagic fever) (6). Generally, rickettsioses is the term used for those diseases which have continuous spectrum of severity of illness and overlapping clinical manifestations. *R. rickettsii*, *R. prowazekii*, *R. conorii*, and *R. typhi* are rickettsial agents with a potential to cause life-threatening diseases (7). The main cause of granulocytic anaplasmosis is *Anaplasma phagocytophilum* which is considered as one of the most important species from humans' point of view because of its zoonotic potential.

Ticks are known as etiological agents of tick-borne fever in ruminants and equine, canine and human granulocytic anaplasmosis (EGA, CGA and HGA, respectively) (8). Genetic diversity has been recognized among various European strains of *A. phagocytophilum* shown through phylogenetically analysis of genes such as groEL (chaperone protein encoding gene) (8) Rickettsiae are commonly defined as genetically related, obligatory intracellular bacteria that reside in an arthropod host during a part of their zoonotic cycle. (9) Ticks as parasites are vectors of many important human and animal pathogens such as Q fever Babesiosis, tick paralysis, haemorrhagic fever, Lyme disease (LD), tick-borne encephalitis and tick-borne muscular fever. Rocky mountain spotted fever, which is caused by *Rickettsia rickettsii*, is a life-threatening, tick-borne disease that occurs throughout much of the United States (11). It has been estimated that 10% of the known tick species act as vectors of the pathogens of above mentioned diseases (12). They also pose a great threat to global animal production in terms of economic expenditure incurred through treatment of various inflammatory and hematologic conditions that occurred in humans and animals through these tick-borne diseases. It has further been suggested that around 80% of cattle production worldwide is at increased risk of tick-borne infections. (6) *Rickettsia* have a comparatively small genome developed through reductive evolution because of their dependence on the host for survival and to carry out essential functions. (18) The genomes of various species of rickettsia have been sequenced such as *Rickettsia prowazekii* and *Rickettsia conorii* (19). There was limited literature available looking at the presence of rickettsial organisms in ticks in lower Sindh. Therefore, this study was aimed to investigate district wise pattern and compare detection of rickettsial agents by using molecular and conventional techniques in blood samples of infected cattles, buffalos, sheep and goats.

## METHODOLOGY

A survey was conducted in lower Sindh including Tharparkar, Badin, Hyderabad, Karachi, Tando Muhammad Khan, Thatta and Mirpurkhas districts. Blood samples were collected randomly from infected Cattle, buffalos, sheep and goats and transported to the Molecular Parasitology laboratory, Sindh Agriculture University, Tandojam, followed by examinations under stereomicroscope and Polymerase Chain Reaction (PCR)(24).

### Blood Collection

Host that carried ticks were selected for blood sampling, 5 ml blood from each infested host was collected from jugular vein or ear vein from large and small animals respectively. The blood transferred to Ethylene diamine tetra acetic acid (EDTA) containing tubes and stored until further diagnosis of pathogen (Viz. Blood Filming and DNA Extraction) was carried out.

### Blood Sampling Procedure

Hairs from collection site were removed using automatic hair shaver. Cotton swab soaked in the antiseptic (alcohol) was applied for disinfection to avoid any secondary contamination in the sample. Ear vein was gently punctured with sterilized needle and blood was allowed to ooze out. A thin and thick blood smear was prepared fixed on spot in 70% alcohol to avoid rupturing of erythrocytes. In case of collection from jugular vein, syringe was gently used and 5ml of blood was drawn and preserved in EDTA tubes. The blood vials were soaked by rotating between palms of two hands for proper mixing of the anti-coagulant. The collection tubes were labelled with the name of owner, type of host and refrigerated at -20° C. Relevant information on

host, sex, age and date of collection was obtained and recorded on a Proforma specifically designed for this project.

### **Blood Smear Method**

Two methods were applied for blood examination viz. thin and thick blood smear(s).

#### **Thin Smear method**

For making thin blood smear, a glass slide was dipped in 95% alcohol. About 2ul of blood was placed on one end of the slide (called microscopic slide). Another slide (called spreader slide) was placed on microscopic slide containing the droplet of blood, positioning it about an inch in front of the droplet. The spreader slide was quickly run on the surface of microscopic slide at angle of 45 degrees. In a smooth motion, the spreader slide was pushed forward to spread the blood in a layer. Prepared blood slide was allowed to air dry for one minute and fixed in absolute alcohol for 5 minutes. Slides were removed from alcohol jars and air dried. Dried slides were stained in freshly prepared Romanowsky stain (commonly called Giemsa's stain) for 5 minutes.

#### **Thick Smear method**

Procedure for making thick blood smear was same except that the spreader slide was moved slowly to make a thick film on microscopic slide.

### **Nucleic Acid Extraction from Blood**

DNA was extracted and obtained from collected blood by commercial kit (GeneJET Genomic DNA purification Kit #K0722, Thermo Scientific, USA) as per manufacturer's instructions. 20 µl of Proteinase-K solution and 400 µl of lysis solution were added to 200 µl of whole blood. The mixture was mixed by vortexing in order to obtain a uniform suspension. It was then kept in incubation at 56° C for around 10 minutes or till the cells were completely hemolysed. Afterwards, ethanol in a quantity of 200 µl was added and vortexed. The solution obtained was then transferred to GeneJET genomic purification column and was centrifuged at 6000xg for up to one minute. The flow through solution in the collection tube was discarded whereas purification column was transferred in a new collection tube. Wash buffer (500 µl) was added to this collection tube which was then centrifuged at 8000xg for one minute. The flow through solution was again discarded while transferring the purification column to a new collection tube to which 500 µl of wash buffer 2 (with ethanol already added) was added and further centrifuged at 12000xg for three minutes. The purification column was transferred into 1.5ml micro tube whereas collection tube containing flow through solution was again discarded. 200 µl of elution buffer was added to 1.5 ml micro tube containing purification column and it was then incubated at room temperature for two minutes and then centrifuged at 8000xg for one minute. The DNA thus extracted is obtained by discarding the supernatant and its concentration was evaluated by spectrophotometer (Thermo scientific Nano drop 1000).

### **PCR Process**

Table-1 shows components and volume used in PCR process, the sample tubes were loaded in Thermal cycles (Applied Bio- system, USA). The cycles were already set. The lid of machine was closed to start the operation. DNA was denatured at 94oC for 5 min. Annealing process took place at 55oC for 1 min. Two complementary copies of DNA were obtained from one DNA at 72oC for I min, the cycle again started from 94oC. The PCR product was subjected to electrophoresis.

**Table -1 Components used in PCR process.**

Components	Volume
Master mix	25 µl
Piro Primer(F)	8 µl
Piro Primer(R)	8 µl
DNA extract	2 µl
Distilled water	7 µl
Total	50 µl

## Method

All primers were diluted with 20ul of TE Buffer. Piro primer (F) =8µl was added in 25 µl of master mix in a small tube (Neptune company). Piro primer (R) =8µl was added in 25 µl of master mix in a small tube (Neptune company). 2µl of DNA extract were added in Piro (F) and (R) primers respectively.

### Agarose Gel (1%)

Agarose gel powder was taken in a quantity of 0.5 grams in a conical flask. 50 ml of 0.5 TAE buffer was then added to the agarose powder and microwaved for about one minute in order to dissolve the powder. It was then allowed to cool down to 60° C. afterwards, 2 µl of ethidium bromide was added to gel solution and it was then poured down slowly into the tank. Comb was correctly positioned in the tank and it was then left for at least 30 minutes to solidify. Before using the gel, it was submerged in 0.5 TAE buffer in the tank.

**Table-2 Primers used**

Primers	Nucleotides	Species	References
PIRO-F	AATACCCAATCCTGACACAGGG	All Piroplasms	Karimi <i>et al.</i> ,2012
PIRO-R	TTAAATACGAATGCCCCAAC	All Piroplasms	Karimi <i>et al.</i> ,2012
Bi-F	AATAACAATACAGGGCTTTCGTCT	Babesia bigemina	Kim <i>et al.</i> , 2007
Bi-R	ACGCGAGGCTGAAATACAAC	Babesia bigemina	Kim <i>et al.</i> , 2007
<i>T. annulata</i> -F	CACCTTCGACAAGAAAGAAGTCGG	Theileria	Designed in Mathe: USA
<i>T.annulata</i> -R	TGAGAAGACGATGAGTACTGAGGC	Theileria	Designed in Mathe: USA

### Sample loading

Before loading the samples in the gel, DNA ladder (Fermantas EU) was loaded down in the very first well of agarose gel in order to quantify the size of the samples. Afterwards, 4 µl of each sample was loaded in the subsequent wells. After all samples have been added, electrophoresis unit was allowed to run with 80 volts and 100 amperes for 30-45 minutes allowing samples to travel a sufficient distance.

### Gel Documentation

After electrophoresing the samples, the gel was removed and put in gel documentation system (Clever Scientific, Ltd, UK) in order to visualized the bands of samples and to determine their size by comparing them with the ladder.

### Statistical methods

Statistical package for Social Sciences (SPSS Version 21) was used for results analysis. Frequencies and percentages were analysed and presented.

## RESULTS

Blood samples were collected from different districts of lower Sindh, in order to compare sensitivity of polymerase chain reaction with that of blood smear method, Table-3 and 4 reveals the detection of rickettsial agents through blood smear method then blood samples were subjected to PCR for detection of rickettsial infection. Table-5,6 and 7 reveals that PCR is more sensitive diagnostic method as samples that were negative via blood smear method were found positive when diagnosed through PCR. Presence of rickettsial agents through blood smear test was confirmed under high power magnification, whereas through PCR the detection was confirmed by looking at the bands that appeared at 405, 150, 170 and 290 base pairs on gel documentation.



(Figure-1-3), Table- 3 to 6 shows data on Cattle, Buffalo, Sheep and Goat that were diagnosed positive for rickettsial agents via blood smear test and PCR, the highest ratio of differences in districts was found in District Tharparkar 83:83, 81:87, 66:66, 57:71 in cattle, buffalo, sheep and goat, whereas lowest ratio of differences is found in district Karachi was 36:30, 45:41, 25:25, 28:42 in cattle, buffalo, sheep and goat respectively.

**Table 3. Detection of rickettsial agents in Cattle and Buffalo through blood smear method in lower Sindh**

Districts	CATTLE				BUFFALO			
	Observed	Infested	Random blood samples	Infected samples (%)	Observed	Infested	Random blood samples	Infected samples%
Karachi	96	33	12	36.36	117	24	11	45.83
Hyderabad	27	20	8	40	67	33	14	42.42
Badin	27	17	6	35.29	26	15	7	46.66
Tharparkar	10	6	5	83.33	20	16	13	81.25
T.M Khan	59	24	10	41.66	119	35	16	45.71
Mirpurkhas	37	24	11	45.83	73	29	14	48.27
Thatta	16	10	4	40.00	44	11	5	45.45
Total	272	134	56	41.79	466	163	80	49.07

**Table 4. Detection of rickettsial agents in Sheep and Goat through blood smear method in lower Sindh**

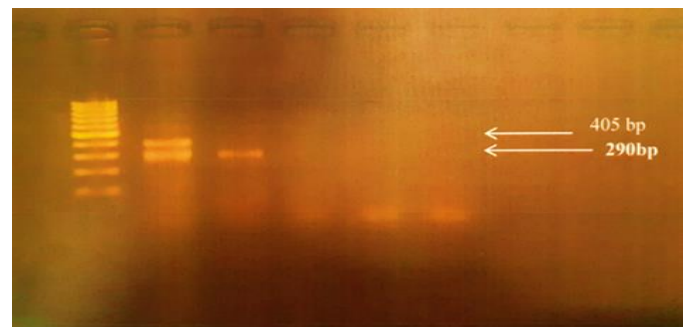
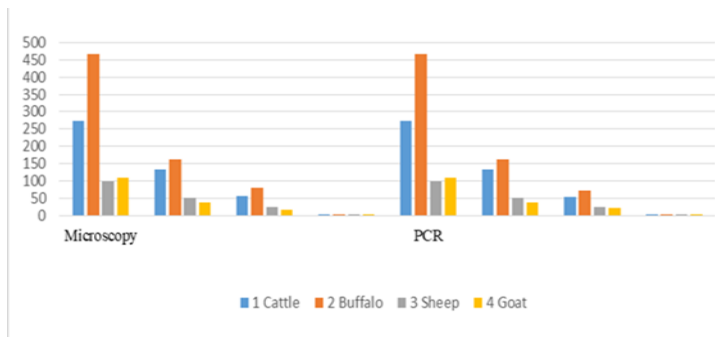
Districts	SHEEP				GOAT			
	Observed	Infested	Random blood samples	Infected samples %	Observed	Infested	Random blood samples	Infected samples %
Karachi	11	4	1	25	32	7	2	28.57
Hyderabad	15	6	2	33.33	10	3	1	33.33
Badin	18	14	5	35.71	16	5	2	40
Tharparkar	12	3	2	66.66	13	7	4	57.14
T.M Khan	15	5	2	40.00	16	5	2	40
Mirpurkhas	9	3	1	33.33	12	4	2	50.00
Thatta	14	4	2	50.00	10	5	2	40.00
Total	94	39	15	38.46	109	36	15	41.66

**Table 5. Detection of rickettsial agents in Cattle and Buffalo through PCR in lower Sindh**

Districts	CATTLE				BUFFALO			
	Observed	Infested	At Random blood samples	PCR detection(%)	Observed	Infested	At Random blood samples	PCR detection%
Karachi	27	20	6	30	67	33	9	27.27
Hyderabad	96	33	10	30.30	117	24	10	41.66
Badin	27	17	7	41.17	26	15	6	40
Tharparkar	10	6	5	83.33	20	16	14	87.5
T.M Khan	59	24	9	37.5	119	35	13	37.14
Mirpurkhas	37	24	10	41.66	73	29	12	41.37
Thatta	16	10	6	60.00	44	11	7	63.63
Total	272	134	53	39.55	466	163	71	43.55

**Table 6. Detection of rickettsial agents in Sheep and Goat through PCR in lower Sindh**

Districts	SHEEP				GOAT			
	Observed	Infested	At Random blood samples	PCR detection%	Observed	Infested	At Random blood samples	PCR detection%
Karachi	11	4	1	25	10	3	1	33.33
Hyderabad	15	6	2	33.33	32	7	3	42.85
Badin	18	14	5	35.71	16	5	3	60
Tharparkar	12	3	2	66.66	13	7	5	71.42
T.M Khan	15	5	2	40.00	16	5	3	60
Mirpurkhas	9	3	1	33.33	12	4	2	50.00
Thatta	14	4	2	50.00	10	5	3	60.00
<b>Total</b>	<b>94</b>	<b>39</b>	<b>15</b>	<b>38.46</b>	<b>109</b>	<b>36</b>	<b>20</b>	<b>55.55</b>

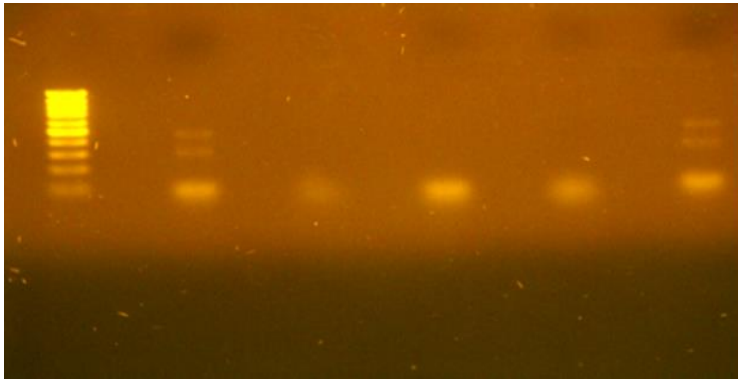


**Graph-1 showing the comparison between Microscopy and PCR** **Figure-1 Gel electrophoresis of amplified PCR products of rickettsial agents of buffalo blood DNA**

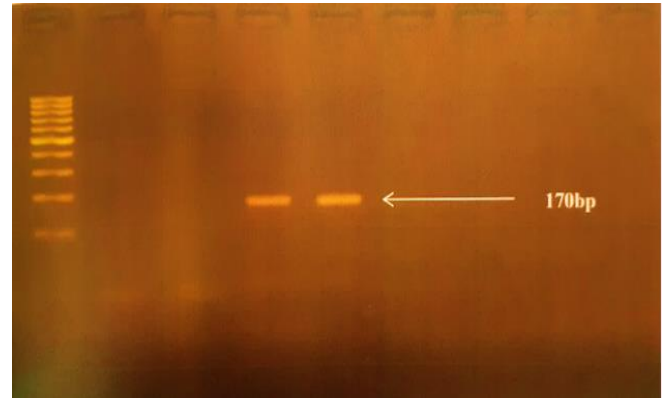
Table-7 shows the pooled data of all districts indicates that through blood smear test Buffaloes were (49.09%) more susceptible to rickettsial infection as compared to sheep (46%), cattle (41.79%) and goats (41.66%), Whereas through PCR, the data indicates that in case of goats 55.55% were more susceptible to rickettsial infection as compared to sheep (46%), Buffaloes (43.55) and cattle (39.55%).

**Table 7. Pure and mixed infection of rickettsial agents in cattle, buffalo, sheep and goat**

S. No.	Name Of Animal	Microscopy				PCR			
		Total no. of observed	Total no. of infested	At Random blood samples	% of infected samples through Smear method	Total no. of observed	Total no. of infested	At Random blood samples	% of infected samples PCR
01	Cattle	272	134	56	41.79%	272	134	53	39.55%
02	Buffalo	466	163	80	49.09%	466	163	71	43.55%
03	Sheep	99	50	23	46%	99	50	23	46%
04	Goat	109	36	15	41.66%	109	36	20	55.55%
<b>Total</b>		<b>946</b>	<b>383</b>	<b>174</b>	<b>45.43%</b>	<b>946</b>	<b>383</b>	<b>167</b>	<b>43.60%</b>



**Figure-2 Gel electrophoresis of amplified PCR product of rickettsial agents of Cattle blood DNA**



**Figure-3 Gel electrophoresis of amplified PCR product of rickettsia agents of Buffalo blood DNA**

## DISCUSSION

The blood samples of tick-carrying cattle, buffalo, sheep and goat conventionally confirmed blood samples were subjected for PCR detection of Piroplasmids, for this purpose, DNA was extracted from positive blood samples and quantified on Nano-drop spectrophotometer. Primers used for PCR reaction are described in Table 2. Different concentrations of MgCl<sub>2</sub> were used for PCR reaction i.e. 5ul & 6 ul for *T. annulata* whereas for *B. bovis* and *B. bigemina* concentration of MgCl<sub>2</sub> was 3ul. PCR was done for 30 cycles with following conditions: Denaturation at 94° C for 5 min, 94° C for 30 sec. Temperature was lowered for several minutes to allow both forward and backward (right or left) primers to anneal with the complementary sequences. At this stage three conditions 50° C, 55° C and 60° C for 30 secs were checked for each primer set. Finally, extension was carried out 72° C for 45 secs. Analysis of amplified product by electrophoresis was done with a 1% agarose gel. The results were photographed with Gel Documentation System (Gel Doc USA). In order to compare sensitivity of polymerase chain reaction, with that of blood smear method, blood samples were subjected to PCR detection of rickettsial infection. (2) gave findings take out at Maharashtra (India) by (3) *Boophilus*, *Haemaphysalis*, *Hyalomma*, *Amblyomma*, *Nosoma* and *Rhipicephalus* were found tick infesting in subfamily Bovinae animals, which includes cattle, buffalo, and kudus at 40, 16.96, 20.14, 10.22, 4.56, and 1.96 percent attentiveness, correspondingly. He discovered 8 different tick genera of ticks to be precise as *Boophilus*, *Rhipicephalus*, *Hyalomma*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, and *Aponoma* from many segments of Pakistan. The lessen quantity of the genera perceived possibly would be the reason of looked-for the partial region stipulated for the present investigation, also in a partial investigation takeout by (4) and (5) stated 4 genera, even if dissimilar from every one, for the tick troublesome invasion resident of a tract of land on which crops and often livestock are raised for livelihood in their particular investigation.

## CONCLUSION

Information regarding to cattle, buffalo, sheep and goat farms (946 observed animals out of which 383 were the infested animals and 45.43% were infected animals through Microscopy and 43.60% were infected through PCR) According to Microscopy Buffaloes were more susceptible to rickettsial infection as compared to cattle, sheep and goat. According to PCR Goats were more susceptible to rickettsial infection as compared to cattle, buffalo and sheep, Highest rate of rickettsial infection is found in district Tharparkar. Lowest rate of rickettsial infection is found in district Karachi.

**Ethical Consideration:** The study was approved by the Ethical committee of xxx

**Conflict of Interest:** There is no conflict of interest.

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## IMPACT OF MANDIBULAR RESECTION GUIDANCE PROSTHESES ON ORAL HEALTH RELATED QUALITY OF LIFE (OHRQoL) - A PROSPECTIVE STUDY

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### ABSTRACT

This prospective study was aimed to measure the impact of mandibular resection guidance prostheses on oral health-related quality of life (OHRQoL). A total of 35 patients with mandibulectomy defects were included. All the patients were rehabilitated with mandibular resection guidance prostheses respectively. These patients were subjected for assessment of OHRQoL. The assessment was done by using OHIP-Edent-19 and a novel scale—maxillofacial prosthesis performance scale (MFPPS). The assessment was done on all the patients at two weeks and three months of prosthesis function. The scores of OHIP-Edent, and MFPPS for mandibular resection guidance prostheses after two weeks were 33.20 and 18.74 respectively. The scores of OHIP-Edent and MFPPS for mandibular resection guidance prostheses at three months were 27.71 and 15.20 respectively.

These findings show significant improvements of prosthesis in terms of functional, physical, psychological and social parameters after long-term follow-up (3 months).

**Key Words:** Maxillofacial Defects, Oral Health Impact Profile (OHIP), Intra- Oral Prostheses, Guide flange prostheses

### INTRODUCTION

Maxillofacial defects result from both congenital and acquired causes leading to significant changes in the anatomic structures of the maxillofacial region<sup>1, 2</sup>. Maxillofacial defects can be classified as maxillary defects and mandibular defects, both need surgical correction and rehabilitation with prostheses. Mandibulectomy involves extensive loss of tissues and associated functions resulting in an inability to masticate efficiently. Functional activities such as mastication, deglutition, phonetics, mandibular movements, control of saliva and psychic functioning are adversely affected post mandibulectomy<sup>3</sup>. The greatest challenges faced by maxillofacial Prosthodontist in rehabilitating these conditions include the rate of disease occurrence and financial constraints. They are frequently associated with Functional, Physical, Psychosocial and Esthetic Impairments<sup>4</sup>.

Brown's classification proposal depends upon the standard shape of the mandible having four corners: two vertical corners making the angles of the mandible, and two horizontal corners that are centred at the canine teeth on each side in the dentate mandible, and are roughly 7 mm anterior from the mental foramen in the edentulous jaw. Our proposed mandibular defect classification is logical and simple, and groups defects into categories that can be compared in an understandable way. The use of the corners of the mandible at the angles and the canine regions make this classification system a rational approach to categorise defects, increasing in size and complexity from class I (a simple lateral defect not including the condyle involving the angle or vertical corner) to class IV (which involves at least three corners) and class IVc (which includes total mandibulectomy). The proposed classification system shows the increase in morbidity in terms of aesthetics



and function from class I to class IV. This morbidity is likely to increase with non-reconstructed cases in line with the size of the defect, increasing from 70 mm in class I, 85 mm in class II, 100 mm in class III, and 152 mm in class IV. In modern years, Oral Health –Related Quality of Life has gained enormous impact in cancer patients. Evaluation of the treatment success is greatly influenced by the physical and mental strength of the patient<sup>5</sup>. This investigated the oral health related quality of life of patients with Mandibular Resection Prosthesis. However, only a few cross-sectional studies have evaluated the change in quality of life in maxillofacial defect patients with intra oral prosthesis. Thus this study was designed to establish and evaluate the Specific Questionnaire Scale system for Maxillofacial Defects (mandibular defects), and to evaluate the Impact of Mandibular Resection guidance Prosthesis on oral health related quality of life (OHRQol) in mandibular defects (Brown’s Classification) by Oral Health Impact Profile (OHIP-Edent) and Maxillofacial Prosthesis Performance Scale.

## **METHODOLOGY**

This was a prospective study including patients with acquired mandibular defects having provision of mandibular resection guidance after 3 months, aged between 30 to 75 Years. During the period between 2015 to 2019, a total of thirty-five (35) mandibulectomy patients according to Brown Classification were selected for this study. Informed consent was obtained for all participating patients. Mandibular Resection Prostheses were fabricated in the Author’s Department.

The OHRQol was evaluated by means of the Oral Health Impact Profile (OHIP-Edent-19), Obturator functioning scale (OFS-15) and Maxillofacial Prosthesis Performance Scale (MFPPS-10) with standardized questionnaire subsequently at two weeks and three months of prosthesis function for all rehabilitated patients. Patients were asked questions by using all three scales. Answers were recorded by a single operator.

The OHIP-Edent comprises of 19 statements derived from the OHIP using an item impact method (Table 1 ). Oral Health Impact Profile (OHIP-Edent) which includes seven subscales: Functional limitation, Physical pain, Psychological discomfort, Physical disability, Psychological disability, social disability & Handicap.

To assess the oral health related quality of life for patients with maxillofacial defects Novel scale known as Maxillofacial Prosthesis Performance Scale (MFPPS) was developed. Validity and reliability was tested.

Maxillofacial Prosthesis Performance Scale comprises of 10 statements which includes Functional Discomfort, Retention/Stability, Phonetics, Esthetics, Oral Hygiene, Saliva, Taste Ability, Psychology and Satisfaction (Table 2 ). Internal consistency of the questions was assessed by Alpha Cronbach’s Test.

Total OHIP –EDENT scores ranged from 1- 95. A five point Likert scale is used and the highest score indicates, function of the obturator prostheses with greater difficulties. Lower scores indicating Maxillo facial prosthesis enhance the oral health related quality of life. Maxillofacial Prosthesis Performance Scale (MFPPS) and the subscales would be calculated by summing the score of the responses to the 10 items and items corresponding to the subscales. Total MFPPS scores range from 1-50. This scales suggest that lower scores indicate significant improvement of oral health related quality of life (OHRQol) with maxillofacial prostheses in terms of their functional, physical, psychosocial and aesthetics parameters.

The collected data were analysed with IBM.SPSS statistics software 23.0 Version. To describe the data descriptive statistics, frequency analysis and percentage analysis were used for categorical variables. Mean & standard Deviation (S.D) were used for continuous variables. The Cronbach's alpha was used to check the reliability, to find the significant difference between the bivariate samples in Paired groups the Paired sample t-test and the Wilcoxon signed rank test was used & for Independent groups the unpaired sample t-test was used. In all the above statistical tools the probability value .05 is considered as significant level.

**Table 1: Oral Health Impact Profile –Edentulous (OHIP-Edent)**

Domain /Item	S.no	Subscale	5 point Likert				
							5
Functional limitation (FL –3)	1.	Have you had difficulty chewing any foods because of problems with your prosthesis?					
	2.	Have you had food catching in your prosthesis?					
	3.	Have you felt that your prosthesis not been fitting Properly?					
Physical pain (P1-4)	4.	Have you had painful aching in your mouth?					
	5.	Have you found uncomfortable to eat any foods because of problems with your prosthesis?					
	6.	Have you had sore spots in your spots?					
Psychological Discomfort (P2-2)	7.	Have you had uncomfortable dentures					
	8.	Have been worried by dental problems					
Physical disability (D1-3)	9.	Have you been self-conscious because of your prosthesis?					
	10.	Have you had to avoid eating some foods because of your prosthesis?					
Psychological disability (D2-2)	11.	Have you been unable to eat with your prosthesis because of problem with them?					
	12.	Have you had to interrupt meals because of your prosthesis?					
Social disability (D3-3)	13.	Have you been upset because of your prosthesis?					
	14.	Have you been bit embarrassed because of your prosthesis?					
Handicap (H-2)	15.	Have you avoided going out because of your prosthesis?					
	16.	Have you been less tolerant to your partner or family because of your prosthesis?					
TOTAL OHIP-Edent Score RANGE (1-95)	17.	Have you been irritable with others because of your prosthesis?					
	18.	Have you been unable to enjoy other people company as much because of your prosthesis?					
	19.	Have you felt that life in general less satisfying because of your prosthesis?					

**Table 2 : Maxillofacial Prostheses Performance Scale (MFPPS -10)**

Item	Sr. No	Subscale	Measures: 5 point Likert scale				
			1	2	3	4	5
Functional Discomfort	1.	With respect to chewing, have you had difficulty of Chewing any foods?					
	2.	With respect to swallowing, have you felt any leakage of foods Underneath the prosthesis?					
Problems related to Retention & stability	3.	Do you feel uneasy during meals due to loose & Unstable prosthesis?					
Speech problems	4.	Have you had difficulty speaking in public					
Aesthetics Problems	5.	Have you had any problem With the appearance?					
Problems with Oral hygiene	6.	Have you had any problem with cleaning /maintaining your prosthesis					
Problems related to Salivary control	7.	Have you had problem With drooling of saliva?					
Problems related to Taste ability	8.	Has your prosthesis altered your taste sensation?					
Problems related to Psychological aspect	9.	Have you had any problem with prosthesis that affects your mental well-being?					
Problems associated with General satisfaction	10.	Do you have any dissatisfaction with overall Performance of the prosthesis?					

**RESULTS**

The mean score of 35 mandibular resected patients rehabilitated with mandibular resection prosthesis after two weeks and three months of follow up were 33.20 and 27.71 respectively. After 2 weeks of follow up, the mean score of OHIP-Edent subscales such as functional limitation (M=5.77/ 38%), physical pain (M=6.29/ 31.4%), psychological discomfort (M=4.26/42.6%), physical disability (M=5.66/37.6%), psychological disability (M=2.86/ 28.6%), social disability (M=4.57/30.4%) and handicap (M=3.8/ 38%) were observed.

Based on the observation, the most prevalent impact on OHRQol by OHIP –subscale; psychological discomfort (42.5%) specified that nearly half of the patients were upset with dental problems and self-conscious about the prosthesis even after the 2 weeks of follow up. Despite the discomfort in psychological subscale, there was significant improvement in functional limitation (30%), physical pain (26%), psychological disability (28%) and social disabilities (25.8%) after long term follow up. When Analysing OHIP scale, there was highly significant difference (p value = 0.005) were observed in all parameters except for psychological disability, no significant differences (p value = .157) after 2 weeks and 3 months of mandibular resection prosthesis function. On long term assessments, OHIP scale scores revealed significant progress on oral health. Psychological discomfort was the most prevalent OHRQol impairment with 36% of the patients followed by handicap (31.7%) and functional limitation (30%). A summary of the results is given in Table 3.

**Table 3: Mean, standard deviation of OHIP –Edent scale after 2 weeks and 3 months of Mandibular Resection Prosthesis Function**

		Mean	N	Std. Deviation	P value	Std. Error mean
Pair 1	FUNCTIONAL LIMITATION 2W	5.1	35	1.942	.00	.3
	FUNCTIONAL LIMITATION 3M	4.4	35	1.615	.00	.2
Pair 2	PHYSICAL PAIN 2W	6.1	35	2.080	.00	.3
	PHYSICAL PAIN 3M	5.1	35	1.471	.00	.2
Pair 3	PSYCHOLOGICAL DISCOMFORT 2W	4.4	35	1.314	.00	.2
	PSYCHOLOGICAL DISCOMFORT 3M	3.4	35	1.140	.00	.1
Pair 4	PHYSICAL DISABILITY 2W	5.1	35	1.924	.00	.3
	PHYSICAL DISABILITY 3M	4.4	35	1.704	.00	.2
Pair 5	PSYCHOLOGICAL DISABILITY 2W	2.4	35	.974	.00	.1
	PSYCHOLOGICAL DISABILITY 3M	2.4	35	.901	.00	.1
Pair 6	SOCIAL DISABILITY 2W	4.4	35	1.539	.00	.2
	SOCIAL DISABILITY 3M	3.4	35	1.301	.00	.2
Pair 7	HANDICAP 2W	3.4	35	1.132	.00	.1
	HANDICAP 3M	3.4	35	1.098	.00	.1

p value <.005 significant

**Table 4: Mean, standard deviation of MFPPS after 2 weeks and 3 months of Mandibular Prosthesis**

		Mean	N	Std. Deviation	Std. Error mean	P value
Pair 1	F/C-2 MFPPS 2W	3.71	35	1.526	.258	.0005
	F/C-2 MFPPS 3M	3.03	35	1.014	.171	.0005
Pair 2	R/S -1 MFPPS 2W	1.89	35	.718	.121	.0005
	R/S -1 MFPPS 3M	1.46	35	.505	.085	.0005
Pair 3	Ph-1 MFPPS 2W	1.86	35	.845	.143	.0005
	Ph-1 MFPPS 3M	1.57	35	.698	.118	.0005
Pair 4	AEST-1 MFPPS 2W	1.74	35	.505	.085	.0005
	AEST-1 MFPPS 3M	1.29	35	.458	.077	.0005
Pair 5	OH-1 MFPPS 2W	2.14	35	.355	.060	.0005
	OH-1 MFPPS 3M	1.43	35	.558	.094	.0005
Pair 6	SALIVA-1 MFPPS 2W	2.11	35	.583	.098	.0005
	SALIVA-1 MFPPS 3M	1.74	35	.657	.111	.0005
Pair 7	TASTE-1 MFPPS 2W	1.89	35	1.132	.191	.0005
	TASTE-1 MFPPS 3M	1.60	35	.736	.124	.0005
Pair 8	PYCHO-1 MFPPS 2W	1.71	35	.825	.139	.0005
	PYCHO-1 MFPPS 3M	1.54	35	.611	.103	.0005
Pair 9	SATIS-1 MFPPS 2W	1.69	35	.471	.080	.0005
	SATIS-1 MFPPS 3M	1.54	35	.505	.085	.0005

## DISCUSSION

Masticatory muscle balance and mandibular movements were adversely affected by mandibulectomy, leading to altered masticatory movement and deviation of residual fragment towards the surgical side. Other observed dysfunction was mastication, speech and swallowing and Angular path of opening and closing mandibular pattern. Less precise envelope of motion occurs towards the surgical site during mastication.<sup>6-8</sup> Rehabilitation of mandibular defects after tumour resection is one of the most challenging problems facing maxillofacial prosthodontist. Swallowing, mastication, speech, control of saliva and psychic functioning are most commonly seen adverse effects by Mandibulectomy patients<sup>9-12</sup>. Based on the clinical observation of Rehabilitated Mandibular Patients, the most prevalent impact on OHRQoL by OHIP –subscale; Psychological Discomfort (42.5%) specified that nearly half of the patients were upset with dental problems and self-conscious about the prosthesis even after the 2 weeks of follow up. Despite the discomfort in psychological subscale, there was significant improvement in functional limitation (30%), physical pain (26%), psychological disability (28%) and social disabilities (25.8%) after long term follow up.

Psychological discomfort was the most prevalent OHRQoL impairment with 36% of the patients followed by handicap (31.7%) and functional limitation (30%). Results obtained by the Rehabilitated Mandibular Resected Patients OHIP-Edent scores were also similar to that of Definitive Obturator Prosthesis.

The most ubiquitous impact on OHRQoL was problems with appearance. Since a considerable period of time had elapsed after the resection, the acceptance of the guidance appliance was much more difficult for the patient.

Guidance therapy improves form and function of the individuals and it serves as an interim basis for neuro muscular adaptation to correct the existing deranged occlusion. In addition to the above, other factors such as masticatory muscle pull, mandibular deviation and uncoordinated masticatory movements also influence facial disfigurement. Facial aesthetics and oral functions are essential for social interaction and have an impact on individual's OHRQoL.

The locations and sizes of the mandibular defect in the present study did not significantly affect HRQoL. On the contrary, Young et al. noted that the site of resection appears to have an impact with posterior resections involving the mandibular angle having the most adverse effects on appearance and those involving the parasymphysis (lateral) having the most deleterious effect on overall QoL. Young et al. and Rogers et al have also concluded that resections involved in parasymphysis deleterious effect on mastication, lip support and aesthetics. Our study observations were similar with Young and Rogers et al studies and it is likely that these factors contributed to significant impairment in OHRQoL<sup>13-15</sup>. When analysing the MFPPS score of rehabilitated Mandibulectomy patients after 3 months' improvement for various dimension were, psychological aspects (M=1.54/30.8%), aesthetics (M=1.29/25.6%), taste ability (M=1.60/32%) oral hygiene (M=1.43/28.4%), general satisfaction (M=1.54/30.8%) and saliva control (M=1.74/34%). Highest score was recorded for problems with saliva control (34.8%) followed by general satisfaction and psychological aspects (30.8%). This may be due to problems in speaking and chewing and alterations in appearance may have been frequently reinforced by a range of strained and negative social interactions with others. Due to loss of function and unpleasant appearance which leads to markedly restrict patient's normal social activities.

When comparing maxillofacial defect rehabilitation with conventional prosthesis, it may possess many challenges to enhance the oral health quality of life in restoring the maxillofacial defect. This may be successfully achieved through sound theoretical knowledge, appropriate surgical technique, and surgical skill of the operator, maxillofacial prosthetic experience and team approach. Precise treatment planning and designing in fabrication of intra oral prostheses could certainly enhance the Quality of life of patients with maxillofacial defects. There is significant improvement in mastication, speech, deglutition and appearance

after rehabilitation with maxillofacial prostheses. Therefore, it is an essential pre-requisite for oro-facial defects which in turns support the patient in resuming their normal social life.

## CONCLUSION

The study concludes a remarkable improvement of prosthesis in terms of functional, physical, psychological & social parameters after long term follow-up (3 months). After 2 weeks and 3 months' follow-up, where Mandibular Resection Guidance Prosthesis showed highly significant differences were found in all three scales. There was a significant improvement of oral health related quality of life in terms of Functional, Physical, Psychological and social aspects. Within the confines of this study, a highly positive association exists between OHRQoL and Maxillofacial Prosthesis

**Ethical Consideration:** The Study protocol and informed consent form were approved by the ethical committee of the Vinayaka Mission's Research Foundation, Salem, (OHIP-Edent-19) and Tamilnadu, India. (Ref: VMSDC/IEC/Approval no.069, dated: 15/7/2016)

**Conflict of Interest:** There is no conflict of interest.

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