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THE STAGING AND PATTERNS OF PRESENTATION OF GALLBLADDER CANCER

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Declaration

I, Meshack Nkosinaye Motha Student number 0704198F, declare that this research report is my own work. It is being submitted for the Degree of Master of Medicine in Surgery at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any other degree or examination at any other University.



August 2024, Johannesburg.

Dedication

I dedicate this thesis to my parents (Elizabeth and Isaac Motha) and my siblings. Mom thank you for the unwavering support and sacrifices you have made for me throughout my career. Dad I know you not with us anymore but thank you for shaping the man I have become. I miss you and hope you are proud of your son.

Publications and Presentations Arising From this Study

This research has been be presented at:

1. 2024 Surgical Research Society of Southern Africa (SRS) annual meeting,
Bloemfontein, South Africa, June 2024.

Acknowledgements

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Lastly, I would like to thank Dr N Lahoud and Sr Jolene Kobue who assisted immensely and gave guidance with parts of the data collection.

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Abstract

Introduction: Gallbladder cancer is often a silent yet lethal disease. It is the most common biliary tract malignancy. The recognition of risk factors as well as prognostic features are of utmost importance. Early detection and surgery is associated with better outcomes.

Aim: To describe the stage at presentation and presenting symptoms of gallbladder cancer at a tertiary institution, Chris Hani Baragwanath Academic Hospital (CHBAH) in South Africa.

Methods: This was a retrospective review that evaluated patients who presented with gallbladder cancer at CHBAH from 2016-2019.

Results: A total of 269 suitable patients were included in our study. Diagnosis of gallbladder cancer was confirmed with histology or radiologically. Majority of our patients were female and the mean age at presentation was above the 5th decade. The predominant presenting sign was jaundice at presentation and only 22% of patients had associated gallstones. The majority of the patients (93.7%) presented with advanced disease and the median (IQR) days from diagnosis to death was 27 (13-44) days.

Conclusion: Early detection and diagnosis of gallbladder cancer is crucial for better outcomes. The true incidence and burden of gallbladder cancer in South Africa is yet to be determined. Large prospective and collaborative studies are required to determine the true incidence and burden of GBC in a South African cohort.

List of Abbreviations

ALP – Alkaline Phosphatase

AST – Aspartate Aminotransferase

ALT - Alanine Aminotransferase

BMI – Body Mass Index

CB – Conjugated Bilirubin

CHBAH - Chris Hani Baragwanath Academic Hospital

CRP - C-Reactive Protein

CT – Computer Tomography

ERCP - Endoscopic Retrograde Cholangio-pancreatography

GBC – Gallbladder Cancer

GGT – Gamma-glutamyl Transferase

HB – Hemoglobin

HIV – Human Immunodeficiency Virus

TB – Total Bilirubin

TP – Total Protein

WCC – White cell count

INTRODUCTION

Gallbladder cancer (GBC), when compared to gastro-intestinal tumours, is regarded as a rare cancer. However, it is the most common cancer arising from the biliary tree as it accounts for approximately 80-95% of all biliary tract cancers globally (Lazcano-Ponce et al., 2001; Hundal and Shaffer, 2014). GBC is often associated with a poor prognosis and this is due to its aggressive and silent progression (Hundal and Shaffer, 2014). The five-year survival rate is estimated to be ~65% in localised (stage 1 and 2), ~30% in regional (stage 3) and distant (stage 4) cancers (Cancer Research UK, 2024).

Most GBCs are either diagnosed incidentally on cholecystectomy specimen or as advanced disease which is attributed to the non-specific symptoms and aggressive nature. The proposed pathogenesis is the metaplastic transformation of epithelial cells to, progression to dysplasia and then carcinoma. This process is thought to develop over a period of five to 15 years. Because the gallbladder lacks a serosal layer on the hepatic surface, the progression to local invasion or nodal spread is believed to be relatively quick (Hundal and Shaffer, 2014).

The identification of risk factors for GBC is of utmost importance, as it helps us gain valuable knowledge and understanding of the pathogenesis of this disease (Pilgrim et al., 2013). There are several risk factors that have been identified that are associated with GBC. These include: age, gender, gallstones >2cm, gallbladder polyps, geographical location, ethnicity, genetics, poverty, chemical exposure and congenital developmental disorders. (Pilgrim et al., 2013).

GBC is associated with significant morbidity and mortality (Hondt et al., 2013). Surgical treatment remains the main modality of treatment, with better prognosis if performed early (Hondt et al., 2013). For those patients with locally advanced disease, neoadjuvant chemotherapy has been shown to improve prognosis. Ozer et al (2022), showed higher overall survival in patients with locally advanced disease treated with neoadjuvant chemotherapy.

At Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa, we have observed a notable increase in gallstone disease and in the number of patients presenting with GBC. Musas et al., (2023), Master of Medicine final report from the University of the Witwatersrand, unpublished data) reported a fivefold increase in the cholecystectomy rate at CHBAH for 2017-2019 when compared to previously published data from 1983-1985, from 39 to 216 patients per annum, respectively. Following this steep incline in the incidence of gallstones which are a precursor to GBC, the aim of this study was to evaluate the stage at presentation and the presenting factors of patients with GBC within the South African cohort presenting to CHBAH. Furthermore, we wanted to correlate the presence gallstones in patients presenting with gallbladder cancer.

METHODS

This was a descriptive study that evaluated data retrospectively on patients who presented with GBC at CHBAH from 2016 to 2019. Ethical Approval was previously obtained from the University of the Witwatersrand Human Research Ethics Committee (M180284 & M230780).

Study Population

All adult patients aged 18 years or older who presented with GBC at CHBAH during the study period were included in the study. Patients were excluded if significant data were missing from their hospital files/records. Data was collected from our Hepato-Pancreato-Biliary (HPB) database maintained at our institution. All patient demographics were collected and expressed in tables and graphs. Admission laboratory/biochemistry results and histology reports, if done, were collected and verified with the National Health Laboratory Services (NHLS). Anaemia was defined as having haemoglobin (Hb) level below 13.5 g/dL in men and below 12.0 g/dL in women. A normal

platelet count range was $171\text{--}388 \text{ L} \times 10^9$. A normal total and conjugated bilirubin range was $5\text{--}21 \mu\text{mol/L}$ and $0\text{--}3 \mu\text{mol/L}$ respectively as per NHLS. CT scan results were obtained from the radiology department of CHBAH PACS system. Reports from the MDT with a radiologist were used to verify the diagnosis of GBC. The 8th AJCC staging criteria was used for clinical and radiological staging; the staging for gallbladder cancer is well described by Cancer Research UK (<https://www.cancerresearchuk.org/about-cancer/gallbladder-cancer/stages/number>; Accessed on 31st July 2023).

Data Collection & Analysis

De-identified data was captured in an Excel spreadsheet and data analysis performed using STATA statistical software version 16. The Shapiro-Wilk test was applied to determine the distribution of continuous data, such as age and biochemistry measurements. Statistical tests included standard descriptive statistics with means and standard deviations (SD) or medians and interquartile ranges (IQR) reported, as appropriate, for continuous variables. Categorical variables were described using frequencies and proportions. Association tests were applied using the T-test or Mann-Whitney U test, and the Chi-Squared test or Fisher's exact test, as appropriate. A P value of <0.05 was considered statistically significant. Mortality analysis was performed using the Kaplan-Meier method.

RESULTS

A total of 301 patients met the inclusion criteria during our study period of whom 32 patients were excluded because of significant data missing from their patient files. Therefore, 269 patients were included in this study and the diagnosis of GBC was confirmed either histologically and/or radiologically by CT scan.

Table 1 shows the demographic and clinical characteristics of the study population. The mean ($\pm$ SD) age at diagnosis was 59.4 ($\pm$ 12.7) years. There was no significant age difference in patients who presented with early disease 6% (TNM stage 1-3, as per Cancer Research UK, 2024) versus metastatic disease 94% (stage 4). A significant proportion (69.5%) of our study population were female. The majority of our patients were of African descent (90.3%), and the remaining 9.7% were either of White, Indian and Coloured decent. This is in keeping with the demographic pattern of patients who present to CHBAH. A large proportion of the patients were native South Africans (95.5%) and a small percentage (4.5%) of our patients were migrants from neighbouring countries such as Zimbabwe, Mozambique and Congo. Geographically most of our patients resided in the Gauteng province (Table 1). A small subset of our patients were from the Northwest Province (14.9%), which is predominantly a large mining and farming region.

Of the 154 (57.2%) study patients who had a known HIV status, 32.5% were HIV positive. Importantly, 22.7% of our GBC patients presented with associated gallstone disease. Most of the patients (91.8%) presented with obstructive jaundice. Only 44.6 % patients had fever documented at presentation.

In total, 141 patients were documented to have undergone drainage procedures and included 65 (46.1%) endoscopic retrograde cholangio-pancreatography (ERCP), 58 (41.1%) percutaneous trans-hepatic biliary drainage and 18 (12.8%) combined procedures.

Table 1. Demographic and clinical characteristics of the study population by TNM stage*

Variable	All patients (n=269)	TNM Stage 1-3 (n=17)	TNM Stage 4 (n=252)	P-value
Age, years (mean±SD)	59.4 (12.7)	58.6 (15.4)	59.5 (12.5)	0.90
Sex, n (%)				
Male	82 (30.5)	8 (9.8)	74 (90.2)	0.17
Female	187 (69.5)	9 (4.8)	178 (95.2)	
Ethnicity, n (%)				
Black	243 (90.3)	16 (6.6)	227 (93.4)	>0.99
White	14 (5.2)	1 (7.1)	13 (92.4)	
Coloured	7 (2.6)	0 (0.0)	7 (100)	
Indian	5 (1.9)	0 (0.0)	5 (100)	
Country of origin, n (%)				
South Africa	257 (95.5)	16 (6.23)	241 (93.8)	0.55
Zimbabwe	7 (2.6)	1 (14.3)	6 (85.7)	
Congo	4 (1.5)	0	4	
Mozambique	1 (0.4)	0	1	
Geographical location, n (%)				
Gauteng	224 (83.3)	15 (6.7)	209 (93.3)	0.48
Northwest	40 (14.9)	1 (2.5)	39 (97.5)	
Not indicated	5 (1.9)			
HIV Positive, n (%)	50/154 (32.5)	4 (8.0)	46 (92)	0.73
Gallstones, n (%)	61 (22.7)	7 (41.2)	54 (21.4)	0.07
Obstructive Jaundice, n (%)	246/268 (91.8)	14 (5.6)	232 (94.1)	0.15
Fever, n (%)	120 (44.6)	4 (23.5)	116 (46.0)	0.08
Site of GBC, n (%)				
Body	20 (7.4)	1 (5.0)	19 (95.0)	#
Fundus	13 (4.8)	3 (23.1)	10 (76.9)	
Body/Fundus	9 (3.4)	0 (0.0)	9 (100)	
Neck	7 (2.6)	1 (14.3)	6 (85.7)	
Neck/Body	2 (0.7)	0 (0.0)	2 (100)	
Unknown	218 (81.0)	12 (5.5)	206 (94.5)	
Procedure, n (%)	n=141			
ERCP	65 (46.1)	3 (4.6)	62 (95.4)	>0.99
PTBD	58 (41.1)	3 (5.2)	55 (94.8)	
ERCP/PTBD	18 (12.8)	1 (5.6)	17 (94.6)	

*Staging according to Cancer UK. #No statistic, too small numbers across numerous categories. Abbreviations: ERCP, Endoscopic retro-grade cholangio-pancreatography; GBC, gallbladder cancer; PTBD, percutaneous trans hepatic biliary drainage.

Histopathology

Figure 1 shows the histopathological subtypes of GBC in this study population, of whom 111 patients had reports available. Adenocarcinoma was the most common GBC subtype 91% Squamous cell carcinoma and neuroendocrine only accounted for a small proportion of the patients (4.5% each). Our patients presented with large and metastatic disease. Hence, the site of GBC was only clearly located in 19 % of the patients, specifically in 29.4% of TNM stage 1 to 3 and 18.3% in TNM Stage 4 disease patients.

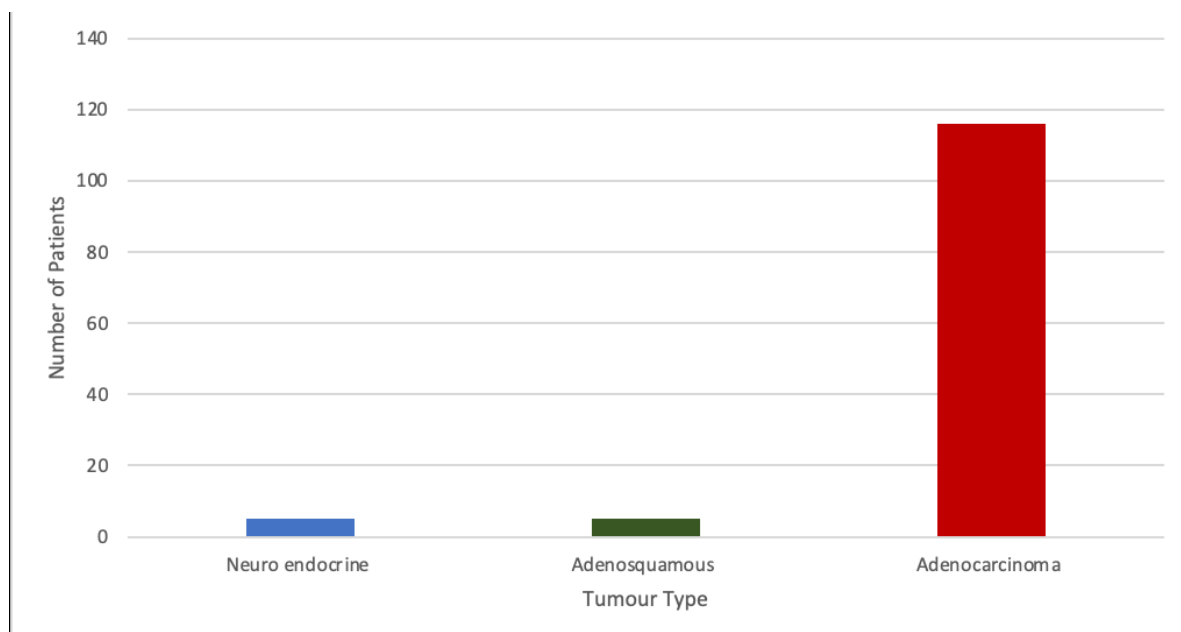


Figure 1. GBC histopathological tumour subtypes.

Staging

A significant proportion of our patients presented with advanced disease, with 93.7% (n=252/269) presenting with Stage 4 cancer (Table 1). The remaining small subset of patients 6.3% (n=17/269) had early stage disease of whom only 5 patients (29.4%) had surgery. When evaluating the T staging at presentation, 143 (53.2%) of patients presented with T4, 90 (33.5%) with T3, 24 (8.9%) with T2b, 8(3%) with T2a and only 4 (1.5%) with T1 disease. Similarly, the majority of patients had advanced nodal disease.

Biochemistry

Table 2 shows the biochemistry measurements according to early vs advanced GBC stages. Due to the skewness of data, these were all reported as median and interquartile ranges (IQR). We observed raised inflammatory markers including CRP and WCC in all patients with GBC. Anaemia was a common finding across the stages of GBC, with a median (IQR) Hb level of 9.9 (8.4-11.9) g/dL. Albumin at a median (IQR) of 29 (23-33) g/L was low in all patients who presented with GBC. When we assessed the liver function test, the median (IQR) conjugated bilirubin level was high at 99 (34-226) $\mu\text{mol/L}$ in all study patients, and even more so in the stage 4 GBC. Furthermore, the liver enzymes AST and ALP were also raised in all patients who presented GBC. However there were no statistically significant differences in the biochemistry measurements between the early and advance stage GBC groups.

Table 2. Biochemistry measurements by TNM stage in GBC patients*

Parameter**	All patients	TNM Stage 1-3	TNM Stage 4	P-value
WCC, $\times 10^9/\text{L}$	11.0 (7.9-14.2)	10.9 (7.8-14.0)	11.0 (7.9-14.3)	0.67
HB, g/dL	9.9 (8.4-11.9)	9.0 (7.4-13)	9.9 (8.5-11.9)	0.82
Platelets, $\times 10^9/\text{L}$	334 (214-435)	265 (203-432)	334 (215-438)	0.58
CRP, mg/L	98 (54-176)	57 (12-204)	98 (55-176)	0.12
TP, g/L	66 (58-72)	70 (55-74)	66 (59-71)	0.91
Albumin, g/L	29 (23-33)	26 (22-35)	29 (23-29)	0.85
AST, U/L	95 (51-199)	151 (46-240)	94 (52-179)	0.61
ALP, U/L	468 (250-759)	348 (202-479)	525 (251-771)	0.08
CB, $\mu\text{mol/L}$	99 (34-226)	24 (8-173)	102 (41-230)	0.06
EGFR, mL/min/1.73 m ²	59 (51-60)	60 (47-60)	59 (51-60)	0.92
Creatinine, $\mu\text{mol/L}$	68 (56-89)	79 (67-101)	67 (56-89)	0.08
Urea, mmol/L	5.7 (3.8-7.9)	5.7 (4.2-8)	5.6 (3.7-7.9)	0.70

*Staging according to Cancer UK. **All values given as median and interquartile ranges (IQRs).

Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase; CB, conjugated bilirubin; EGFR, estimated glomerular filtration rate; CRP, C-reactive protein; HB, haemoglobin; TP, total protein; WCC, white cell count

Outcomes

Figure 2 shows the 30-day Kaplan Meier survival estimates of all GBC study patients, according to early vs advanced stage disease. Overall, 50% of patients demised by day 26, with early stage cancers (TNM 1-3) having a 30-day survival estimate of 55% compared to 42.5% in advanced cancer (TNM 4). The majority of our patients were palliated and only a small subset received chemotherapy. None of the routine biochemistry markers measured in this study could prognosticate mortality in this cohort of GBC patients.

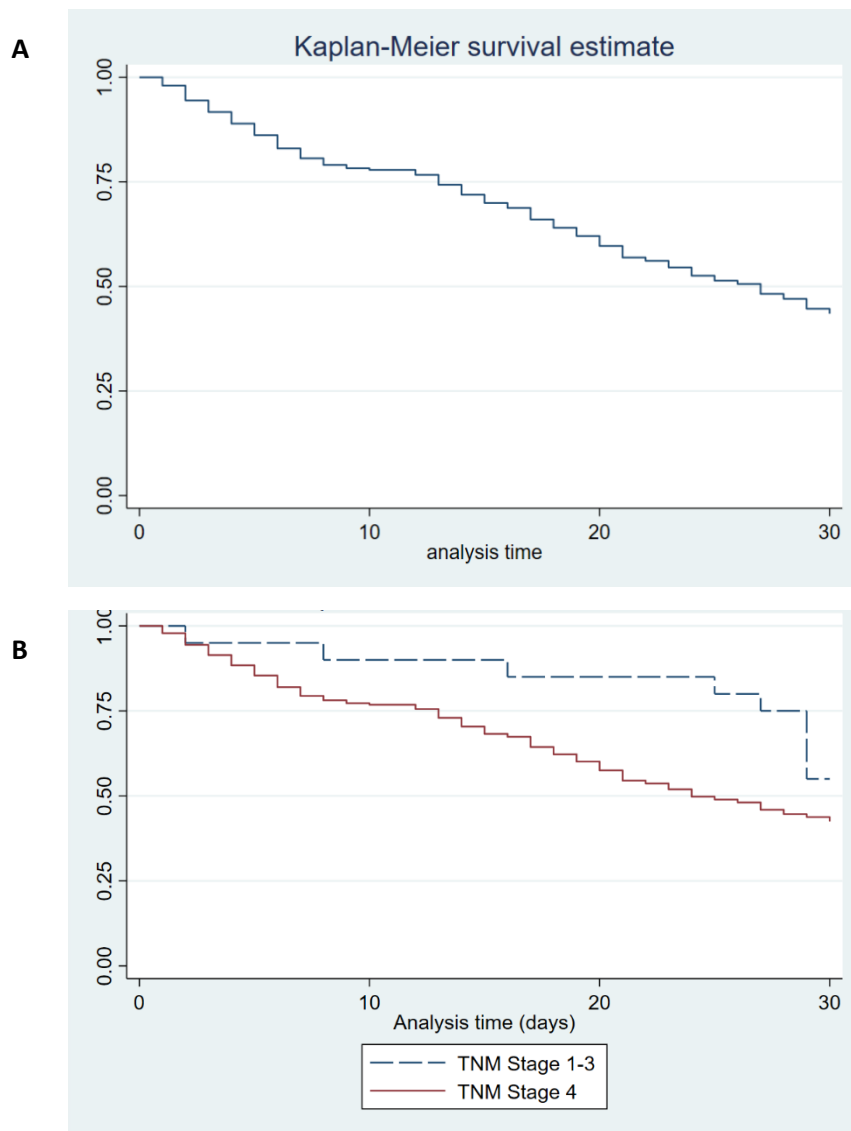


Figure 2. Kaplan Meier Survival estimates in GBC study patients: A. All patients; B. Early (TNM Stage 1-3) vs advanced (TNM Stage 4) disease.

DISCUSSION

Our study is the first to describe the staging at presentation of GBC in a South African cohort. The presenting signs and symptoms of GBC are non-specific. Furthermore, there are very limited symptoms reported by patients in the early phase of the disease. Hence, patients may be asymptomatic for a long duration. In this study the main presenting signs and symptoms reported were jaundice and fever. Khan et al. (2010), reported abdominal pain, abdominal mass and weight loss as the other signs and symptoms in patients presenting with advanced GBC.

The mean age at presentation in our study population was 59.4 years. Compared to other studies published in the USA and Western European Countries with a mean age above 70 years old and a higher incidence of GBC, our patients presented at a younger age (Duffy et al., 2008; Rawla et al., 2019). Our Findings are similar to those reported by Dutta's group (Dutta et al., 2005) who assessed the epidemiology of GBC in an Indian Population. In Europe the incidence of GBC seems to increase with advanced age. However, majority of these patients have more than one risk factor that makes them susceptible to GBC.

There was a strong predominance of females in our study who presented with GBC and this ratio did not differ in early vs advanced disease stages. These findings are similar to other international studies (Gupta et al., 2012; Schmidt et al., 2019), often reporting the ratio of female: male between 3:1 and 4:1. This high incidence of GBC in females is attributed to multiple factors. These include exposure to high levels of sex hormones, obesity and high propensity to develop of gallstones (Mhatre et al., 2021; Li et al., 2016).

When we explored ethnicity, the patients of African descent made up the majority of patients in this study, a direct reflection of the demographic of patients presenting to CHBAH. Geographically

the majority of our patients were from the Gauteng province in South Africa, with only a small subset from the North-West mining province. However, patients in South Africa tend to move from rural areas or other provinces to Gauteng in seek of medical attention and employment. Hence, this data does not show a true reflection of geographical distribution of GBC patients in South Africa at large.

The association of GBC with gallstones has been well documented globally. In South Africa we have noted an increase in the incidence of symptomatic gallstone disease (Khan et al., 2020). However, in our study only 22.7% of GBC patients also presented with gallstones. This was low compared to other international studies that documented 70-90% of GBC patients as having gallstones (Batra et al., 2005, Humdani et al., 2012). The majority of our patients had advanced disease, and the cross sectional imaging that was frequently performed was a CT scan. CT scan has low sensitivity in detecting stones compared to ultrasound especially where there is large mass arising from the gallbladder.

Obstructive jaundice is a well-known late sign of gallbladder cancer. There are several mechanisms which cause obstructive jaundice. These include obstruction of the common hepatic duct by tumour, extrinsic compression of the bile duct by large metastatic lymph nodes and metastatic disease to the liver. Hence, it is usually associated with locally advance or metastatic disease. In our study, obstructive jaundice was a common sign (91.8%) as a majority of our patients presented with stage 3 and stage 4 disease. There are several complications associated with obstructive jaundice. Biliary sepsis or cholangitis is a lethal complication in these patients, hence they require biliary drainage. The most commonly used modality of biliary drainage would either be ERCP or PTPD (Niemela J et al 2003; Xie J et al 2022). In our study 41.1% had undergone PTPD, 46.1%

ERCP and 12.8 had a combination as a palliative procedure. Mortality in GBC patients is usually as a result of biliary sepsis (MahdaviFar et al 2018).

The most commonly seen GBC histological subtype is adenocarcinoma. It accounts for over 80% of all GBC. A small subset will include, squamous, adenosquamous and neuroendocrine. In our study, adenocarcinoma was the predominant subtype accounting for over 90%. Cariati et al (2014) suggested that some risk factors are associated with specific GBC subtypes. Adenocarcinoma maybe associated with gallstone disease. However we had a low rate of gallstones on in our study population.

Malignancies of the gastrointestinal tract are strongly associated with an inflammatory response. Neutrophils are the first responders to cellular damage, while the lymphocyte initiate cytotoxic cell death of the tumour (Weigelin et al., 2021). Hence, there has been a pursuit to use circulating biomarkers to prognosticate GBC (Mady et al., 2020). In our study, we demonstrated that the majority of our patients presented with lymphocytosis. However, we could not find any association with mortality or staging of disease. The latter may be due to the fact that a large proportion of our study population had advanced disease. Previous studies have used other haematological biomarkers such as platelets, haemoglobin and liver function test to prognosticate GBC (Velasco et al 2024). Similarly in our study the vast majority of our GBC cohort demonstrated to have anaemia, thrombocytosis and abnormal LFTs. However, we could not find a significant association between these markers and our outcomes for staging and mortality.

Staging of GBC remains pivotal in guiding surgical treatment options for patients. Early disease T1a is usually treated with a simple cholecystectomy, while there is still controversy about the T1b

lesion. There is very limited treatment options for curative intent in patients with advanced disease (stage 2 and 3). Unfortunately majority of our patients presented with advanced disease and this may be attributed to the non-specific symptoms of GBC. Furthermore, the poor help seeking behaviour and late referral from our referral hospitals may have contributed to the late presentation of the majority of our study population. Hence, only small portion of our study population underwent surgical resection and a large number underwent palliative procedures.

Study Limitations

This was a retrospective study which is subjected to selection bias. Our data collection was limited to a single centre in South Africa (public academic tertiary institution), which was a referral centre. There are patients who presented to other institutions including regional hospitals, with GBC that would not have been included in this study. Furthermore, there are patients who presented to our HBP clinic with advanced disease that may have been referred back to their base hospital for palliation that are not included in this study. We also had missing data in the sub-analysis groups.

CONCLUSION

GBC is a silent yet deadly disease. Early diagnosis and surgery are of utmost importance for better outcomes. In our study the average age of patients was before the 6th decade of life, with the majority of patients presenting with advanced disease, most commonly adenocarcinoma, with very limited treatment options. Importantly, only 22% of our patients had documented gallstones. Late referrals, poor help seeking behaviour and the non-specific clinical symptoms may contribute to advanced stage presentations in our population with a limited life expectancy. The true incidence and burden of disease still needs to be established in South Africa. This can only be done through the establishment of multicentre collaboration and a national GBC registry.

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APPENDIX 1.



PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Meshack Nkosinaye Motha (Student number: 0704198F) am a student registered for the degree of Master of Medicine in Surgery in the academic year 2024.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
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APPENDIX 2

HREC Clearance Certificate



R49 Professors D Kruger and M Smith; Dr J Devar

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) CLEARANCE CERTIFICATE NO. M230780

NAME: Professors D Kruger and M Smith; Dr J Devar
(Principal Investigator)

DEPARTMENT: School of Clinical Medicine
Department of Surgery
Medical School
University

PROJECT TITLE: *Presentation and demographic characteristics of patients in hepatobiliary units in Johannesburg academic hospitals and Wits Donald Gordon Medical Centre*

DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS: Renewal of M18/02/84 - expires on 2023/08/22

NOTE: If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Not applicable

APPROVED BY: _____
Professor P Ruff, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2023/08/18 **EXPIRY DATE:** 2028/08/17

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

18 August 2023

Date

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG



RESEARCH PROPOSAL FOR A Masters in Medicine

ON

The staging and patterns of presentation of gallbladder cancer

By

Dr Meshack N Motha

Supervisors: Dr John Devar

Department of Surgery
School of clinical Medicine
University of Witwatersrand



1. Introduction

1.1. Overview

Gallbladder cancer, when compared to gastro-intestinal tumours is regarded as a rare cancer. However, it is the most common cancer arising from the biliary tree. It accounts for approximately 80-95 percent of all biliary tract cancers globally (Lazcano et al 2001) (Hundal et al 2014). It is often associated with a poor prognosis and this is due to its quick and discreet progression (Hundal et al 2014). The five year survival rate is estimated to be 32 percent in T1 and T2 lesions (Hondt et al 2013). Moreover, the one year survival rate for patients with lesions that extend beyond the gallbladder is a dismal 10 percent (Hendson et al 1992), (Hondt et al 2013).

Most gallbladder cancers are diagnosed incidentally or as advanced disease. This is attributed to the non-specific presentation and aggressive nature. The proposed pathogenesis, is the transformation of epithelial cells to metaplasia, progression to dysplasia and carcinoma. This process is thought to develop over a period of 5 to 15 years. Because the gallbladder lacks a serosal layer on the hepatic surface, the progression to local invasion or peri-cholecystic lymphatics is thought to be relatively quick (Hundal et al 2014).

1.2. Risk Factors

The identification of risk factors for gallbladder cancer is of utmost importance, as it helps us gain valuable knowledge and understanding of the pathogenesis of this disease (Pilgrim *et al* 2013). There are several risk factors that have been identified that are associated with gallbladder cancer. These include: age, gender, gallstones >2cm, geographical location, ethnicity, genetics and poverty, chemical exposure, congenital developmental disorders. (Pilgrim *et al* 2013)

1.2.1. Age

Studies published In the United States show an increase rate of gallbladder cancer with advancing age. The median age of 67 has been reported by Duffy et al 2008 in their single centre study of 436 gallbladder cancer patients. More importantly, there is an increase in mortality rates associated with advance age, especially above the age of 75 years (Duffy *et al* 2008), (Hundal *et al* 2014).

1.2.2. Gender

Gallbladder cancer is noted to have a female predominance. It affects females almost two to six times more than males (Konstantinidis *et al* 2009). It is postulated that female hormones have a vital role in biliary tract malignancies (Gupta *et al* 2012). Epidemiological studies suggest that pregnancy influences the development of bile stasis, weight gain and hypercholesterolaemia (Hundal *et al* 2014). This then permits for the formation of gallstones which are a risk factor for gallbladder cancer.

1.2.3. Gallstones

Gallstones are well established as a risk factor for gallbladder cancer. They are found in a significant proportion of patients with gallbladder cancer (Hundal *et al* 2014). The

presence of gall stones in specific ethnic group females like the Mapuche Indians, Pima Indians and North American Indians increases the risk of cancer (Everhart *et al* 2002). There are certain characteristics of the gallstones that are associated with an increased risk which include stones greater than 2 cm and cholesterol stones. The proposed pathophysiology is that of chronic irritation and long standing inflammation that leads to malignancy (Lowenfels 1989).

1.2.4. Chronic Inflammation

As mentioned above, chronic inflammation is another well-established risk factor for this malignancy. The inflammation may not only be due to gall stones but as result of infection and primary sclerosing cholangitis as well. There are two pathways in which chronic inflammation is carcinogenic. Firstly, the repeated insult to tissue leads to damage to DNA which is mediated through chronic release of inflammatory cytokines. Inflammatory cytokines, prostaglandins, reactive oxygen and nitrogen species, micro RNA are all implicated in DNA mutation, DNA methylation and promoting angiogenesis (Rashid *et al* 2002). Secondly chronic inflammation leads to the deposition of calcium on the gallbladder. This leads to the formation a porcelain gallbladder (hard and brittle) which is associated with malignancy (Schnelldorfer 2013). More recently we have discovered that it is more stippled calcification that carries a higher risk of malignancy rather than just the porcelain state (Kanthan 2015).

1.2.5. Chronic Infection

Chronic infection with organism such as *salmonella typhi* and *helicobacter* species have been associated with the development of gallbladder cancer (Gonzalez *et al* 2011). This is mediated through chronic inflammation, alteration of tumor suppressor genes such as *p53* and proto-oncogenes such as *k-ras*. Furthermore, bacterial colonisation with these organisms in the biliary system leads to the breakdown of bile acid into carcinogenic metabolites (Rashid *et al* 2002).

1.2.6. Carcinogens

There are various other carcinogens that we are exposed to in our different environments that are associated with development of gallbladder cancer. Darby *et al* 2005 showed that there is an increased risk of gallbladder cancer in minors exposed to radon. Although unclear, exposure of workers to carcinogens like nickel and cadmium in the metal industries has also been implicated in gallbladder carcinoma (Pandey 2006). Exposure to nitrosamines and 3-methylcholanthrene in animal studies has been shown to cause gallbladder carcinoma, however this is still to be proven in human studies.

1.2.7. Congenital Abnormalities

On the other end of the spectrum of risk factors, congenital abnormalities has also been associated with gallbladder cancer. Precisely the anomalous pancreaticobiliary junction accounts for approximately ten percent of all gallbladder cancer (Nomura *et al* 2002). This congenital anomaly is more common in the Western and Asian population (Hundal *et al* 2014). In this congenital malformation the pancreatic duct drains directly into the common bile duct and there is a long common channel of greater than 2 cm. This leads to

pancreatic juice reflux into the common bile duct, which leads to chronic inflammation and irritation, hence predisposing to malignant transformation (Nuzzo *et al* 2005).

Gallbladder cancer is associated with a significant morbidity and mortality (Hondt *et al* 2013). The prognosis for most patients is poor as they are diagnosed at an advanced stage (Khan *et al* 2010). Surgical treatment remains the main modality of treatment, with better prognosis if performed early (Hondt *et al* 2013).

1.3. Justification of the study:

In the 1950's there was on average only one cholecystectomy per year done at Chris Hani Baragwanath Academic hospital for calculous cholecystitis (Bremner *et al*). We have noted an explosion of gallstone disease in the last decade and we have also noted at Chris Hani Baragwanath Academic Hospital a significant increase in the number of patients presenting with GBC. Not all patients have gallstones and there may be a geographic distribution. To our knowledge, the epidemiology has never been described in our population. With this steep incline in the incidence we are obliged to investigate and study the Epidemiology of this disease.

1.4. Aim of the Study

The aim of this study is to evaluate the stage at presentation and the prognostic factors of patients with gallbladder carcinoma within the South African cohort presenting to CHBAH and Wits Donald Gordon.

2. Objectives

Primary

- To describe the epidemiology of gallbladder carcinoma in a cohort of 200 patients
- Stage all patients with Gallbladder Carcinoma at presentation to CHBAH and WDGMC
- To determine the risk factor profile, presenting symptoms and routine biochemistry and determine their relationship, if any,

Secondary

- To evaluate the outcomes of patients treated for gallbladder cancer at our institutions
- To determine the HIV Status of all patients presenting with gallbladder Carcinoma
- To Peg the Geographical location of patients presenting with gallbladder cancer

2. Methods

2.1. Study Design

This will be a retrospective observational study.

2.2. Study Population:

Sampling will be done from the admission records of all patients diagnosed with GBC including those that undergo surgical resection with curative intent and those that undergo palliative care. The sample population will come from the Hepatobiliary units at Chris Hani Baragwanath Academic hospital and the Wits Donald Gordon medical center. Convenience sampling will be used whereby all patients admitted to these units between 2016 and 2019 will be included. The cohort will consist of roughly 200 patients. Please refer to the attached data collecting sheet.

2.3. Data Collection:

All data will be collected from our HPB database at our two institutions (CHBAH, DG)

Data will be extracted from the databases of the two sites which include: Patient demographics (age, gender and ethnicity); tumour stage and histology; patients and overall survival

2.4. Data Analysis:

Statistica 12, software developed by *StatSoft*, will be used to analyse data. All categorical variables which include: patient ethnicity, gender, histology will be presented as percentages. The continuous variables which include patient age, biochemistry will be presented as means and standard deviations if nominally distributed and by medians and interquartile range if not nominally distributed.

- All patients demographics will be collected
- Data will be expressed in tables and graphs
- Admission laboratory results, staging CT scans, and histology if done will be collected
- Surgical procedure data will be collected
- The 6th AJCC staging criteria will be used for clinical and radiological staging
- Statistical analysis using *t* test and Chi Square for univariate data
- *P* values > 0.05 will be considered significant

3. Ethical considerations

Human ethics application will be submitted to the University of the Witwatersrand Human Ethics Research Council

4. Time Line:

	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May
Protocol writing and assessment													
Collection of Data													

Data												
Analysis												
Write Up												

5. Budget:

No cost to the state nor any public hospital will be incurred. Realistically the budget will require minimal funds for printing and stationary to complete the project. This will be funded by the primary investigator.

6. Study Limitations:

Data may lacking and files record

Patients seen as outpatients for upfront palliation may not be included

7. References

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