Study on MAD and BUB1 genes of spindle assembly checkpoint with response to primary adjuvant chemotherapy in advanced epithelial ovarian cancer

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2. T. Mistry, S. Ghosh, P. Sahoo, S. Mahata, R. Pal, **S. Sarkar**, T. Choudhury, N. Alam, S. Mandal, V.D. Nasare, ***Single nucleotide polymorphisms of ABCB1 (rs1128503) and ABCC2 (rs145008610) genes and its clinical impact in ER & PR positive breast cancer patients in a tertiary care hospital of India,*** *European Journal of Cancer***,** Volume 175, Supplement 1,2022, Page S79, ISSN 0959-8049, https://doi.org/10.1016/S0959-8049(22)01565-9. (IF: 10.002)

"STATEMENT OF ORIGINALITY"

I, **Sinjini Sarkar** registered on **20th June, 2019 (Index No. 285/19/Ph)** do hereby declare that this thesis entitled "**Study on MAD and BUB1 genes of spindle assembly checkpoint with response to primary adjuvant chemotherapy in advanced epithelial ovarian cancer**" contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 1 %.

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TABLE OF CONTENTS

	Page No.
Preface	i-ii
List of abbreviations	iii-vii
List of Tables	viii-ix
List of Figures	x-xi
1. Chapter 1: Introduction and Objectives	1-3
2. Chapter 2: Literature Review	4-27
3. Chapter 3: Materials and Methods	28-38
4. Chapter 4: Results	39-75
5. Chapter 5: Discussion	76-85
6. Chapter 6: Conclusion	86-87
7. References	88-111
8. Annexures	112-125
Annexure I: Ethical Clearance Certificate	112-113
Annexure II: Patient Information and Informed Consent Form	114-119
Annexure III: Pain Evaluation Forms	120-122
Annexure IV: Fact-O Questionnaire	123-125
9. Publications	

PREFACE

Ovarian cancer is a multifactorial disease with very few early detection markers that results in late-stage disease presentation and poor chances of successful treatment and survival time. Since the GOG158 (Ozols et al, 2003) clinical trial at Carboplatin and Paclitaxel is the preferred treatment for ovarian cancer patients with primary and surgically resected advanced stage (III & IV) tumors. The trial established the above regimen to be non-inferior and less toxic when compared to Cisplatin and Paclitaxel. The trial data had represented a follow up of 6 years and hence for the last 30 years there has been negligible changes in the first-line treatment of advanced epithelial ovarian carcinoma. Despite of the success of the trial patients can be categorised as platinum sensitive (responders) partially platinum sensitive (partial responders) and platinum resistant/refractory (non-responders). Thus, we aim to identify tumor markers that can predict and stratify between the responders, partial and non-responders. With this aim we have also observed, identified, and analysed the clinical characteristics and disease outcomes specific to the subcontinental population.

While study designing, we found that spindle assembly checkpoint (SAC) was activated by both Carboplatin and Paclitaxel that triggers mitotic arrest and subsequent apoptosis in ovarian cancer cells *in vitro*. So, the major components of the of the SAC i.e., MAD1, MAD2, BUB1 and BUB3 expression were analysed. Similarly, from literature search, several single nucleotide polymorphisms in the SAC components were found to be associated with cancer development, aneuploidy, mosaic variegated disease and worse outcome in cancer patients. Hence, a few SNPs in MAD1, MAD2, BUB1 and BUB3 were selected and correlated with clinical response, safety of chemotherapy and 2-year survival outcomes.

The methodology included clinical assessments at different time intervals, histology, immunohistochemistry (IHC) and PCR-RFLP. Additionally, sanger sequencing was performed along with qRT-PCR for the mRNA expression to correlate with the IHC results along with investigation of miRNA involvement in upregulation/downregulation of SAC components.

Majority of the patients were in the age range of 41-60 years, from rural setup, presented with abdominal complaints, nausea, vomiting, fever, and pelvic symptoms. Serous was

the most observed histological subtype (81%), with space occupying lesion more than 5cm (73·2%). Most of the tumors were large at the time of diagnosis and platinum sensitive (ORR= 77·27%) however, the recurrence was inevitable. 41, 44 and 25 patients were categorized as responders, partial responders, and non-responders. The standard regimen was well tolerated but Grade 3/4 toxicities in anemia, anxiety/depression, diarrhoea and constipation were observed. Patients had poor physical, functional, social well-being with worsening of disease- specific concerns. They experienced significant neuropathy and movement-associated pain. The overall survival for responders, partial responders and non-responders were significantly different ($p<0·05$) with CA125, age, parity, menopausal age and occupation being significant baseline risk factors (HR>1).

The findings of this study highlight the downregulation of MAD1 and BUB1 with upregulation of MAD2 and BUB3 as the signature feature in advanced ovarian cancer pathology suggestive of impaired SAC pathway in the tumor cells. MAD2, BUB1B and BUB3 SNPs had significant relevance to chemo-induced toxicity. In addition, MAD2 expression was significantly associated with poor OS. Association of the miRs analysed showed significant impact on survival outcome either independently or in correlation with a SAC component.

All the findings need to be further validated in a larger cohort. However, with the results of the present study a dysregulated SAC can have important clinical utility to stratify patients who may become non-responders to Paclitaxel-Carboplatin therapy. The present research has demonstrated the tip of the iceberg as SAC signalling remains to be a mysterious pathway affecting several physiological, pathological, and pharmacodynamic mechanisms.

LIST OF ABBREVIATIONS

OC	:	Ovarian Cancer
EOC	:	Epithelial Ovarian Cancer
GOG	:	Gynecologic Oncology Group
PFS	:	Progression free survival
QOL/Qol	:	Quality of Life
SAC	:	Spindle assembly checkpoint
BUB1	:	Budding uninhibited by benzimidazole 1
BUB2	:	Budding uninhibited by benzimidazole 2
BUB3	:	Budding uninhibited by benzimidazole 3
MAD1	:	Mitotic arrest deficiency 1
MAD2	:	Mitotic arrest deficiency 2
MAD3	:	Mitotic arrest deficiency 3
MTA	:	Microtubule targeting agents
DDR	:	DNA damage response
CA-125	:	Cancer antigen 125
ILs	:	Interleukins
TNF	:	Tumor Necrosis Factor
IL-8	:	Interleukin 8
CCL2	:	Chemokine (CC-motif) ligand 2
MCP-1	:	Monocyte chemoattractant protein 1
CCL5	:	Chemokine (CC-motif) ligand 5
RANTES	:	Regulated upon activation, normal T cell expressed and secreted
BARD1	:	BRCA 1 associated ring domain 1
RAD51C/D	:	RAD51 recombinase homolog C/D
BRIP1	:	BRCA1- interacting protein 1

PALB2	:	Partner and localizer of BRCA2
MMR	:	DNA Mismatch repair
BRCA1	:	Breast cancer gene 1
BRCA2	:	Breast cancer gene 2
MDM2	:	Murine double minute 2
MDM4	:	Murine double minute 4
HGSOC	:	High grade serous ovarian cancer
HR	:	Homologous Recombination/ Hazard ratio
DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid
AURKA	:	Aurora Kinase A
ERBB3	:	Human epidermal growth factor receptor 3
CDK2	:	Cyclin-dependent kinase 2
mTOR	:	mammalian target of Rapamycin
BRD4	:	Bromodomain protein 4
MYC	:	Master Regulator of Cell Cycle entry and proliferative metabolism
FOXM1	:	Forkhead box protein M1
PI3K	:	Phosphoinositide 3-kinase
RAS	:	Rat sarcoma virus
MEK	:	Mitogen activated protein kinase kinase
PARP	:	Poly (ADP-ribose) polymerases
PTEN	:	Phosphatase and TENsin homolog deleted on chromosome 10
KRAS	:	Kirsten rat sarcoma virus
BRAF	:	v-raf murine sarcoma viral oncogene homolog B1
CDKN2A	:	Cyclin dependent kinase inhibitor 2A
RNF43	:	Ring finger protein 3

ELF3	:	Early flowering 3
GNAS	:	Guanine nucleotide binding protein
KLF5	:	Kruppel-like factors 5
SMARCA4	:	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
RASSF1A	:	Ras association domain-containing protein 1
HNF1B	:	Hepatocyte nuclear factor 1 homeobox B
RAR	:	Retinoic acid receptor
MGMT	:	O6-Methylguanine-DNA methyltransferase
APC	:	Antigen presenting cell
GSTP1	:	Glutathione S-transferase pi gene
ARID1A	:	AT-rich interactive domain-containing protein 1A
HDAC	:	Histone deacetylase
MHC	:	Major histocompatibility complex
DNMTi	:	DNA methyltransferase inhibitors
HDACi	:	Histone deacetylase inhibitors
CSF-1	:	Macrophage colony stimulating factor 1
IL-6	:	Interleukin 6
NF-kB	:	Nuclear factor kappa B
TAMs	:	Tumor-associated macrophages
ATP7A/B	:	Copper transporting P-type ATPase
MRP1	:	Multidrug resistance protein 1
NER	:	Nucleotide excision repair
ERCC1	:	Excision repair cross complementation group 1
FDA	:	Food and Drug Administration
NSCLCs	:	non-small-cell lung cancers

GTP	:	Guanosine triphosphate
APC/C	:	Anaphase-promoting complex/cyclosome
CYP	:	Cytochrome P450
G-CSF	:	Granulocyte-colony stimulating factor
CPC	:	Chromosome Passenger Complex
CENP-A	:	Centromeric Protein A
KMN network	:	KNL1/Mis12 complex/ NDC80 complex
CDC20	:	Cell division control protein 20
Mps1	:	Monopolar spindle gene 1
BUBR1	:	BUB related protein 1
KNL1	:	Kinetochore scaffold 1
MELT	:	Met-Glu-Leu-Thr repeats
CIN	:	Chromosomal instability
DMBA	:	Dimethylbenzanthracene
HSIL	:	High grade intraepithelial lesion
LSIL	:	Low grade intraepithelial lesion
TTK	:	Threonine tyrosine kinase
PRP4K	:	pre-mRNA splicing factor 4 kinase
SKOV3	:	Ovarian cancer cell line
CCNG1	:	Cyclin G1 (CCNG1)
PLK1	:	Polo-like kinase 1
USG	:	Ultrasonography
CECT	:	High dose contrast-enhanced computed tomography
FIGO	:	The international Federation of Gynecology and Obstetrics
CTCAE	:	Common terminology criteria for adverse events
SGOT	:	Serum glutamic-oxaloacetic transaminase

ACT	:	Adjuvant chemotherapy
NACT	:	Neo-adjuvant chemotherapy
BSO	:	Bilateral salpingo-oophorectomy
TAH	:	Total abdominal hysterectomy
GCIG	:	Gynecologic cancer InterGroup
NRS	:	Numerical Rating Scale
NPSI	:	Neuropathic pain system inventory
PBS	:	Phosphate buffered saline
NBF	:	Neutral buffered formalin
HRP	:	Horseradish Peroxidase
DAB	:	3,3'- Diaminobenzidine
DPX	:	Distyrene, plasticizer, xylene
TE	:	Tris EDTA
TAE	:	Tris-acetate-EDTA
PCR	:	Polymerase chain reaction
RFLP	:	Restriction fragment length polymorphism
PAGE	:	Polyacrylamide Gel Electrophoresis
GAPDH	:	Glyceraldehyde-3- phosphate dehydrogenase
ECOG	:	Eastern cooperative oncology group
CA19·9	:	Cancer antigen 19.9
CEA	:	Carcinoembryonic antigen
ORR	:	Objective/Overall response rate
CIPN	:	Chemo-induced peripheral neuropathy

LIST OF TABLES

Table 3.1: Genes, designed primers, annealing temperatures and product sizes

Table 3.2: Genes, restriction enzymes and expected patterns after digestion

Table 3.3: Primers used for amplification of mRNA and miRs

Table 4.1: Sociodemographic characteristics of the ovarian cancer patients

Table 4.2: Gyne-obstetric history of the patients

Table 4.3: Clinical features and clinical response of the patients

Table 4.4: Significance of survival distributions among response categories analysed by Log Rank test

Table 4.5: Common clinically observed toxicities

Table 4.6: Association of neuropathic pain occurrence in adjuvant and neoadjuvant chemotherapy groups

Table 4.7: Mean NRS scores in responders, partial responders, and non-responders at various time intervals

Table 4.8: Patient distribution having different types of neuropathic pain at various time intervals

Table 4.9: FACT-O scores at various time intervals among responders, partial responders, and non-responders

Table 4.10: Survival probability in relationship with QoL at different time points and chemotherapy

Table 4.11: Immunohistochemical expression of MAD1 in proportion of OC tumours

Table 4.12: Immunohistochemical expression of MAD2 in proportion of OC tumours

Table 4.13: Immunohistochemical expression of BUB3 in proportion of OC tumours

Table 4.14: Immunohistochemical expression of BUB3 in proportion of OC tumours

Table 4.15: Ct median for the mRNA and miR

Table 4.16: Distribution of alleles in the ovarian cancer patients observed by RFLP

Table 4.17: Association of polymorphisms with clinical response, survival outcome and risk to survival

Table 4.18: Association of MAD1L1(rs1801368) with toxicities

Table 4.19: Association of MAD2L1(rs1972014) with toxicities

Table 4.20: Association of BUB1B (rs28989181) with toxicities

Table 4.21: Association of BUB1B (rs28989186) with toxicities

Table 4.22: Association of BUB3 (rs11248416) with toxicities

Table 4.23: Association of BUB3 (rs11248419) with toxicities

Table 4.24: Association of BUB3 (rs6599657) with toxicities

LIST OF FIGURES

Figure 1.1: Different states of chromatid-kinetochore attachments that activate the spindle assembly checkpoint (Sarkar et al., 2021)

Figure 1.2: Schematic diagram of the on and off states of spindle assembly checkpoint (Sarkar et al., 2021)

Figure 3.1. Experimental Design

Figure 4.1: Initial symptoms

Figure 4.2: Gross structure of low grade (A), high grade (B), large (25cm approx) malignant tumors. C: Papillary structures visible after cutting through the ovarian capsule of a 10% NBF fixed hemorrhagic malignant ovarian tumor

Figure 4.3: A: Enbloc uterus and ovarian tumors (shown by black arrows); B: mild pleural effusion of Stage IV ovarian cancer patient; C: Pre NACT CECT of abdomen shows gross ascites, omental cake, multiple enhancing peritoneal nodules seen; D: Post NACT CECT shows liver, gall bladder, spleen, pancreas (within normal limit), mild omental thickening.

Figure 4.4: Kaplan Meier Survival Curve plotted for Responders, Partial Responders and Non-Responders

Figure 4.5: Survival risks associated with patient characteristics

Figure 4.6: Mean QoL scores for all segments of Fact-O evaluation at different time points

Figure 4.7: Histopathology of A, High grade serous papillary carcinoma (20X); B, Mucinous carcinoma (20X); C, Endometroid carcinoma of ovary; D, Ascitic fluid cytology showing adenocarcinoma deposits (40X)

Figure 4.8: High grade serous papillary carcinoma stained with MAD2 antibody, A (20X), B (40X). The images show high nuclear immune-positivity of MAD2.

Figure 4.9: IHC expressions of MAD1 observed in advanced ovarian carcinoma tissues. Brown colour indicates very low to moderate intensity immunopositivity in both nucleus and cytoplasm, A (20X), B (40X).

Figure 4.10: IHC expressions of BUB1 observed in ovarian cancer tissue, A (20X), B (40X).

Figure 4.11: IHC expressions of BUB3 observed in ovarian cancer tissue, A (10X), B (20X), C (40X), D (100X).

Figure 4.12: Melt curve of the qPCR

Figure 4.13: Survival curves with related to MAD2 expression (high, moderate, low) in ovarian cancer patients.

Figure 4.14: A: PCR amplification; B: RFLP of PCR products of MAD1L1(rs1801368)

Figure 4.15: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs1972014)

Figure 4.16: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs1546120)

Figure 4.17: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs3752830)

Figure 4.18: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs121908981)

Figure 4.19: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1B (rs28989181)

Figure 4.20: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1B (rs28989186)

Figure 4.21: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1 (rs121909055)

Figure 4.22: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs11248416)

Figure 4.23: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs11248419)

Figure 4.24: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs6599657)

CHAPTER 1

1.1. INTRODUCTION

Cancer is a group of diseases characterized by uncontrollable pathological cell division which favors tumor growth with evasion of apoptosis and metastatic dissemination (Fouad and Aanei, 2017). In 2020, the global burden of cancer was 19.3 million new cases and 10 million deaths worldwide (Ferlay et al., 2020). According to American Cancer Society (2022) ovarian cancer (OC) is the fifth cause of cancer deaths among women (American Cancer Society, 2022). OC accounts for approximately 45,701 new cases and 32,077deaths every year in India (Ferlay et al., 2020). The disease is widely metastatic in the abdomen and late diagnosis is the reason of the high death rates of this malignancy (Jayson et al., 2014). The most relevant and important symptoms of epithelial ovarian cancer (EOC) include persistent abdominal swelling, bloating, altered bowel habit, pelvic pain, vaginal bleeding, indigestion, and loss of appetite (Brain et al., 2014; Gajjar et al., 2012). The current standard of care for OC is a combination of surgical staging and judicious use of chemotherapy. The primary adjuvant therapy for stages 2 to 4 involves intravenous regimens of carboplatin + paclitaxel and intravenous/intraperitoneal regimen of paclitaxel + cisplatin (Armstrong et al., 2021; Armstrong et al., 2006; Ozols et al., 2003). GOG 158 clinical trial compared these two therapy arms in eligible patients with residual stage III disease. The carboplatin group was found to be non-inferior and more GI, renal and metabolic toxicity, grade 4 leukopenia with cisplatin + paclitaxel was observed. Thus, the paclitaxel + carboplatin regimen became a new standard of care without fully replacing cisplatin (Ozols et al., 2003). Post-surgical adjuvant chemotherapy of paclitaxel and carboplatin for advanced stages of EOC does not seem to increase the relative 5-year survival rate or the median progression free survival (PFS) compared to results of earlier studies. In India, a single study by Shamsunder et al., (2000) at AIIMS, Delhi on patients with recurrent EOC concluded that cisplatin-based chemotherapy was beneficial in recurrent disease but there was no discussion on clinical efficacy with respect to primary adjuvant therapy. Despite all the improvements of treatment with dose optimization, and efficacy; toxicity remains an important issue. Cisplatin and carboplatin along with paclitaxel continue to produce a lot of drug-induced adverse effects such as nausea, vomiting, neutropenia, thrombocytopenia, anemia, neuropathy, abdominal pain, and diarrhoea (Bajpai et al.,

2017; Koo et al., 2015). Thus, clinical efficacy, safer doses, effective treatments and improved QoL are still a requirement for advanced ovarian cancer patients.

Loss of mitosis regulation is a common feature of cancer cells which leads to aberrant cell division with inaccurate chromosome segregation and aneuploidy. The Spindle Assembly Checkpoint (SAC) or the Mitotic Checkpoint functions to preserve the genome from chromosome imbalances by delaying the anaphase onset until all the chromosomes are bi-oriented and properly attached to the mitotic spindle (London and Biggins., 2014). It was first discovered in the budding yeast genetic screens experiment, which identified some major components of SAC; budding uninhibited by benzimidazole genes (BUB1, BUB2, BUB3) (Hoyt et al., 1991) and mitotic arrest-deficient genes (MAD1, MAD2, MAD3) (Li et al., 1991). Several evidences suggest that mutations in SAC components or their reduced expressions may lead to tumorigenesis (Percy et al., 2000; Tsukasaki et al., 2001). Hence, SAC had been one of the most targeted pathways in anti-cancer drug development. The spindle poisons or the microtubule targeting agents (MTA) influence the microtubule dynamics and consequently, SAC is activated and cells undergo a prolonged mitotic arrest followed by apoptosis. Paclitaxel and Vinka alkaloids are prototypes of MTAs, which are used in the first line treatment of several cancers of breast, ovarian, non-small cell lung and, head and neck (Rowinsky and Donehower, 1995). Despite being one of the most successful chemotherapeutic agents, resistance poses a significant hindrance to their clinical efficacies. Underlying mechanisms of resistance include mitotic slippage even with an active checkpoint and variations in the SAC signaling strength (Henriques et al.2019, London and Biggins., 2014). Among many other cancers, altered expression of SAC components is observed in epithelial ovarian carcinoma which may be the reason of extensive aneuploidy and chromosomal instability in the said cancer (Capo chichi et al., 2016). Since spindle microtubules are the primary drug targets for paclitaxel, key SAC proteins such as MAD2 and BUB1 have been important predictors for paclitaxel response (Hao et al., 2010; Fu et al., 2007; Fang et al., 2006).

A weakened spindle checkpoint with reduced expression of BubR1 (Bub related protein 1), is associated with acquired paclitaxel resistance in OC cells (Fu et al., 2007) and reduced levels of MAD2 is associated with poorer outcome (Byrne, 2017). In addition, spindle checkpoint activation by MAD2 is shown to induce mitotic arrest in response to

DNA damage, indicating overlapping roles of the SAC and DDR (Lawrence et al., 2015) and a novel role of MAD2 in rendering sensitivity towards platinum compounds. However, the relationship between the SAC components and platinum/taxane has not been fully understood. A few polymorphisms of MAD1, MAD2, BUB1, BUB3, have been identified (Akhoundi et al., 2016) and strongly linked with development of different cancers like breast (Wang et al., 2014) and lung (Guo et al., 2010). The most studied MAD1 1673G > A polymorphism plays a role in recurrence (Cruz et al., 2017) and is also reported to be associated with a worse response to chemotherapy in patients with OC (Santibanez et al., 2013). Genetic variations in SAC components and their expression may contribute to compromised efficacy and toxicity of platinum/taxane therapy in ovarian cancer patients.

Thus, in this study we aim for the assessment of clinical efficacy of Carboplatin-Paclitaxel and further study the expressions & polymorphisms of MAD1, MAD2, BUB1 and BUB3 genes with the hope to identify clinical utility of this pathway in order to manage ovarian cancer better.

1.2. OBJECTIVES

- To assess the clinical efficacy of carboplatin and paclitaxel chemotherapy in advanced epithelial ovarian cancer patients.
- To assess the immunohistochemical expressions of MAD1, MAD2, BUB1 and BUB3 in ovarian cancer patients.
- To investigate the effects of single nucleotide polymorphisms of MAD1, MAD2, BUB1 and BUB3 and their allele frequencies in response to combinational chemotherapy in ovarian carcinoma.
- Analysis of data for prediction and clinical management of epithelial ovarian carcinoma patients.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Ovarian cancer

The ovaries are one the most important female reproductive organs of round shape that are about 3 x 1.5 cm in length and 1 cm thick. The ovarian surface layer is formed of simple squamous or cuboidal epithelium called the germinal epithelium, that is enclosed by a thicker layer of connective tissue called tunica albuginea. The cortical part of the ovary houses the ovarian follicles that encase the oocytes, while the medullary region has a dense vascular bed. The ovarian stroma is made up of reticular fibres, ground material, and fibroblasts with the distinctive spindle shape that respond to hormonal stimuli (Hoffman and Whitridge, 2012).

Epithelial ovarian cancer (EOC) is a heterogeneous malignancy made up of tumors with various histology, grades, genetic, epigenetic, and microenvironmental characteristics. These factors all affect therapy options and the course of the disease. In order to treat ovarian cancer successfully, prompt detection, access to the right surgery, and systemic chemotherapy are essential. Even in high-resource nations like the USA and Canada, the 5-year survival rate for ovarian Vs breast malignancies is at 47% Vs 85% (Howlader et al., 2020).

Ovarian cancer (OC) is the 8th most frequent cancer and the high mortality rate makes it the 2nd most common cause of gynecologic cancer death globally. With 152,000 deaths (4.3% of all cancer deaths) and 239,000 new cases (3.6% of all cancer cases) annually; the mortality-to-incidence ratio of OC is >0.6; 1 in 6 deaths within 90 days from diagnosis were reported from US and UK. Along with the potential high mortality the disease presents itself with very high morbidity in advanced stages and barriers to effective therapy, (Lhereux et al., 2019). The mainstays of therapy have continued to be cytoreductive surgery and combined platinum-taxane chemotherapy for many years.

2.2. Screening, Diagnosis and Clinical manifestations

These dismal statistics are partly attributable to the absence of reliable screening tools to identify ovarian cancer at an early stage and the absence of early, specific warning signs or symptoms that cause a diagnostic delay (Della et al., 2015).

It's possible that ovarian cancer does not first manifest any symptoms. When ovarian cancer symptoms appear, they are frequently mistaken for those of other, more prevalent diseases. The transvaginal ultrasound and Cancer antigen 125 (CA-125) blood biomarker are majorly used to diagnose/ suspect ovarian cancer followed by histological analyses. Ovarian cancer symptoms and signs can include: changes in bowel habits, such as constipation; a frequent need to urinate; acidity; bloating or swelling in the abdomen; weight loss; pelvic discomfort (Matulonis et al., 2020).

2.3. Types and Stages

EOC is histologically divided into five main subtypes: mucinous ovarian cancer, endometrioid cancer, high-grade serous cancer, and low-grade serous cancer. Histological grading, which relates to prognosis, is used to further subclassify epithelial malignancies of the ovary and fallopian tube. The nonepithelial tumors are not included in this grading scheme. Despite being rare, nonepithelial tumors are quite significant. These include lymphomas, sarcomas, granulosa cell tumors, and germ cell cancers. In accordance with their respective sites of origin, metastatic neoplasms to the ovary are graded and staged, including tumors in the breast, lower reproductive tract (cervix or uterine carcinomas), and gastrointestinal tract (signet ring cell [Krukenberg] carcinomas, low grade appendiceal or pancreaticobiliary mucinous tumors, among other neoplasms).

Stage I OC is defined as when the cancer cells are confined within the ovaries only. It can be present in either one (IA) or both (IB) the ovaries, or IB + presence of cancer cells in the abdominal fluid either due to burst of fluid filled tumor or leakage during surgical removal (IC). Stage II OC is defined when the cancer has spread to nearby organs like the uterus, fallopian tubes (IIA) or to the rectum or colon, and urinary bladder (IIB). Stage III (A/B/C) OC is determined by the presence of cancer cells in stomach lining which is visible under microscope, in the nearby lymph nodes, reaching to the liver and spleen (lesions approx. 2cm or more). Stage IV is the most advanced stage when the cancer has spread to distant organ. Once cancer cells are detected in the pleural fluid

cavity but no other places than the abdominal cavity, it is Stage IVA. Stage IVB is when there are distant metastases in lung, brain, breast, and skin (Prat, 2014).

2.4. Pathophysiology

One of the most sinuous types of cancer in humans is possibly the EOC. Among the few theories to determine the aetiology of EOC, the gonadotrophin theory and the hypothesis of continuous ovulation dominate the most. The incessant ovulation hypothesis, put forth in the early 1970s, claims that the development of EOC is caused by repeated damage of the ovarian surface epithelium during cyclical ovulation and its subsequent repair, which may increase the possibility of replicative DNA errors being integrated in ovarian epithelial tissue (Fathalla, 1971). On the other hand, the gonadotrophin hypothesis is suggestive of exposure to excessive gonadotrophins leading to enhanced epithelial cell proliferation and malignant transformation (Cramer and Welch, 1983). A third theory also states that hormonal influences of androgen and progesterone, may majorly impact on cell replicative signaling in the ovarian surface epithelia and, hence, EOC (Risch, 1998).

2.4.1. Inflammation

The surface epithelium of the ovary is directly exposed to inflammatory, metabolic, xenobiotic and environmental stress existing in the peritoneal cavity. Seeding of malignant ovarian cell is further facilitated by the constant flow of peritoneal fluid (Naora and Montell, 2005). This particular so called "open" environment has resulted in a myriad of heterogenetic features specific to epithelial OC with unique formation of ascites and faster metastases in distant organs (Risch, 1998).

The process of ovulation, was discovered to be pro-inflammatory and perhaps mutagenic more than two decades ago (Ness and Cottreau, 1999). The series of events that take place during ovulation are multiplication and apoptosis of proximal ovarian cells, followed by follicular expansion and thinning of theca externa and tunica albuginea, finally extrusion of ovum. These ovulatory processes, are characterized by the production of large number of chemokines or cytokines and matrix remodeling enzymes, such as bioactive eicosanoids, prostaglandins, plasminogen activators, collagenases, interleukins (ILs), tumor necrosis factors (TNF), and various growth factors. The panel of inflammatory modulators strongly associated with EOC include but not limited to IL-

8, CCL2/MCP-1, CCL5/RANTES. Both pathological and physiological ways of inflammatory assaults might expose the fallopian tube as well. Under normal circumstances, the retrograde flow of endometrial fluid causes the fallopian tube to become acutely inflamed by the exposure to a variety of inflammatory molecules, such as TNF-α, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF), all of which have been shown to be elevated in ovarian tumor specimens (Strandell et al., 2004, Maisey et al., 2003, McGee et al., 1999). Additionally, the functional tubal fimbria, that has two epithelial surfaces made up of peritoneal mesothelium and ciliated epithelium (endosalpinx), may be a location of ongoing abrasion, stress-induced inflammation, and ultimately, aiding to the start of malignant changes (Carvalho et al., 2008).

Studies that support the gonadotrophin theory and the hormonal hypothesis, respectively, claim that elevated levels of estrogens and androgens can enhance immune responses by attracting molecular effectors and pro-inflammatory cells.

2.4.2. Genetics

The genetic changes related to DNA repair in ovarian tumors have received the most research interest. Approximately 1/3rd of OCs, encompassing both serous and non-serous histology, have been found to contain germline or somatic mutations in homologous recombination (HR) genes (clear-cell and endometrioid carcinomas, as well as carcinosarcoma). The genes involved in Fanconi anemia pathway (BARD1, RAD51C/D, BRIP1, and PALB2,) DNA mismatch repair (MMR), and the BRCA1, BRCA2 genes are the most frequently implicated hereditary genes (Castilla et al., 1994).

TP53 is the most frequently altered gene in HGSOC. Mis-sense or nonsense mutations, in-frame and frameshift insertions and deletions, as well as TP53 alterations, are all possible. TP53 mutations can occur in areas of the gene encoding the non-DNA-binding domains as well as the DNA-binding domains. Tumors without TP53 mutations exhibit symptoms of p53 malfunction through an increase in the copy number of MDM2 or MDM4, two genes whose products are involved in the control and degradation of p53 itself. Studies revealed defective homologous recombination in ~50% of analyzed HGSOCs. HR defects are associated with mutations and alterations in BRCA (germline and somatic), and DNA damage repair pathway genes. BRCA1 functions in DNA repair,

remodeling of chromatin and transcriptional regulation, cell cycle checkpoint control, mitosis; BRCA2 is crucial for homologous recombination and DNA repair (Alsop et al., 2012). Additional recurrent genetic defects were found in AURKA, ERBB3, CDK2, MTOR, BRD4 and MYC genes and molecular alterations in HGSOC were identified forkhead box protein M1 (FOXM1), Notch, phosphoinositide 3-kinase (PI3K), and RAS–MEK signalling pathways, with an increased somatic copy number alterations in the respective (Walsh et al., 2011, Ketabi et al., 2011, Bell et al., 2011, Reyez-Gonzalez et al., 2013). These genes and pathways could be future therapeutic targets as they are reported to activated and important for cancer cell survival in HGSOC cell lines. With the aid of information from gene expression profiling, HGSOC has been further segmented. Based on gene expression, the Cancer Genome Atlas identified four subtypes of HGSOC: differentiated, immunoreactive, mesenchymal, and proliferative. These subtypes differ in clinical prognosis; however, this information has not been therapeutically helpful for patient care. Integrated genomic analysis using several platforms have been used in attempts to more precisely characterise the HGSOC subgroups. For instance, a network controlled by microRNAs (miRNAs) was discovered and linked to the mesenchymal subtype of HGSOC as well as poor clinical outcomes (Wang et al., 2013). According to certain studies employing gene expression profile, treatment resistance, responsiveness to platinum-based chemotherapy, and PARP inhibitors, the prognosis of patients with advanced-stage HGSOC has been predicted. However, because the focus of these studies was on retrospective analysis, prospective data from randomised trials are still needed to show the effectiveness of expression assays in patient subtyping. The molecular diversity of HGSOC at the time of diagnosis, its progression and change over time, the lack of many druggable driver mutations, and the high rate of copy number changes in genes of various signalling pathways are characteristics of this cancer's genomic complexity. In fact, this chemical complexity explains why it might have been difficult to develop effective treatments for HGSOC. Even though ovarian cancer patients have recurrent mutations, according to genomic data, some tumors, particularly those of the HGSOC subtype, are genetically heterogeneous (Matulonis et al., 2016).

Apart from the serous histological subtype, others can also harbour mutation in PTEN, PIK3CA and ARID1A. KRAS, BRAF mutations are frequent in low grade serous cancers. Endometroid ovarian cancers have ARID1A, KRAS and PIK3CA mutations,

with PTEN loss. Clear cell carcinomas can also harbour KRAS. The most common mutational characteristic of mucinous carcinomas has been demonstrated to have C>T transitions in a NpCpG trinucleotide context, indicating deamination of methylcytosines. TP53 mutations are present in about 50% of mucinous carcinomas, while KRAS, BRAF, CDKN2A, RNF43, ELF3, GNAS, ERBB3 and KLF5 mutations are also common. Small-cell carcinomas linked to hypercalcaemia have somatic or germline mutations in SMARCA4.

2.4.3. Epigenetics

Epigenetic changes like hypermethylation were reported to be significantly higher in BRCA1 and RASSF1A promoter regions in ovarian cancer tissues. Silencing of these genes through hypermethylation drives genomic instability and promotes cell-cycle progression (Ibanez et al, 2004). Although it has been demonstrated in a sizable fraction of HGSCs, hypermethylation of the BRCA1 promoter has no effect on overall survival and prognosis.

Gloss et al. 2014 demonstrates silenced the lincRNA ZNF300P1 in serous ovarian cancer by hypermethylation, which is a crucial regulator of cell motility ad cycle. This is an illustration of an epigenetic change that has the potential to serve as a biomarker for disease. Clear cell ovarian cancer included 22 distinct CpG sites that were hypermethylated and linked to 9 different genes (Cicek et al., 2013). In HGSOC but not in the clear cell subtype, transcription factor HNF1B is methylated (Shen et al., 2013). Furthermore, reports of promoter hypermethylation of a group of genes associated with cancer (H-cadherin, RAR, p16, E-cadherin, MGMT, APC, GSTP1) were shown to be substantially more common in invasive carcinomas than in benign cystadenomas and non-invasive malignancies (Markala et al., 2005). Chromatin modifying enzymes like H3K9 methyltransferase G9a, that functions to add methyl groups to histone, was found to be present in high levels in HGSOC that correlates with late-stage and shorter survival (Hua et al., 2014). Stem-like cells or bivalent chromatin loci with activated and transcribed H3K4me3 and silenced, non-transcribed H3K27me3 were observed in high-grade cancer cells than nonneoplastic cells. The ARID1A is a chromatin remodeler is mutated in more than 50% of ovarian clear cell carcinomas (Bitler et al., 2015). The histone deacetylase (HDAC) enzymes downregulate gene transcription by deacetylation of positively charged histones which remains tightly bound to the negatively charged

DNA thereby promoting a closed chromatin structure. Apoptotic and tumor suppressor capabilities of P53 are restored when HDAC6 is inhibited in tumors with ARID1A mutations. Pan-HDAC inhibitors have demonstrated notable effectiveness against ARID1A-mutated tumor in preclinical models of clear cell carcinoma in addition to specialised HDAC inhibitors Fukumoto et al.2018. By triggering an interferon response through the overexpression of surface tumor antigens and important immunomodulatory proteins such major histocompatibility complex (MHC) components, DNMTis and HDACis can reverse immune evasion and sensitise to subsequent immune checkpoint inhibition (Jones et al, 2016)

2.4.4. Immune cells and microenvironment

A recent topic of research in the aetiology of ovarian cancer is the function of the immune system and the tumor microenvironment. Numerous studies have demonstrated a link between ovarian cancer cytotoxic T cell infiltration and an increase in overall survival. For instance, peripheral blood, ovarian cancer tissue, and ascites can all contain antitumor immune responses made up of tumor-reactive T cells and tumor-specific antibodies. Furthermore, cytotoxic T cell infiltration in ovarian cancers has been linked in multiple studies to an improvement in overall survival.

The tumor microenvironment is made up of a range of cells and substances that suppress immune responses, support cancer cell survival, support angiogenesis and metastasis, and ultimately support the growth and proliferation of the tumor (Jones et al, 2016). The critical role of macrophages has been identified in several ovarian cancer pathological processes, such as tumor growth, tumor cell proliferation, angiogenesis, invasion, and metastasis. By modulating an immunostimulatory phenotype, suppressing tumor growth, and enhancing tumor cells' response to both conventional and novel chemo-therapeutics and targeting macrophage-produced factors like CSF-1, IL-6, NF-kB, or even macrophages may be a successful cytotoxic approach against ovarian cancer. Additionally important participants in the development of an immunosuppressive tumor microenvironment are macrophages. However, a fuller understanding of macrophages will be essential if they are to be used clinically, particularly as a diagnosis and prognosis marker in ovarian cancer. However, there are still several issues that require further study. For instance, the precise molecular mechanisms by which TAMs suppress the immune system are unknown. Furthermore, it's unclear exactly what M1 and M2

macrophages contribute to the tumor microenvironment. Even though it is well known that M2 macrophages promote tumor progression and M1 macrophages suppress tumor growth, it has been demonstrated that M1 cells can also hasten the metastatic process in OC. Therefore, it appears that tumor-related suppressive factors have an impact on macrophage (M1 and M2) function in the tumor microenvironment in order to promote tumor growth (Matulonis et al., 2016, Lu et al., 2007, Hwang et al., 2012).

2.5. Chemotherapy of Primary epithelial ovarian cancer

2.5.1. Pharmacology of Platinum Co-ordination Complexes

The platinum coordination complexes include cisplatin and carboplatin that are divalent, water- soluble and inorganic compounds. These platinum drugs enter the cells through the active Cu^{2+} transporter CTR1 and quickly destroy the transporter (Kruh, 2003). Varying expressions of the Cu^{2+} transporters (ATP7A and ATP7B) and multidrug resistance protein 1 (MRP1) and their influences on other cellular mechanism might be a cause of clinical resistance (Dolan and Fitch, 2007). The three platinum analogues; i.e., Cisplatin, carboplatin and oxaliplatin, have their chloride, cyclohexane, or oxalate ligands displaced by water molecules inside the cell respectively. This results in positively charged and extremely reactive platinum moieties. These aquated species of the drugs then interact with nucleophilic sites on the DNA and proteins to begin their cytotoxic reaction. The aquation of cisplatin is favoured with low chloride levels in urine and inside cell. High chloride amount in the drug has a stabilizing impact in the body; hence, chloride diuresis can stop nephrotoxicity. The activated platinum complexes are required to create intrastrand and interstrand crosslinks with the DNA and can interact with electron-rich groups like sulfhydryls in various DNA locations. N-7- Guanine is a particularly reactive location, that results in platinum cross-links to develop GG intrastrand bonds on the same DNA strand. Similarly, cytotoxic Guanine-Adenine cross-links may also occur. Interstrand cross-links are less frequently formed. The "DNA-platinum adducts" thus produce single- and double-stranded breaks, that are capable of activating DNA damage response (DDR) in the cells however, upon failure to reverse the undesirable cross-links, replication, and transcription are prevented; apoptosis is induced when p53 and other checkpoint proteins detect them. The shape and bulk of the DNA-Pt adducts for each analogue varies in the impact of DNA distortion. For example, oxaliplatin has a bulkier DNA adduct than cisplatin which are more difficult to repair,

and cisplatin creates the DNA crosslinks faster than the rest two. A distinct H-bonding pattern with nearby DNA segments is also exhibited by these drugs (Sharma et al., 2007).

Typically, the cell cycle specificity of cisplatin activity varies among different cell types but only most noticeable during the S-Phase. Despite falling under the pharmacological classification of "anti-cancer agents", the platinum coordination complexes have the ability to cause cancer, mutations, and teratogenicity. A 4-fold greater incidence of subsequent leukaemia is linked to chemotherapy for ovarian cancer that uses the cisplatin or carboplatin molecule.

Development of platinum resistance is not a straight-forward mechanism and involves activation of innumerable signalling and factors like, intracellular levels of sulfhydryl (metallothionein), glutathione that bind to inactivate the drug, intracellular uptake, drug accumulation, efflux transporters, and efficiency of DNA repair signals. The three compounds have separate cross-resistance mechanisms as well. Carboplatin and cisplatin share cross resistance in most experimental tumors, but oxaliplatin does not (Meijer et al., 1990); and rates of repair of DNA adducts. Participation of the NER pathway is required to repair platinum-DNA adducts. It was observed that lack of NER pathway improves sensitivity to cisplatin on patients with ovarian cancer while upregulation of this pathway renders poor response to platinum-based chemotherapy in gastric, colon and lung carcinomas (Paré et al., 2008). Higher expression of NER component ERCC1 in peripheral white blood cells and cancer cell correlate with lower response to cisplatin (Dolan and Fitch, 2007).

The proteins related to MMR (hMLH1, hMLH2, or hMSH6), which identify platinum-DNA adducts and trigger apoptosis, appear to be partially responsible for resistance to cisplatin but not oxaliplatin. Sensitive cells are unable to replicate or translate damaged DNA strands in the absence of adequate DNA-platinum adduct repair. A few DNA polymerases were evidenced to avoid the DNA adducts particularly caused by cisplatin. However, adducts of oxaliplatin are more difficult to get around. Whether or not these polymerases increase resistance is yet up for debate. In yeast, it has been shown that cisplatin resistance is caused by a loss of active uptake; overexpression of the copper efflux transporters ATP7A/B are associated to a poor prognosis following cisplatin-based therapy for ovarian cancer.

The pharmacodynamic spectrum of carboplatin (CBDCA, JM-8) are like cisplatin. However, the two drugs differ significantly in their chemical, pharmacokinetic, and toxicological properties. Adequate hydration before treatment and chloride diuresis, cisplatin-induced nephrotoxicity has been largely reversed. Diuresis has no effect on the ototoxicity brought on by cisplatin, which manifests as tinnitus and high-frequency hearing loss. With repeated dosages, the ototoxicity become more frequent and severe, especially in youngsters. It might be unilateral or bilateral. Nearly all patients experience considerable nausea and vomiting, which is typically manageable with NK1-receptor inhibitors, 5-HT3 antagonists and high-dose corticosteroids.

Cisplatin induces a progressive peripheral motor and sensory neuropathy with higher doses and repeated cycles of treatment. This condition may develop after the drug is stopped and may be made worse by concurrent or subsequent treatment with taxanes or other neurotoxic medicines. Cisplatin results in temporary leukopenia, thrombocytopenia, and mild to moderate myelosuppression. After several treatment cycles, anemia may start to show itself more noticeably. Electrolyte abnormalities are frequent and include hypocalcemia, hypomagnesemia, hypophosphatemia, and hypokalemia. If left untreated, hypomagnesemia and hypocalcemia resulting from tubular injury and renal electrolyte depletion may cause tetany. It is advised to frequently measure plasma $Mg2+$ concentrations. Rare side effects include hyperuricemia, haemolytic anemia, and heart problems. To combat anaphylactic-like reactions characterised by bronchoconstriction, tachycardia, facial edema and hypotension; epinephrine injection (I.V.) with antihistamines and corticosteroids. Acute myeloid leukaemia can develop after approximately 4 years of cisplatin treatment. Much of the parent form of the drug remains in plasma, unattached to proteins.

Carboplatin is significantly found to be less reactive than cisplatin. Most drugs are excreted through the kidneys, having a plasma half-life of about 2 hours. A small fraction of platinum binds irreversibly to plasma proteins and disappears slowly, with a $t_{1/2}$ of 5 days.

Clinically, carboplatin is tolerated more favourably than cisplatin and results in reduced nausea, neurotoxicity, ototoxicity, and nephrotoxicity. Instead, myelosuppression, particularly thrombocytopenia, is the dose-limiting hazard. The likelihood of a

hypersensitive reaction is higher; in patients who experienced a mild reaction, premedication, graduated drug doses, and longer infusion times led to desensitisation.

The therapeutic uses of cisplatin and carboplatin are for non-small cell lung cancer, advanced, metastatic stages of small cell lung cancer and advanced ovarian cancer. Carboplatin was found to be less effective than cisplatin in cases of head and neck, esophageal and germ cell cancers (Go and Adjei, 1999). Carboplatin is a helpful alternative for patients who are unable to tolerate cisplatin due to disabling toxicities like ototoxicity, renal impairment, refractory nausea, and/ or neuropathy. For renal function, dosages must be altered. Additionally, it can be used in high-dose therapy in conjunction with peripheral stem or bone marrow cell rescue. The dose of carboplatin for people with a CrCl of 60 mL/min should be adjusted according to the decrease in creatinine clearance (CrCl).

The following formula is useful for calculation of dose:

Dose (mg)= AUC× (GFR+25)

where the target AUC (area under the plasma concentration–time curve) is ~5-7 min/mg/mL for acceptable toxicity in patients receiving single-agent carboplatin (GFR = glomerular filtration rate). Using the previously mentioned formula to calculate the dose, carboplatin (PARAPLATIN) is administered as an intravenous infusion over a minimum of 15 minutes once every 21-28 days.

2.5.2. Pharmacology of Taxanes (Paclitaxel)

Paclitaxel, an alkaloid ester was originally extracted from Pacific yew tree, *Taxus brevifolia* (Awada et al., 2014; Zou et al., 2015). But owing to its low concentration in plants; *T. brevifolia* is artificially cultured and the drug is synthesized chemically, synthetically, or semi-synthetically. Currently, the US Food and Drug Administration (FDA) has approved paclitaxel for the treatment of advanced ovarian cancer, colorectal cancer, breast cancer and squamous cell carcinoma of urinary bladder (Swain et al., 1995). Furthermore, it is also in the guideline for treatment of diseases such as small-cell and non-small-cell lung cancers (NSCLCs), head and neck cancers, and AIDS, Kaposi's sarcoma (Chen et al., 2016).

The unique ability of paclitaxel to encourage microtubule synthesis at low temperatures and without GTP sparked interest in the medication. The outcome is the appearance of bundles of microtubules and abnormal structures produced from microtubules during the mitotic phase of the cell cycle. It binds selectively to the -tubulin component of microtubules and inhibits the disintegration of this essential cytoskeletal protein. Following is a mitotic arrest. Both the drug concentration and the length of cell exposure affect cell death. Paclitaxel induces mitotic arrests and subsequent apoptosis by activation of the spindle assembly checkpoint (SAC)/ mitotic checkpoint which is a major control mechanism that delays anaphase onset to prevent chromosome missegregation (Kops et al., 2005; Lara-Gonzalez et al., 2012; Foley and Kapoor, 2013). The replicated pairs of sister chromatids connect to the spindle microtubules at their kinetochores, assembly of protein complexes on the centromeric region of DNA. Improperly attached or unattached kinetochores creates an unequal tension between the spindle poles as a result chromosomes are not bioriented or properly aligned at the equator of the dividing cell. Mitotic poisons like Paclitaxel treatment ceases the cell cycle due to presence of a small number of unattached kinetochore by inhibiting the anaphase-promoting complex/cyclosome (APC/C) (Waters et al., 1998). Cells may develop resistance by upregulating the III-isoform of tubulin because taxanes preferentially bind to the II-tubulin subunit of microtubules (Ranganathan et al., 1998).

Every three weeks, paclitaxel is given as a 3-hour infusion containing 135–175 mg/m2 or as a weekly 1-hour infusion containing 80–100 mg/m2. Longer infusions (up to 96 hours) have been tested and found to be effective in a variety of tumor histologies. About 10% of a dosage is excreted intact in the urine after the drug has undergone significant processing by hepatic CYPs (mainly CYP2C8 with a contribution from CYP3A4). Although 6-OH paclitaxel, the main metabolite discovered thus far, is inactive, plasma contains numerous other hydroxylation products (Cresteil et al., 1994).

The nonlinearity of paclitaxel clearance causes it to decline with increasing dose or dose rate. Hepatic metastases with a diameter more than 2 cm were associated with reduced clearance, high drug concentrations in plasma, and increased myelosuppression in studies using 96-hour infusions of 140 mg/m2 (35 mg/m2/day). With a clearance rate of 15–18 L/hr/m2 and a t1/2 of 10–14 hours, paclitaxel leaves the plasma compartment. Depending

on the length of exposure, the threshold plasma concentration for blocking bone marrow components is probably between 50 and 100 nM. (Huizing et al., 1993).

When compared to CREMOPHOR-solubilized paclitaxel, nab-paclitaxel achieves a higher serum concentration of the medication, but its higher clearance causes a similar level of drug exposure (Gardner et al., 2008). The most common way to administer nab-paclitaxel is intravenously over 30 minutes at 260 mg/m2 once every three weeks, however other dosage schedules are being considered. Nab-paclitaxel shouldn't be administered to patients with an absolute neutrophil count below 1500 cells/mm3, just like with the other taxanes. The pharmacokinetics of docetaxel are comparable to those of paclitaxel, with an elimination half-life of around 12 hours. CYP3A4 and CYP3A5-mediated hydroxylation, which results in inactive metabolites, is the main mechanism of clearance (Clarke and Rivory, 1999). Contrary to paclitaxel, docetaxel's pharmacokinetics are linear at dosages of 115 mg/m2. There have been recommendations for dose reductions in individuals with abnormal serum bilirubin and impaired hepatic function. In presence of hepatic metastases >2 cm; and 50–75% doses of taxanes are advised. Drug clearance and toxicity are considerably altered by substances that either promote CYP2C8 or CYP3A4 or inhibit these cytochromes, such as antifungal imidazoles or phenytoin.

Cyclosporine A and a variety of other medicines used experimentally as P-glycoprotein inhibitors significantly slow down the clearance of paclitaxel. This inhibition could result from effects on biliary excretion of the parent drug or its metabolites, a block in CYP-mediated metabolism, or both. The bone marrow is where paclitaxel's principal toxic effects are felt. Neutropenia often develops 8 to 11 days following a dosage and quickly resolves by days 15 to 21. Peripheral neuropathy becomes dosage limiting when used with filgrastim [granulocyte-colony stimulating factor (G-CSF)] at doses up to 250 mg/m2 over 24 hours. After taking paclitaxel, myalgias commonly last for several days in many individuals. A stocking-glove sensory neuropathy can be incapacitating in high-dose regimes or with prolonged use, especially in individuals with pre-existing diabetic neuropathy or those receiving concurrent cisplatin therapy. Infusions lasting 72 or 96 hours as well as the weekly schedule are characterised by mucositis.

Patients receiving short-term (1-6 hours) infusions of paclitaxel experienced hypersensitivity reactions, however as previously mentioned, these events were largely

prevented by pretreatment with dexamethasone, diphenhydramine, and histamine H2-receptor antagonists. 96-hour infusions do not require premedication. During 3- or 24-hour infusions, many individuals develop asymptomatic bradycardia, and on rare occasions, silent ventricular tachycardia episodes also happen and spontaneously end. When compared to paclitaxel given by CREMOPHOR, nab-paclitaxel induces more peripheral neuropathy but less frequently hypersensitivity responses.

Compared to paclitaxel, docetaxel causes more neutropenia, but less peripheral neuropathy, asthenia, and frequent hypersensitivity. With repeated rounds of docetaxel therapy, fluid retention becomes progressively worse and can result in pulmonary edema, pleural and peritoneal effusion, and peripheral edema. Fluid retention is significantly reduced by 8 mg/day of oral dexamethasone administered starting a day before the medication infusion and continuing for three days. Rarely, docetaxel may cause a progressive interstitial pneumonitis, and if the medication is not stopped, respiratory failure may result (Read et al., 2002). Cisplatin and carboplatin are inorganic metal complexes that bind to DNA by creating intra- and interstrand cross-links, activate the DNA damage response (DDR), and impede DNA synthesis (Frezza et al., 2010).

Docetaxel, which is delivered in polysorbate 80 and is somewhat more soluble than paclitaxel, precipitates lower frequency of hypersensitivity reactions when dissolved in CREMOPHOR than paclitaxel. To avoid increasing fluid retention and lessen the severity of hypersensitivity events, dexamethasone pretreatment for three days beginning the day before medication is necessary.

There have been reported drug interactions; paclitaxel's clearance is decreased and its toxicity is increased when cisplatin is given before it (Donehower, 1995). While docetaxel appears to have little impact on the pharmacokinetics of anthracyclines, paclitaxel reduces doxorubicin clearance and increases cardiotoxicity. The mdr-1 gene and its product, P-glycoprotein, are expressed more frequently in some lines of cultivated tumor cells, which are resistant to taxanes. Other resistant cells may have increase in aurora kinase, tubulin mutations, and these latter cells may exhibit enhanced sensitivity to vinca alkaloids (Cabral, 1983).

It is unknown what causes clinical medication resistance. Apoptosis is the process by which cells die, however the presence of an intact p53 gene product is not necessary for

paclitaxel to be effective against experimental cancers. According to preclinical research, nab-paclitaxel had a greater antitumor impact in breast cancer patients and a higher intratumoral drug concentration than paclitaxel administered by cremophor. The exact cause is unknown, but it may have to do with the drug's ability to remain in the nanoparticle micellar system or with the tumor cells' increased expression of SPARC (also known as osteonectin, a matricellular linkage protein expressed in pro-fibrotic states and associated with a variety of pathologies (Kos and Wilding, 2010; Chlenski and Cohn, 2010), which would increase drug uptake.

2.6. Pharmacodynamics on SAC

The spindle microtubules are the primary targets for paclitaxel, and so, key SAC proteins such as MAD2 and BUB1 have been important predictors for paclitaxel response (Hao et al., 2010; Fu et al.,2007; Fang et al., 2006). The activation of checkpoint of the mitotic spindle assembly induces paclitaxel-initiated apoptosis (Zhou et al., 2010). In addition, MAD2 mediated spindle checkpoint activation is shown to induce mitotic arrest in response to DNA damage, indicating overlapping roles of the SAC and DDR (Lawrence et al., 2015) and suggests a novel role of MAD2 in cellular sensitivity to platinum drugs. However, the association between SAC components and platinum/taxane has not been fully understood.

2.7. Spindle assembly checkpoint

In eukaryotes, cell division is a process that transmits accurate genetic material in daughter cells. Genome replication and segregation are two separate events of S and M phase (DNA synthesis and mitosis) in the architecture of cell cycle. During human cell division, the newly synthesized chromatids in S phase and original chromatid are attached as sister chromatids by cohesin, a protein ring structure encircled around the chromatids. The chromosomes align on the microtubule spindle apparatus at the centre of the dividing cell throughout early mitosis, early G2 and the remainder of S phase, during all of which are chromosomes are connected by cohesin. To ensure accurate chromosome segregation the sister chromatids must be aligned at the metaphase plate, captured by microtubules from the opposite poles. Unattached or improperly attached chromatids may lead to unequal chromosomes, deviating the normal karyotype in the daughter cells. The challenge is overcome by a quality control mechanism called the

spindle assembly checkpoint or the mitotic checkpoint or the M-phase checkpoint. The SAC detects the tension of kinetochore-microtubule attachments rather than the spindle assembly itself. Only when all kinetochores are stably linked to microtubules does the SAC become satisfied; at that point, anaphase inhibition is weakened and the cell cycle can proceed.

The SAC is a complex signaling cascade essential for human cell survival (Kops et al., 2004; Michel et al., 2004). This pathway inspects microtubule attachment or tension of each kinetochore on the mitotic spindle and prevents anaphase onset in the presence of unattached or incorrectly attached kinetochores (Musacchio 2011, 2015). Thus, it ensures proper transmission of genetic materials into the daughter cells and preserves the genetic stability which is essential for cellular fitness (Santaguida and Amon, 2015; Gordon et al., 2012.)

2.8. Chromosome alignment and biorientation

Kinetochore and microtubule interactions are frequently created and demolished until all the chromosomes are bioriented and attachments are stabilized. Aurora B is a serine/threonine protein kinase that orchestrates the correction of improper kinetochore-microtubule attachments by setting off changes in the microtubule dynamics and weakening the affinity of kinetochore for microtubules (Carmena et al., 2012). Aurora B is a subunit of a targeting and activating complex named the chromosome passenger complex (CPC). During mitosis the region between kinetochores have great concentrations of CPC from where Aurora B can phosphorylate kinetochore substrates like the centromeric protein A (CENP-A) in the inner kinetochore and the KMN network at the outer kinetochore (Carmena et al., 2012, van der Horst, A, et al., 2014). Phosphorylation by Aurora B is linked to the state of kinetochore-microtubule attachment and declines after bi-orientation is achieved (Liu et al.2009, Welburn et al., 2010, Tanaka et al., 2010, DeLuca et a., 2011, Emanuale et al., 2008). The decline in the Aurora B dependent phosphorylation results from the inability of Aurora B to reach its substrates rather than a decline in its catalytic activity. But this hypothesis awaits proper validation or rebuttal (Lampson, 2011, Santaguida, 2009, Maresca 2009). Thus, Aurora B plays a complementary role to the SAC by promoting chromosome bi-orientation.

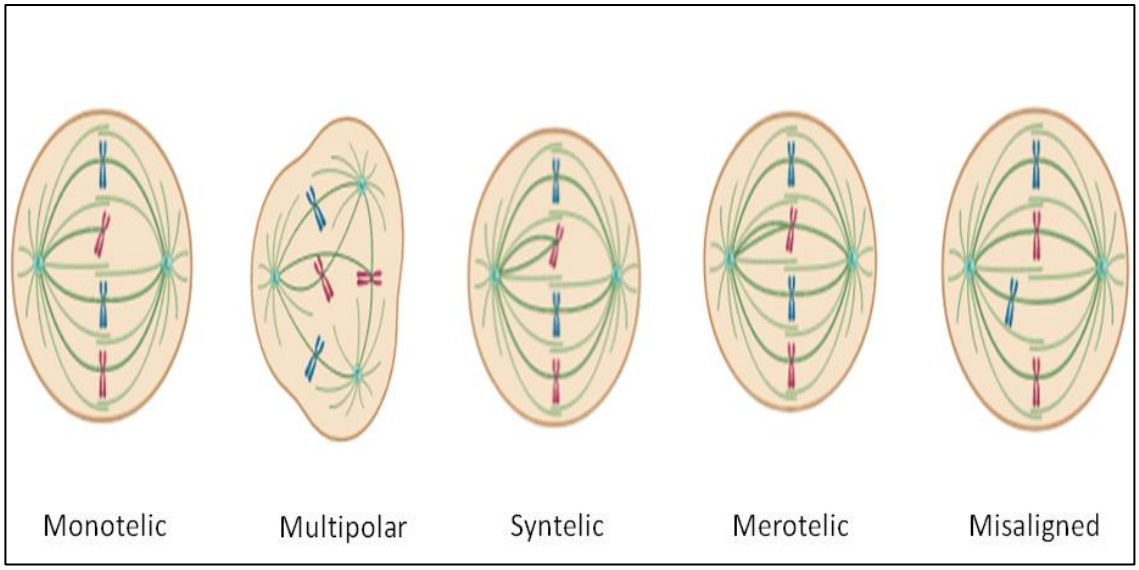


Figure 1.1: Different states of chromatid-kinetochore attachments that activate the spindle assembly checkpoint (Sarkar et al., 2021)

2.9. The mitotic checkpoint signaling cascade

The APC/C (anaphase promoting complex also known as cyclosome), an E3 ubiquitin ligase and its complex with its co-activator cell division control protein 20 (CDC20) activates anaphase onset and controls mitotic exit. The APC/C-CDC20 targets the two key proteins, Securin and Cyclin B for proteasome-dependent degradation (Oliviera, 2010). Prior anaphase the sister chromatids are connected by a proteinaceous bridge which is a multi-protein complex called cohesion. Securin is an inhibitor of Separase which is responsible for proteolysis of cohesin subunit SecI. Securin is ubiquitinated and destroyed by APC/C which leads to Separase activity, cleavage of Sec1, cohesin loss of chromosome and anaphase onset respectively (Nasmyth, 2002). APC/C degrades Cyclin B because timely entry and exit of from mitosis is regulated by CycB-Cdk1 activity which promotes a burst of protein phosphorylation (He´ garat N et al., 2016).

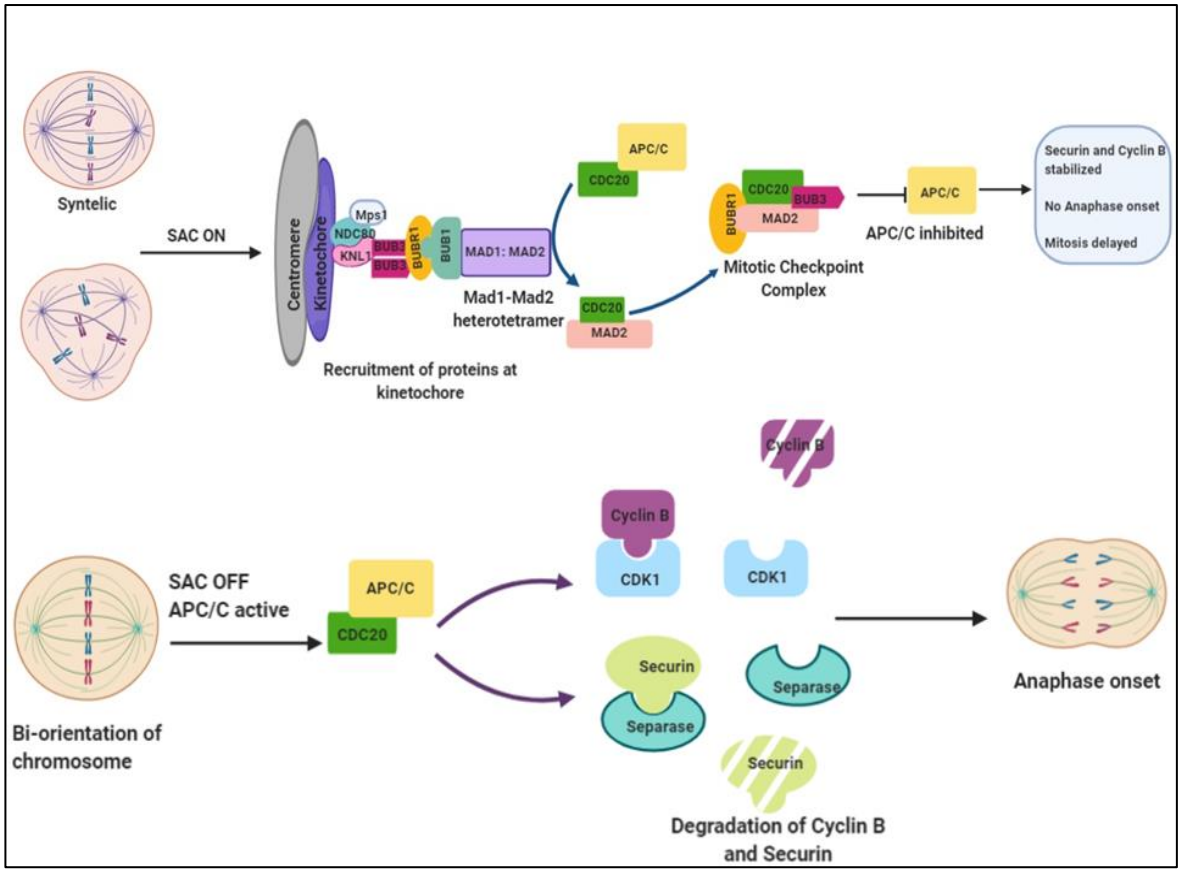


Figure 1.2: Schematic diagram of the on and off states of spindle assembly checkpoint (Sarkar et al., 2021)

The signal generators of the mitotic checkpoint are unattached or inappropriately attached (syntelic, merotelic, monotelic) kinetochores which trigger the recruitment of SAC components and release a diffusible inhibitor of APC-CDC20 complex, thereby preventing the progression to anaphase (Musacchio., 2007). Reider and colleagues showed in their classic experiments that in one cell type, a single unattached kinetochore can delay the mitotic progression for at least 3 hours (Reider et al., 1994). Kinetochore serves as a hub for hierarchical recruitment of SAC proteins (London and Biggins., 2014). In early mitosis, Mps1 is accumulated in the kinetochore and subsequently activated by autophosphorylation (Kang et al., 2007, Mattison et al., 2007). MPS1 associates with the NDC80-C subcomplex of the KMN network (Kemmler et al, 2009, Nijenhuis et al, 2013) followed by activity of Aurora B kinase. Aurora B is a serine/threonine protein kinase that orchestrates the correction of improper kinetochore-microtubule attachments by setting off changes in the microtubule dynamics and weakening the affinity of kinetochore for microtubules (Carmena et al., 2012). The

cascade then hierarchically recruits BUB1-BUB3 complex, followed by BUBR1-BUB3 complex. The KNL1 is a component of the KMN network also known as CASC5 or Blinkin, has an array of large repeat motifs called the MELT motifs. These MELT motifs are phosphorylated by MPS1 and form phospho-docking sites for BUB3. BUB3 carries either BUB1 or BUBR1 with it and bridges Knl1 to the BUB paralogs. This is the recruitment pathway for BUB1 to kinetochore but BubR1 recruitment mechanism is more complex. BUBR1 requires a correct localization of BUB1 in order to be recruited at the kinetochore but not vice versa (London et al., 2012; Shepperd et al., 2012; Yamagishi et al, 2012). Finally, the recruitment of heterotetramer of MAD1-MAD2 takes place. Two distinct conformations are adopted by MAD2; the unbound/ open conformation (O-MAD2) and upon binding with MAD1 or CDC20, the bound/ closed conformation(C-MAD2). Upon mitotic entry, the MAD1-C-MAD2 is localized at the kinetochore. Further the O-MAD2 in the cytosol is then recruited to the kinetochore-bound MAD1-C-MAD2 (Luo et al., 2008). This O-MAD2 bound with MAD1-C-MAD2 then binds with the CDC20. The conformational activation of MAD2 from free 'open' state (O-MAD2) to the CDC20 bound closed form (C-MAD2) is the key step in the formation of the diffusible inhibitor of APC/C, the Mitotic checkpoint complex (MCC) (Skinner et al., 2008). Finally, the MCC is composed of CDC20 in complex with the BUB1-related protein (BUBR1), BUB3 and MAD2. This complex inhibits the APC/C-CDC20 activity and triggers the "wait anaphase" signal until proper chromosome attachment and alignment are achieved.

2.10. Mutations in SAC genes

The mitotic checkpoint signaling is not an all-or-none response because the strength of the checkpoint response varies with the number of unattached kinetochores (Collin et al., 2013). Cahill et al., (1998) first reported that inactivating BUB1 mutations generated a weakened checkpoint response and caused chromosomal instability CIN in a subset of colon cancer cell lines. Lee et al., (1999) reported about acquired mutations in BUB1 and p53 genes and loss of spindle assembly checkpoint in tumors with BRCA2 deficiency from animals. They concluded that these inactivating mutations of the checkpoint genes cooperate with BRCA2 deficiency, thus contributing to tumorigenesis in inherited breast cancer. The somatic missense mutation in the mitotic checkpoint gene hBUB1, a polymorphism in codon 93 of exon 4, a substitution of guanine-to-thymine, was first

reported in a lung cancer cell line and a primary lung tumor (Gemma et al., 2000). On the other hand, Hernando et al., 2001, screened for various potential mutations in hsMAD2, hBUB1 and hBUB3 genes in bladder cancer, soft tissue sarcomas and hepatocellular cancer tissues as well as cell lines. They found some rare point mutations in the respective diseases which may not be responsible for cancer development. These findings were similar to the other study that showed mutational inactivation of hsMAD2 was less frequent in sporadic digestive tract cancers (Imai et al., 1999). Tsukasaki 2001, examined a total of 133 fresh cancer cells (hematopoietic, prostate, breast and glioblastoma) and 44 cell lines (hematopoietic, prostate, osteosarcoma, breast, glioblastoma and lung) for alterations in MAD1L1 and found eight heterozygous mutations with high mutation frequency in prostate cancer. They placed a truncated MAD1L1 in three different cell lines and observed less inhibitory effect of cell proliferation when compared to the wild type. Thus, they concluded that MAD1L1 has a potentially pathogenic role in carcinogenesis. Only one clinical study observed hBUB1 missense mutation (Ala130Ser) was associated with lymph node metastasis in colon cancer and thus, it is an indicator of disease progression (Shichiri 2002). Guo et al., (2010) reported missense variations of MAD1L1 and MAD2L1 confers susceptibility to lung cancer and weakens the SAC function. Zhong et al., (2015) identified two genetic variations, MAD1L1 Arg558His and MAD2L1 Leu84Met, to be associated with increased risk of colorectal carcinoma in Chinese population. In our departmental clinical study, we have found prevalence of MAD1 and MAD2 mutations in Indian ovarian cancer patients which may carry the risk of developing the ovarian carcinoma (Sarkar. et al., unpublished observations).

2.11. Altered protein expressions are common in cancer cells

Apart from mutational defects, the checkpoint-induced aneuploidy is often associated with deregulation of mRNA and changes in the protein levels of the primary SAC components. Dai et al., (2004) reported that RNA interference mediated downregulation of BubR1, significantly reduced the levels of securin. They also observed rapid development of lung and intestinal adenocarcinomas in BubR1 (+/-) haploinsuffient mice as compared to their wild type littermates, when challenged with carcinogens. Significant increase in number of aneuploid fibroblasts was observed in mice with reduced levels of MAD2 and BUB1B. Tumor incidence increased up to 6% in mice with

severely reduced BUBR1 and mice heterozygous for functional BUBR1 had a tendency of developing colon and lung carcinomas after treatment with AOM and DMBA respectively. 28% of mice developed papillary lung adenocarcinomas (Dai et al., 2004; Michel et al., 2001). Similarly, overexpression of CDC20 was observed in oral cancer cell lines and primary tumors that were associated with premature anaphase onset and chromosomal abnormalities in oral squamous cell carcinomas. (Mondal et al.,2007). The cytokinesis and mitotic exit are the final stages of mitosis. Cytokinesis is inhibited when MAD2 is overexpressed which stabilizes securin and cyclin B. Hence, tumors with overexpressed MAD2 contribute to cytokinesis failure leading to both aneuploid and tetraploid cells (Hernando et al., 2004; Sotillo et al., 2007). High CDC20 expression was correlated with high tumor grade in ovarian cancer (Gayyed et al., 2016). The pro-tumorigenic effect can be observed here as overexpression of this SAC component deregulates the timing of APC/C and induces aneuploidy. In addition, MAD2 overexpression specifically can increase the susceptibility to hepatoma, hepatocellular carcinoma, lung adenoma, fibosarcoma and lymphoma (Sotillo et al., 2010). MAD2 is overexpressed in several other tumors of different origins like mucinous ovarian, osteosarcoma, endometrial, gastric, soft tissue sarcoma, nasopharyngeal, testicular germ cell, breast, hepatocellular, prostate and lung that are highly proliferative and its expression level typically correlates with ki67 labelling indices in patients (Murray et al., 2012; McGrogan et al., 2014; Bargiela-Iparraguirre et al 2016). Contradictorily, Wang et al., (2019) found that MAD2 was downregulated in cervical cancer and HSIL followed by LSIL and chronic cervicitis. Recently, Huang et al., (2020) reported that TTK or MPS1 upregulation in gastric cancer cells were essential for malignant cell survival and proliferation. It was also reported that TTK regulates the apoptosis and proliferation of tumor cells through Akt-mTOR pathway.

2.12. Spindle Assembly Checkpoint and Ovarian Carcinoma

Increased MAD2 and BubR1 expression in advanced stage ovarian tumors are correlated with increased cellular proliferation. Reduced nuclear intensity of MAD2 identified patients with poorer time to recurrence irrespective of their tumor histologic subtype or treatment received (McGrogan et al., 2014). Santibanez et al., 2013 showed that a MAD1 1673 G→A polymorphism identifies worse response to chemotherapeutic agents. This polymorphism also alters the dynamics of microtubules and affects SAC functionality in

ovarian cancer patients (Santibanez et al., 2013). The miR-493-3p confers resistance to microtubule drugs in cancer cells and high level of this miR-493-3p is associated with reduced survival of ovarian and breast cancer patients undergoing Paclitaxel therapy with aggressive tumors. When overexpressed in cancer cells, this microRNA targets the 3'UTR of the MAD2 mRNA, inhibiting the translation of MAD2 into protein and increasing the likelihood of aneuploidy and cellular senescence. Intratumoral profiling of miR-493-3p and MAD2 may be useful for diagnosing taxane treatment effectiveness (Tambe et al., 2016). The pre-mRNA splicing factor 4 kinase (PRP4K), in mitotic cells has been identified as a novel regulator of the spindle assembly checkpoint. It plays a role in the recruitment of Mps1 kinase, MAD2 and MAD1 to the kinetochore and also in chromosome alignment. It has been seen that loss of PRP4K expression leads to failure of SAC induced by nocodazole (Montembault et al., 2007). A positive correlation between PRP4K and Her-2 status has been found in breast and ovarian cancer. Knock down of PRP4K results in reduced sensitivity to taxanes in ovarian cancer cell lines (Corkery et al., 2015). Peluroside, a microtubule-stabilizing agent, induces aneuploidy in ovarian cancer cells (Chan et al., 2016). High CDC20 expression was correlated with high tumor grade in ovarian cancer (Gayyed et al., 2016). Upregulation of Akt2 mediates paclitaxel resistance in A2780 ovarian cancer cells by inhibiting BUB1 expression (Zhou et al., 2010). Aurora Kinase A synergistically enhances cytotoxicity in ovarian clear cell carcinoma cell lines when treated with cisplatin and ENMD-2076. Aurora kinase A can also be a promising biomarker for predicting patient outcomes (Chiba et al., 2017). In another study, Mad 2 was knocked down by MAD2-specific siRNA in Paclitaxel-sensitive A2780 cells and recombinant eukaryotic expression plasmid pEGFP-MAD2 was transfected into paclitaxel-resistant SKOV3 cells. Results of paclitaxel sensitivity assay revealed that Paclitaxel sensitivity reversed in both the cell lines after transfection in terms of cells arrested at G2/M phase and Bcl-2 expression significantly changed. These results suggested that weakened SAC with reduced MAD2 expression was associated with Paclitaxel resistance in ovarian cancer cells with involvement of Bcl-2 in the process (Hao et al., 2010). Furlong et al. examined five ovarian cancer cell lines in vitro and showed that cells with low MAD2 expression were less sensitive to paclitaxel. They also showed that cells transfected with MAD2 siRNA prevented paclitaxel-induced activation of the SAC and apoptosis. Additionally, they stated that MAD2 expression was down-regulated in pre-miR-433 transfected A2780 cells and that miR-433 had a binding domain in the 3'UTR of MAD2. They also concluded that pre-miR-433-

transfected A2780 cells with lower MAD2 protein expression were less susceptible to paclitaxel (Furlong et al., 2012). MAD2 expression is also correlated with risk for recurrence in ovarian serous adenocarcinoma. The expression was significantly lower in recurrent group than in the relapse-free group. The overall survival was also significantly shorter in the low-expression group than the high-expression group (Nakano et al., 2012). Increased expression of the atypical cyclin Cyclin G1 (CCNG1) causes mitotic arrest brought on by paclitaxel, irrespective of p53 integrity or signaling via the SAC component BUBR1. After exposure to paclitaxel, CCNG1 overexpression promotes cell survival; in contrast, cyclin G1 depletion via RNA interference inhibits mitotic slippage and taxane-induced death. CCNG1 amplification is associated with shorter post-surgical survival in ovarian cancer patients who have received adjuvant chemotherapy with taxane and platinum drugs (Russell et al., 2012). Overexpression of dynein light chain km23-1 suppresses ovarian carcinoma growth *in vitro* and *in vivo*. The underlying mechanism appears to involve a BubR1 related mitotic delay at prometaphase/metaphase (Pulipati et al., 2011). A study on 263 ovarian cancer patients (stages I/II) revealed the association of bad prognosis with high Polo-like kinase (PLK) 1 expression. Strong mitotic arrest in ovarian cancer cell lines was induced by triple treatment with paclitaxel, BI6727 and proTAME which targets the microtubules, PLK1 and Anaphase Promoting Complex respectively. In cell lines and primary patient-derived ovarian cancer cells, this triple therapy induced apoptosis. Cyclin B1 is stabilized by BI6727/paclitaxel/proTAME, which also causes mitotic arrest. Mitotic arrest causes mitochondrial apoptosis to begin by deactivating antiapoptotic Bcl-2 family proteins, which is then followed by caspase-dependent effector pathways. The triple therapy has crucial ramifications for the development of paclitaxel-based combinatorial therapy for ovarian cancer since it has prevented endoreduplication and reduced CIN, the two processes involved in drug resistance (Raab et al., 2019).

2.13. Research lacunae and rationale of the study

A survey of literature reveals that, no study has previously reported on the clinical relevance of the major SAC components in advanced ovarian cancer patients receiving first-line chemotherapy. The study thus identifies scopes of improvement in clinical efficacy, safety and QoL in association to SAC status in the primary ovarian cancer pathology. Therefore, the outcome of the study will be helpful in designing safer, effective dose with reduced drug toxicity in ovarian cancer patients. It is anticipated that with the judicious use of genetic evaluation, the best possible mode of management of ovarian cancer may become achievable with reduced severe adverse drug reactions.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Design

This is a non-randomized, prospective study to evaluate the clinical efficacy of platinum-taxane therapy in Indian women with 77 clinically advanced epithelial ovarian cancer. The required number of patients for this study was 76.99 ~77 with power 81%. The core dataset was compiled with information about age at diagnosis, initial symptoms, religion, occupation, marital status, family history or personal history of cancer, menopausal status, pretreatment CA125, history of pregnancy, contraceptive usage/ hormone replacement therapy, tumor histological subtype (serous, endometroid, clear cell, mucinous, other), tumor grades (well, moderate, poorly differentiated and undifferentiated), any other co-morbidity, post chemo clinical response, toxicity assessment and quality of life (QoL) during the first line treatment. Blood biomarker CA125 was monitored at baseline and after every 3 cycle to evaluate therapy response. USG and CECT (whole abdomen and thorax) data was analyzed at baseline, after 3rd or 6th cycles. Non-responding patients had a change in chemotherapy with alternative regimen after 2/3 cycles of Paclitaxel and Carboplatin, or their chemotherapy was stopped while Responders continued till the 6thcycle **(Figure 3.1)**. There was no blinding or randomization involved in the study design.

3.2. Ethics and Informed consent: The Chittaranjan National Cancer Institute's Institutional Ethical Committee approved the study and it was carried out in compliance with the Declaration of Helsinki [A-4.311/VN/27/06/2018-10]. Ovarian carcinoma patients were recruited in the study only after obtaining written Informed Consents at the study site, Dept. of Gynecologic Oncology, Chittaranjan National Cancer Institute as per pre-defined eligibility.

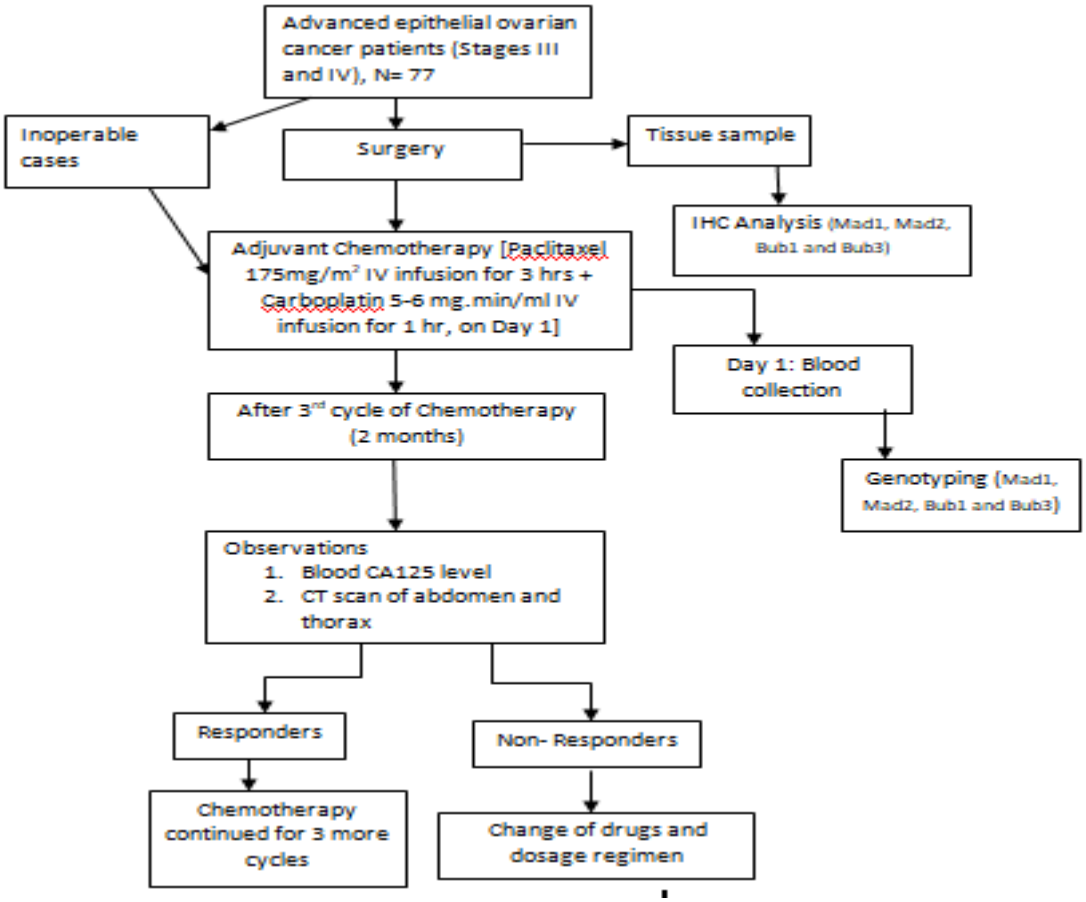


Figure 3.1. Experimental Design

3.3. Patient eligibility

3.3.1. Inclusion criteria

- Patients of age ≥ 20 years newly diagnosed with advanced epithelial ovarian cancer (EOC); FIGO stages IIIA/B/C and IVA/B; yet to receive chemotherapy.
- The histological characters of the primary tumor should match with advanced epithelial ovarian carcinoma.
- Patients with co-existing fallopian tube cancer *in-situ*, will be included as long as the primary origin is ovarian.
- A patient has to have optimum platelet count (≥ 100,000/ μL), blood coagulation parameters, bone marrow function, hepatic condition (CTCAE Grade 1), (SGOT and alkaline phosphatase ≤ 2.5 x ULN), and neurologic function.
- Estimated life expectancy of patients must be a minimum of 12 months.

- Patient or a concerned guardian must sign a written informed consent form and authorization permitting release of personal health information.
- Patients may receive chemotherapy with platinum compounds and taxanes as first-line treatment after surgery.

3.3.2. Exclusion criteria

- Patients <= 20 years.
- Patients who are previously diagnosed and had undergone complete oophorectomy.
- Pregnant/nursing women.
- Women with recurrent ovarian cancer, or other specific, invasive cancer.
- Patients with active infection (bacterial or viral, need parenteral antibiotic treatment), uncontrolled diabetes, hepatitis, serious bleeding & wound-healing disorder, coagulopathy, clinically significant proteinuria and/ or bone fracture.
- Patients with history of or having clinically significant cardiovascular complications which include: myocardial infarction/unstable angina, cardiac arrhythmia and/or uncontrolled hypertension.
- Patients with clinically significant uncontrolled autoimmune disorder.

3.4. Treatment regimen

Patients were recruited from the Out Patient Department and underwent surgery at Gynecological Oncology; received their respective chemotherapy at Dept of Medical Oncology, CNCI. In this study, patients with primary cases of advanced EOC received the classical platinum-taxane chemotherapy. The patients underwent either primary debulking followed by adjuvant chemotherapy (ACT) or underwent neo-adjuvant chemotherapy (NACT) (3 cycles) and interval debulking surgery followed by another 3 cycles of chemotherapy. The surgical treatment included bilateral salpingo-oophorectomy (BSO), omentectomy, total abdominal hysterectomy (TAH), and bilateral pelvic and para-aortic lymphadenectomy with cytoreduction. The chemotherapy treatment plan included intravenous doses of paclitaxel at 175 mg/m2 on Day 1 (3 hours)

and carboplatin at 5 to 6 mg/mL on Day 2 (over an hour). The chemotherapy cycle is repeated every 3 weeks up to a total of 6 cycles (Armstrong et al., 2021; ESGO 2017).

3.5. Clinical Response Evaluation

Tumor burden were assessed by ultrasonographic, radiographic images (CT scan abdomen and thorax) and CA-125 blood biomarker (GCIG) measured at baseline, after 3rd and 6th cycles. Clinical response was evaluated according to GCIG criteria (Rustin 2003; Rustin et al, 2004) for evaluation by CA125 levels and RECIST guideline version 1.1 (Schwartz et al., 2016) was used for the patients receiving neoadjuvant chemotherapy (inoperable tumors). Platinum sensitive patients were considered as Responders (Rs) and platinum resistant patients were categorized as partial responders (PRs). Non-Responders (NRs) included patients with stable disease, progressive disease (platinum-refractory), palliative care patients and patients not evaluable. Survival analysis was done till 24 months since start of the treatment.

3.6. Toxicity Assessment and Quality of Life

Drug related toxicities were recorded for the patients receiving six cycles of adjuvant and neoadjuvant chemotherapy. Blood biochemistry and hematologic parameters were measured before every cycle of chemotherapy and patients also reported their most bothering side effects through questionnaire. Maximum grade of toxicities was noted as per National Cancer Institute (NCI) common toxicity criteria (CTCAE v4) (Dueck et al., 2015). Commonly observed toxicities included anemia, leucopenia, thrombocytopenia, granulocytopenia, nausea, vomiting, anxiety/depression/neurologic conditions, neuropathy, weight loss/gain, diarrhoea, constipation, indigestion/bloating/hyperacidity, abdominal pain, renal toxicity, and mucositis.

Quality of life was assessed using Fact- O questionnaire (FACT-O: For patients with ovarian cancer) combining FACIT-Sp-12 for spiritual well-being. The questionnaire set had a total of 51 questions different segments that included physical, social, emotional, functional, and spiritual factors and a special array of questions as “Additional concerns” designed for specific concerns/symptoms of ovarian cancer patients. QoL was measured at baseline, 2, 4 and 6 months of treatment.

3.7. Pain intensity measurement

Numerical Rating Scale (0-10) was used for spontaneous movement and resting-associated pain measurement. Patients reported pain score 0, as no pain; and pain score 10, as worst pain imaginable. Similarly, sleep-associated pain (Margaret et al., 2008) and neuropathic pain scores (NPSI scale) were measured. NPSI scale included pain parameters like burning, squeezing, pressure, electric shock, stabbing, light touching, cold sensation, pins and needles, and tingling (Bouhassira et al., 2004). The analysis was done at baseline, 2, 4 and 6 months (Saxena et al., 2016).

3.8. Collection of Blood and Tissue samples for molecular analysis

5mg to 1g of solid tumor tissue was collected in 1X PBS (phosphate buffered saline) and 10% NBF (neutral buffered formalin) after primary or interval debulking surgery at Dept. of Pathology, Hospital, CNCI. Peripheral venous blood samples (5mL) were collected in EDTA coated vials. To avoid unnecessary discomfort, the blood collections were performed either during pre-op preparation of the patient or during routine biochemical tests. Sample collection was supervised by trained medical practitioners.

3.9. Histology

Ovarian carcinoma tissues will be fixed in 10% NBF and then was dehydrated in graded alcohol, acetone and xylene followed by paraffin embedding. After the paraffin blocks are prepared 5 µm thin sections were cut in the microtome and pasted on poly-L-Lysine coated slides. For histopathological analysis the slides were deparaffinized, rehydrated and then stained with hematoxylin (5mins) and Eosin (2mins). The color is developed under running tap water. The slides were then cleared with alcohol and xylene and mounted with DPX. (Fischer et al., 2008).

3.10. Immunohistochemistry

Immunohistochemical staining for MAD1, MAD2, BUB1 and BUB3 protein expression was performed with commercially available IHC Select- HRP/DAB Kit Millipore protocol. Deparaffinisation was done by 4 Xylene changes, graded alcohol (100% to 30%) and then distilled water. Then the slides were heated in 0.01M citrate buffer, pH 6.0 in microwave oven for 10 minutes to retrieve antigen, followed by endogenous peroxidase blocking with 3% hydrogen peroxidase in water for 10 minutes. Non- specific binding was inhibited by applying blocking reagent provided in the kit for 5 mins and

slides were not completely washed down. Monoclonal antibodies against MAD1(1:50), MAD2(1:50), BUB1(1:50) and BUB3 (1:100) was applied and kept at 4ºC overnight in a humid chamber. The next step was to apply secondary antibody followed by Streptavidin HRP (Horseradish Peroxidase) sequentially and incubation for 10 minutes in each step. Then the chromogen DAB (3,3'- Diaminobenzidine) was freshly prepared in a solution for application to the sections (10 mins in dark) and then counterstained with Meyer's haematoxylin for 1 min. The slides were then dehydrated with graded alcohol and xylene and mounted with DPX (McGrogan et al., 2014; Stahl et al., 2017).

The presence of nuclear or cytoplasmic immunolocalization for each antibody, *i.e.*, for MAD1, MAD2, BUB1 and BUB3 was scored as: 3+, strong. 2+, moderate; 1+, weak; The percentage of tumor cells showing intensity score greater than 2+, moderate were then estimated in 10 vision fields at × 40 magnifications **(**Park et al., 2013).

3.11. DNA quantization

DNA from whole blood was isolated by the Phenol-Chloroform method (Green and Sambroo*k*, 2012), dissolved in 1X TE buffer and stored at -20ºC. The DNA will be then analyzed by gel electrophoresis on 1% agarose prepared in 1X TAE buffer and stained with EtBr (10mg/ml) with final concentration of 0.5µg/ml. 1µL Lambda- Hind III was used as the DNA Ladder. DNA concentration was noted from the gel picture.

3.12. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP)

The PCR principle of exponential amplification of gene region of interest was used to amplify genetic polymorphisms of MAD1L1 (rs1801368, rs121908981); MAD2L1 (rs1972014; rs1546120; rs3752830); BUB1 (rs121909055); BUB1b (rs28989181; rs28989186); BUB3 (rs11248416; rs11248419; rs6599657) (Akhoundi et al., 2016; Wang et al., 2014; Santibanez et al., 2013). The amplified products were run on a 2% agarose gel with 100bp DNA marker. For the PCR amplification primers were designed for each gene with the bioinformatics tool BLAST and Primer 3 (**Table 3.1**). The standard thermocyclic conditions followed for each SNP is as follows:

1. 94ºC 7min (Initial Denaturation)
2. 94ºC 30sec (Denaturation)

3. **XºC 45sec (Annealing, variable for all the SNP)**
4. 72ºC 30sec (Extension)
5. Repeat step 2 for 34 cycles
6. 72ºC 7min (Final extension)
7. Hold at 4 ºC

The Master Mix preparation for 25µL reaction will be

10X Buffer	2.5 µL
10mM dNTPs	1 µL
Forward Primer	0.25 µL
Reverse Primer	0.25 µL
Standard Taq DNA polymerase	0.25 µL
DNA Template	Variable (50-100ng)
Nuclease Free water	Volume make up till 25 µL

The amplification products were analyzed for genomic alterations (mutations, deletions, translocations) by RFLP using specific restriction enzymes that were selected and custom digested from the tool NEBcutter. The digested products were run on 10-12% Native PAGE (Polyacrylamide Gel Electrophoresis) with 50bp DNA marker for further analysis.

Table 3.1: Genes, designed primers, annealing temperatures and product sizes

Sl No.	Genes	Primer sequence	Annealing Temp/Time for PCR	PCR Product size
1.	MAD1L1 (rs1801368)	Forward primer: GCAGGGTGACTATGACCAGA Reverse primer: AACCCTCTGGGGATGACAGG	56ºC /45 sec	327 bp
2.	MAD1L1 (rs121908981)	Forward Primer: CTCACCGGCTACCAGATCG Reverse Primer: AGGGCTACGGTCGGATCTC	57 ºC /45sec	169bp
3.	MAD2L1 (rs1972014)	Forward primer: CATCTCCAGTCCACTTTCCG Reverse primer: AGGAGCCAGACCATGCAAAG	53ºC /45sec	157 bp

Sl No.	Genes	Primer sequence	Annealing Temp/Time for PCR	PCR Product size
4. .	MAD2L1 (rs1546120)	Forward primer: ACTAAACCGTCCAGCCAGAA Reverse primer: ACGGAAAGTGGACTGGAGAT	54 ºC /45sec	237bp
5.	MAD2L1 (rs3752830)	Forward primer: TCCTCAGTCACCTATGGAAAAG Reverse primer: GTGTCTTAGGAAGTAGATGGCA	53.5ºC /45sec	274bp
6.	BUB1 (rs121909055)	Forward Primer: GTTTCAGGCTCCTACACTTCCT Reverse Primer: ACAAGCTAATCAAGGGCAGGG	55 ºC /45sec	198bp
7.	BUB1B (rs28989181)	Forward Primer: TGTTTCAACCTGCCAGCCATA Reverse Primer: GCACAAATCTCTCTACTTCAGGA	56 ºC /45sec	271bp
8.	BUB1B (rs28989186)	Forward Primer: CAGGGGCGTTTATGCAATGAG Reverse Primer: GCCAATCCACCAGAAAGCACT	55 ºC /45sec	295bp
9.	BUB3 (rs11248416)	Forward Primer: CTGGCCAGCGTTTCATTAGG Reverse Primer: TTGGGCTCCAGTCCAATCTC	56 ºC /45sec	312bp
10.	BUB3 (rs11248419)	Forward Primer: GGCTTGTGTAAGGCAAAACTCG Reverse Primer: CATCAACACGGGGATGCACA	57 ºC /45sec	322bp
11.	BUB3 (rs6599657)	Forward Primer: ACCTGCAAAGGCCAGTACCT Reverse Primer: GCACAAAGACTTTCAGGGACA	56 ºC /45sec	555bp

Protocol for Restriction digestion:

PCR product 10µL

Buffer 1.5µL

Restriction Enzyme 0.4µL

Distilled water 0.1 µL

Table 3.2: Genes, restriction enzymes and expected patterns after digestion

Sl No.	Genes	Restriction enzyme to be used	PCR Product size	Expected patterns to be observed after enzyme digestion
1.	MAD1L1 (rs1801368)	BstUI	327 bp	GG- 327bp; AG- 327+ 142+81+50+42+12 or GC- 327+142+123+50+12; CC or AA- 142+81+50+42+12 or 142+123+50+12
2.	MAD1L1 (rs121908981)	AciI	169bp	CC-169; GC- 169+128+41; GG- 128+41
3.	MAD2L1 (rs1972014)	AluI	157 bp	GG- 157 bp; AG- 157+129+28 bp; AA 129+28 bp
4.	MAD2L1 (rs1546120)	Hind III	237bp	No allele specific enzyme found for the SNP.
5.	MAD2L1 (rs3752830)	BstIMutI	274bp	AA or TT- 274, AG-274+117+157; GG- 157+117
6.	BUB1 (rs121909055)	BsrD1	198bp	AA or TT- 198; GA or GC- 198+142+56; GG- 142+56
7.	BUB1B (rs28989181)	HpyAV	271bp	CT or AT -271+ 221+50; CC or TT- 221+50
8.	BUB1B (rs28989186)	BslI	295bp	AC or AG- 295+ 197+98; TT- 197+98
9.	BUB3 (rs11248416)	ApoI	312bp	TT- 312; CT or GT- 300+262+50; CC or GG- 262+38
10.	BUB3 (rs11248419)	HpAII	322bp	AA or TT- 322; AG- 322+278+44; GG- 278+44
11.	BUB3 (rs6599657)	Nla III	555bp	AA or CC- 555; AG or CG- 555+485+70; GG or TT- 485+70

3.13. RNA quantization and qRT-PCR

TRIzol reagent (ThermoFischer Scientific, India) was used for total RNA extraction from tumor tissues according to the manufacturer's instructions and RNA concentrations were measured using nano-spectrophotometer (Nabi Genetix, United States). Complementary DNA (cDNA) was synthesized by cDNA synthesis Kit (ABM) according to the manufacturer's instructions. SYBR Green PCR kit (ABM/Taurus India) was used to run qRT-PCR to detect the mRNA expression of MAD2, BUB1, CDC20 along with miR-143, miR-495, miR-125b and miR-659. The primers used are described in **Table 3.3**. GAPDH were used as control. Ct-values and $2^{-\Delta\Delta Ct}$ method were used for the qRT-PCR analysis. The qPCR reaction program was: 95°C 10 mins; followed by 95°C for 15s; 60°C for 60s; 72° C, 1min; 40 cycles.

Table 3.3: Primers used for amplification of mRNA and miRs

Target	Primers	Sequence
MAD1 mRNA	Forward	CTCCTGACATTTGTCCCGCT
	Reverse	CTTCATCCTGCCCGGTCTTT
MAD2 mRNA	Forward	AGATGACAGTGCACCCAGAG
	Reverse	AACTGTGGTCCCGACTCTTC
BUB1 mRNA	Forward	GACACCCCGGAAAATGTCCT
	Reverse	TCACCAAGAGGGTCATTGCC
BUB3 mRNA	Forward	ACTGTGCCAATTCCATCGGT
	Reverse	GGATGATTAGGTGGACTTGGGT
CDC20 mRNA	Forward	GTAGCTCTGGCCTTCTTCCTG
	Reverse	CTTTTGCGTGCCTTCCACC
hsa-miR-125b	Forward	TTTCCTAGTCCCTGAGACCC
	Reverse	TAGGTCCCAAGAGCCTGACTT
hsa-miR-143	Forward	TGGGAGTCTGAGATGAAGCAC
	Reverse	CACTTACCACTTCCAGGCTGA
hsa-miR-495	Forward	ACCTGAAAAGAAGTTGCCCAT
	Reverse	TACCGAAAAAGAAGTGCACCA
hsa-miR-659	Forward	CATGAGGACATTGTTGGGGAC
	Reverse	GCTTTACCGACCCTCGATTTG
GAPDH	Forward	GACAGTCAGCCGCATCTTCT
	Reverse	GCGCCCAATACGACCAAATC

3.14. Statistical analysis

Descriptive statistics were used to analyze the frequencies of patient demographics, clinical characteristics, response outcomes, and toxicities. Two-way repeated measure analysis of variance (ANOVA) was used for tests of within subject effect for pain evaluation, QoL domains vs groups (Rs, PRs and NRs/ adjuvant and neoadjuvant chemotherapy). Greenhouse-Geisser and Wilk's Lambda significance values ($p < 0.05$) were taken into consideration. Cross-tabulation was applied to find out association (chi-square, $\chi 2$) of different toxicities with QoL domains, SNPs, and protein expressions. Kaplan Meier survival analysis was performed after monitoring survival for 24 months.

It was also used for analysis of survival associated with varying expression of MAD2. Cox regression analysis was used to determine the risk (Hazard Ratio) of death associated with the patient history; QoL domains at baseline and after first-line chemotherapeutic treatment between the adjuvant and neoadjuvant chemotherapy groups, as well as SNPs & protein expression.

CHAPTER 4

RESULTS

4. 1. Clinical Evaluation

4.1.1. Demographic details of the patients

110 participants were included in this prospective study after complying with the inclusion criteria at Gynecological Oncology, Chittaranjan National Cancer Institute (Hospital), Kolkata. Most of the patients were in the age range of 41-60 years with overall mean age of 49.15±10.8 years, had a mean body weight of 46.18 ± 9.39 kg. The women were mostly school-educated (54%), unemployed/homemakers (73.5%), belonging from rural locations of West Bengal, Jharkhand, and Bihar (**Table 4.1**).

Table 4.1: Sociodemographic characteristics of the ovarian cancer patients

Characteristics (N=110)		**Frequency (%)**
Age (Years)	20-40	24(21.8)
	41-60	76(69.09)
	61-80	10(9.09)
Education	Illiterate	39 (34.5)
	School education	61 (54.0)
	Graduates and above	10 (9.09)
Religion	Hindu	90 (81.81)
	Muslim	20 (18.18)
Marital status	Unmarried	7(6.2)
	Married	86(78.18)
	Widowed/ Divorced	17(15.4)
Occupation	Unemployed/ Housewife	83(75.45)
	Self-employed/ business	8(7.1)
	Professional/ Desk job	7(6.2)
	Laborer	7(6.2)
	Farmer	5(4.4)

Characteristics (N=110)		Frequency (%)
Setup	Urban	37(32.7)
	Rural	73(66.3)
Monthly Income	< INR 2000	26(23.6)
	INR 2001 to Rs 5000	75(68.2)
	INR 5001 and above	9(8.2)
Family History of cancer	Yes	11 (10)
	No	99(90)
N= Number of Patients; % - Percentage		

4.1.2. Gynecologic and obstetric history

The participants were mostly menopausal women, with history of regular menstruation, poor menstrual hygiene. Significant percentage (33.6%) of them recalled having used contraception methods (oral pills, Copper T and ligand) but majority had never used any such intervention. The obstetric history mainly finds majority had their first pregnancies during 15-20 years and substantial amount did not know or did not wish to reveal their age at first conception. Most of them were nulliparous and did not have abortion history (**Table 4.2**).

Cox regression and hazard analysis revealed age at first pregnancy, parity and mean menopausal age (44·27 years) poses a risk to survival (HR=1·076) outcome in patients with the advanced disease (**Figure 4.5**). On the other hand, contraceptive usage carries a moderate risk.

Table 4.2: Gyne-obstetric history of the patients

Gynecological Characteristics (N=110)		Frequency (%)
Menstrual cycle	Regular	96(87.27)
	Irregular	14(12.72)
No. of periods (N=109)	12/ year	93(82.3)
	6-9/year	14(12.4)
	<6/year	2(1.8)

Gynecological Characteristics (N=110)		**Frequency (%)**
Menstrual Hygeine	Poor	90(79.6)
	Good	20(17.7)
Menopause	Yes	66(60.0)
	No	44(40.0)
Contraceptive usage	Yes (oral contraceptives)	25(22.1)
	No	75(68.1)
	Copper T	8(7.1)
	Ligation	2(1.8)
Obstetric characteristics (N=110)		
Age at first pregnancy	15-20	54 (47.8)
	21-25	18(15.9)
	26-30	4(3.5)
	31-37	5(4.4)
	Do not know	29(25.7)
Parity	0	26 (23.0)
	1	19 (16.8)
	2	29(25.7)
	3	17(15.0)
	4	11(9.7)
	>=5	8(7.1)
Abortion	0	84(74.3)
	1	21(18.6)
	2	4(3.5)
	4	1(0.9)
Breast feeding history	Yes	82(72.6)
	No	28(24.8)
N= Number of Patients; % - Percentage		

4.1.3. Clinical presentation and clinical response

The patients initially presented with abdominal swelling, pain, bloating that persisted for several months, nausea/ vomiting, pelvic pain, and several other issues but very few had gynecological disturbances (**Figure 4.1**).

The patients were evaluated to have ECOG 1 performance at the time of diagnosis which later increased at the end of 24-month follow-up. 81% of the tumors were of serous histological subtype with solid- cystic gross appearance (58.8%). The largest ovarian tumor size had a length of 25.5cm reported. The fallopian tube was involved in most of the cases (**Figure 4.2**). There were 3 cases of primary peritoneal cancers and one of the cases had Krukenberg tumor. The most common metastatic sites were liver, small & large intestine, colon, diaphragm, peritoneum, omentum and fallopian tube. Other than that, metastases were seen in renal canaliculi, gall bladder, breast, biliary tract, appendix and uterine cervix. There were 91 (Stage III) and 19 (Stage IV) patients among whom 55 underwent primary debulking surgery with adjuvant chemotherapy and 55 patients had interval debulking surgery with neoadjuvant chemotherapy (**Figure 4.3**). The median serum albumin of patients was 3·7g/dL. The median of baseline CA125, CA19·9, and CEA were 480 U/mL, 18·96 U/mL and 2·44ng/mL respectively. The highest CA125 detected at diagnosis was 9400U/mL. 41, 44 and 25 patients were categorized as complete responders, partial responders, and non-responders after evaluation and follow up till 1 year from the start of first-line treatment. The objective response rate (ORR) was achieved by 77.27% of the patient population (**Table 4.3**)

Among 110 patients, there was no significant difference of response among the adjuvant chemotherapy and neoadjuvant chemotherapy arms ($p>0·05$). The overall survival (OS) curves for responders to non-responders after 24-month follow up are shown in (**Figure 4.4**). The log-rank test of the survival among responders, partial responders and non-responders was significant ($p=0.019$) that means survival distributions of different groups are not equal in the participants (**Table 4.4**). The mean PFS was found to be 6 months for the patients included in the study.

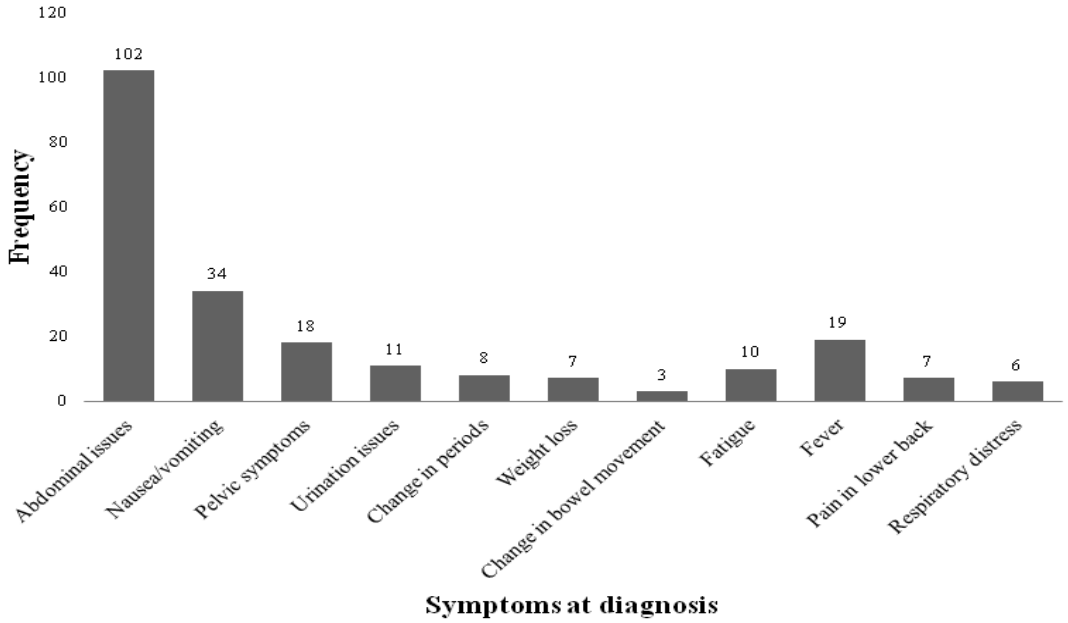


Figure 4.1: Initial symptoms

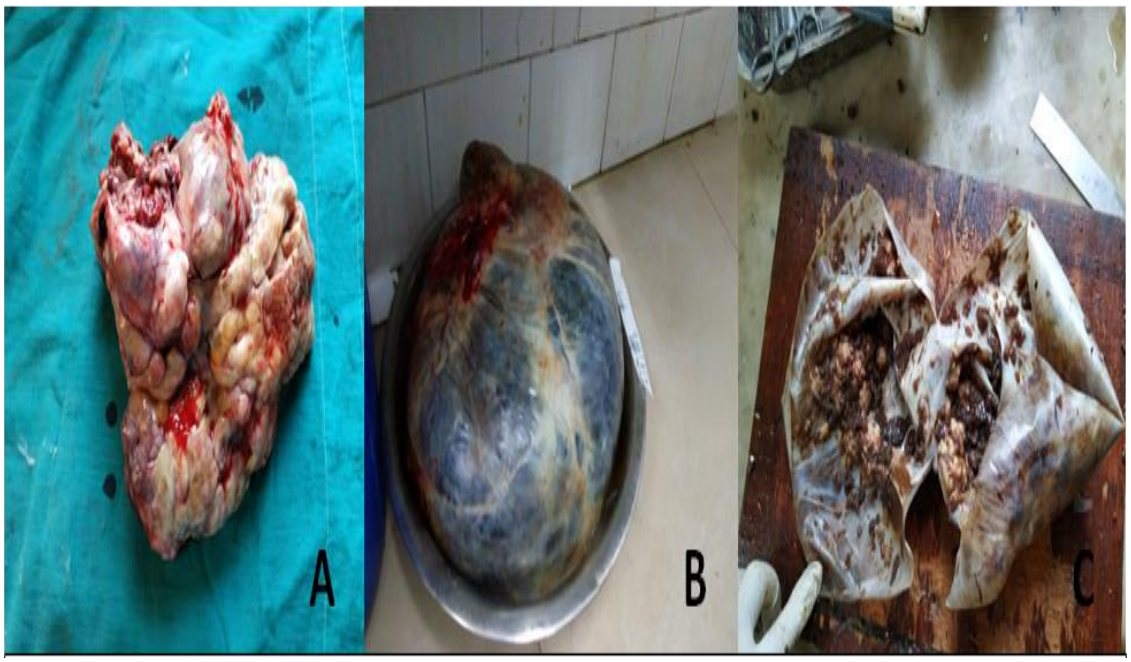


Figure 4.2: Gross structure of low grade (A), high grade (B), large (25cm approx) malignant tumors. C: Papillary structures visible after cutting through the ovarian capsule of a 10% NBF fixed hemorrhagic malignant ovarian tumor**.**

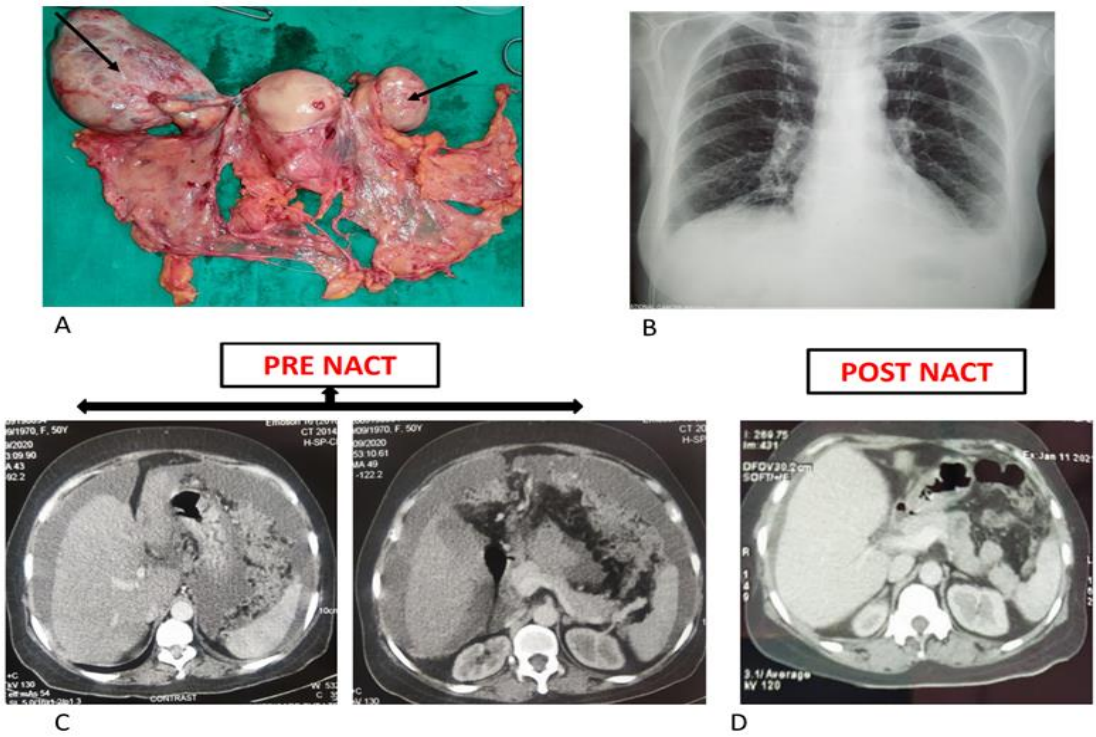


Figure 4.3: A: Enbloc uterus and ovarian tumors (shown by black arrows); B: mild pleural effusion of Stage IV ovarian cancer patient; C: Pre NACT CECT of abdomen shows gross ascites, omental cake, multiple enhancing peritoneal nodules seen; D: Post NACT CECT shows liver, gall bladder, spleen, pancreas (within normal limit), mild omental thickening.

Table 4.3: Clinical features and clinical response of the patients

Characteristics (N=110)	Group	Frequency (%)
ECOG performance status at study entry	0	20 (18.9)
	1	68(62.2)
	2	15 (13.5)
	3	7(5.4)
FIGO	III	91(82.02)
	IV	19(17.98)
Tumor histology	Serous	81(81.0)
	Other	29(26.9)
Gross type	Solid Cystic	65(58.8)
	Cystic	32(29.4)
	Solid	13(11.8)
Size of tumor mass (pre-treatment)	>5 cm	80 (73.2)

Characteristics (N=110)	Group	Frequency (%)
	<5 cm	30(26.8)
Pre-treatment CA125 (units/mL) (median)	480	
Post-Treatment CA125 (units/mL) (median)	42.7	
CEA(ng/mL) (median)	2.44	
CA 19.9(units/mL) (median)	18.96	
Pre-treatment serum albumin (g/dL) (median)	3.7	
Surgeries	Primary debulking	55
	Interval debulking	55
Clinical Response [Non-Responders include Stable disease, Progressive, Time to treatment, Palliative care and Not evaluable] ORR=77.27%	Complete	41 (37.3)
	Partial	44(40)
	Progressive	6(5.5)
	Palliative care	16(14.5)
	Time to treatment	1(0.9)
	Not evaluable	2(1.8)
N= Number of Patients; % - Percentage		

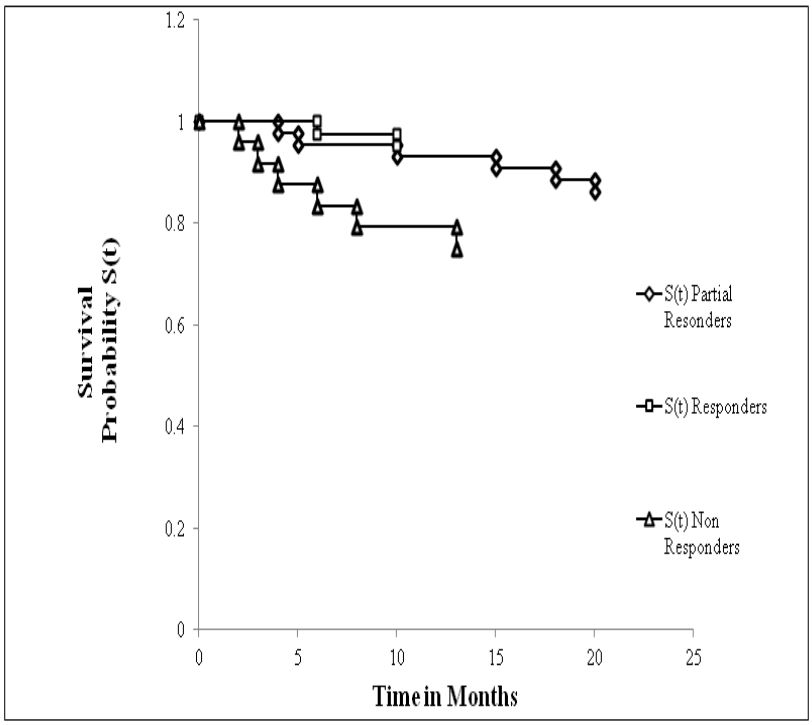


Figure 4.4: Kaplan Meier Survival Curve plotted for Responders, Partial Responders and Non-Responders

Table 4.4: Significance of survival distributions among response categories analyzed by Log Rank test

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	7.909	2	0.019
Test of equality of survival distributions for the different levels of Clinical Response.			

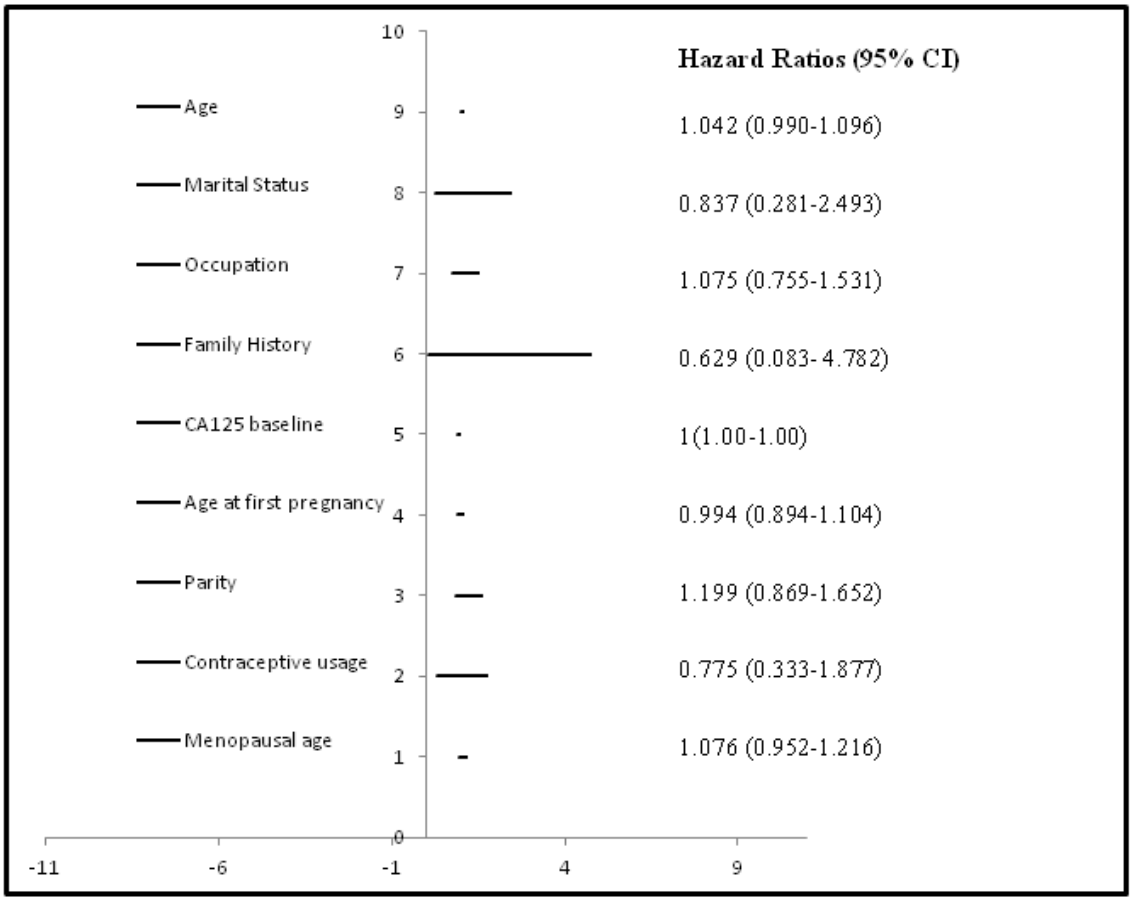


Figure 4.5: Survival risks associated with patient characteristics

4.1.4. Common toxicities

Adverse events of grades 3-4 in anemia (8·1%), leukopenia (0·9%), nausea (1·8%), vomiting (3·6%), anxiety/depression (5·4%), neuropathy (2·7%), diarrhoea (5·4%), constipation (8·1%), abdominal pain/swelling (1·8) and renal toxicity (0·9%) were observed. Most of the adverse events of grades 1-2 were observed in anemia, followed by indigestion, neuropathy, constipation, and diarrhoea (**Table 4.5)**. Patients receiving neoadjuvant chemotherapy had significantly (p=0.001) more occurrences of chemo-induced neuropathy than that of patients receiving adjuvant chemotherapy (**Table 4.6**).

36.36% patients experience grades 1-2 neuropathy whereas only 2.7% patients had grades 3-4 neuropathic pain.

Table 4.5: Common clinically observed toxicities

Sl No.	Adverse Effects	Frequency (%) of Grades 1-2	Frequency (%) of Grades 3-4
1.	Anemia (n= 65)	56(50.9)	9(8.1)
2.	Leukopenia (n=16)	15(13.6)	1(0.9)
3.	Thrombocytopenia (n=6)	6(5.45)	0(0)
4.	Granulocytopenia (n=3)	3(2.7)	0(0)
5.	Nausea (n=18)	16(14.5)	2(1.8)
6.	Vomiting(n=26)	22(20)	4(3.6)
7.	Anxiety/Depression(n=37)	31(28.1)	6(5.4)
8.	Neuropathy(n=43)	40(36.36)	3(2.7)
9.	Weight Loss(n=30)	30(27.2)	0(0)
10.	Diarrhoea(n=35)	29(26.3)	6(5.4)
11.	Constipation(n=40)	31(28.1)	9(8.1)
12.	Indigestion(n=47)	40(36.36)	7(6.3)
13.	Abdominal pain/swelling (n=33)	31(28.1)	2(1.8)
14.	Renal Toxicity(n=25)	24(21.8)	1(0.9)
n= Number of Patients with reported toxicity			

Table 4.6: Association of neuropathic pain occurrence in adjuvant and neoadjuvant chemotherapy groups

Adverse effect grades	0	1	2	3	Total	Pearson Chi-Square
Adjuvant Chemotherapy	42	7	3	3	55	**p= 0.001**
Neoadjuvant Chemotherapy	31	19	2	3	55	

4.2. Pain evaluation

The mean pain scores were calculated for the total 110 patients at different time intervals using NRS and NPSI scales. In this present study, no statistically significant differences were found in pain at baseline and after 6 cycles of chemotherapeutic treatment ($p>0.05$) and, no significant difference was found between the responder and non- responder categories. Movement associated pain had a strong positive correlation ($R^2=1$) with physical and functional wellbeing of the patients. Most of the patients were prescribed to take paracetamol, pethidine, gabapentin, vitamin B12, diclofenac (topical) and tramadol for rescue analgesia (**Table 4.7**). In this study, 57 (51.8%), 49 (44.5%) and 44 (40%) patients reported resting, movement, and sleep-associated pain at the time of diagnosis and study table entry. Upon follow up, after six cycles of chemotherapy the frequencies of resting and sleep-associated pain remained same but movement associated pain increased by 6.4% (56, 50.9%) patients. Patient frequencies experiencing different types of neuropathic pain at different time points are mentioned in (**Table 4.8).** There was no significant decline observed.

Table 4.7: Mean NRS scores in responders, partial responders, and non-responders at various time intervals

Resting stage					
Groups (n=110)	**Baseline**	**2nd month**	**4th month**	**6th month**	**p-value‡ (within groups)**
Rs (n=41)	3.00±3.30	3.07±3.27	3.34±3.36	3.15±3.36	0.385
PRs(n=44)	2.93±3.40	2.57±3.2	2.48±3.34	2.86±3.38	
NRs(n=25)	3.84±3.78	3.72±3.72	2.96±3.47	3.40±3.62	
p-value*(between groups)	0.343				
Movement stage					
Groups	Baseline	2nd month	4th month	6th month	**0.032**
Rs (n=41)	2.37±3.11	2.46±3.09	2.63±3.23	2.76±3.12	
PRs(n=44)	2.16±3.02	2.30±3.08	2.36±3.12	3.09±3.48	
NRs(n=25)	3.92±4.06	3.88±4.10	2.96±3.55	3.36±3.70	
p-value*(between groups)	0.081				
Sleep interference					
Groups	Baseline	2nd month	4th month	6th month	0.499
Rs (n=41)	2.32±3.36	2.17±3.19	2.51±3.23	2.54±3.37	
PRs(n=44)	2.63±3.61	2.84±3.72	2.37±3.55	2.35±3.30	
NRs(n=25)	3.20±4.03	3.08±4.03	2.80±3.73	2.72±3.69	
p-value*(between groups)	0.603				

Responders (Rs); Partial Responders (PRs); Non Responders (NRs).All values are expressed as mean±SD. The NRS scores of resting, and sleep were non-significant within-subject effect and multivariate analysis.

*Multivariate analysis (Wilk's Lambda)

‡Greenhouse-Geisser

Mauchly's sphericity was significant ($p<0.05$).

Table 4.8: Patient distribution having different types of neuropathic pain at various time intervals

Neuropathic pain type	Baseline	2 months	4 months	6 months
Burning	18	18	15	20
Squeezing	21	16	17	14
Pressure	17	13	10	17
Electric Shock	16	11	10	15
Stabbing	13	11	10	15
Light touch	16	15	11	13
Pressing	18	14	14	20
Cold	18	14	12	24
Pins/Needles	14	19	19	23
Tingling	13	13	16	20

4.3. Quality of Life assessment

The mean and standard deviation scores of the FACT-O questionnaire domains are represented in **Table 4.9**. There were no statistically significant differences found ($p>0.05$) in baseline QoL and in all the QoL domains (spiritual, emotional, social, functional, physical and additional concern) throughout the 6 months. There were also no significant differences between the QoL of the responders, partial responders, and non-responders or between adjuvant and neoadjuvant chemotherapy arms. Combined QoL mean scores are plotted (**Figure 4.6**). Even though there was no significant improvement in the QoL, there was no significant decline noted as well. Relationship with QoL at baseline and after chemotherapy are documented in **Table 4.10**. It is evident from the table that poor physical well-being has the highest risk of death at baseline, but adjuvant chemotherapy arm has a higher risk of death than the neoadjuvant group at 6 months.

QoL associated with different toxicities were also analyzed. Among the adversities, anemia and constipation were found to be significantly associated with the physical ($p=0.003$ & $p=0.004$), emotional ($p=0.016$ & $p=0.000$), functional ($p=0.006$ & $p=0.001$) wellbeing, and additional concerns ($p=0.000$ & $p=0.000$). Additional concerns specific to ovarian cancer was associated ($p=0.000$) with leukopenia, granulocytopenia,

neuropathy, weight loss, indigestion and abdominal pain. Functional well-being was associated with weight loss (p=0.014) and diarrhoea (p=0.008). Emotional wellbeing was significantly associated with granulocytopenia (p=0.000), nausea (p=0.008), anxiety (p=0.006), weight loss (p=0.000), indigestion (p=0.040), and abdominal pain (p=0.000). Social well-being was associated with thrombocytopenia (p=0.017) and weight loss (p=0.005) (data not shown).

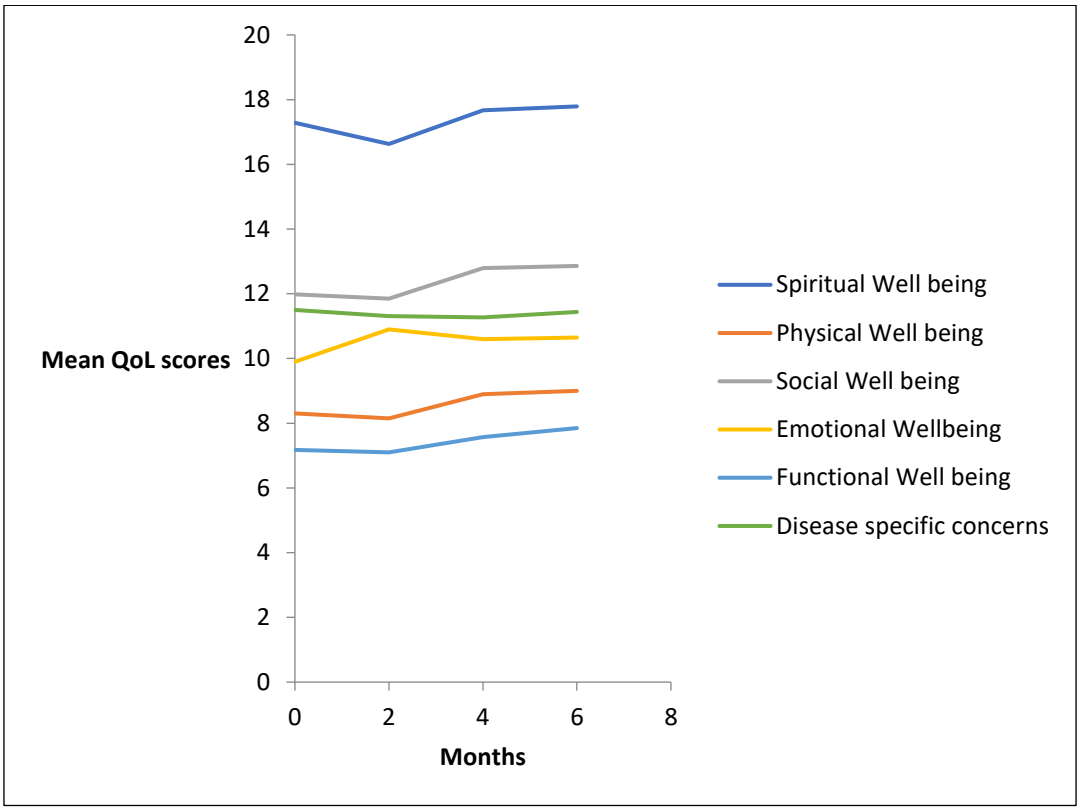


Figure 4.6: Mean QoL scores for all segments of Fact-O evaluation at different time points

Table 4.9: FACT-O scores at various time intervals among responders, partial responders, and non-responders

QoL Domains	Groups	Baseline	2nd month	4th month	6th month	*p-value*ǂ
Spiritual	Rs (n=41)	20.22±13.37	20.10±13.39	21.20±12.27	21.49±11.68	0.662
	PRs(n=44)	16.23±14.00	16.30±13.95	17.73±13.80	17.39±13.25	
	NRs(n=25)	14.32±14.14	11.56±14.02	11.80±14.26	12.44±14.06	
*p- value**	0.635					
Emotional	Rs (n=41)	10.22±7.51	9.71±7.35	10.78±7.64	11.10±7.54	0.472
	PRs(n=44)	11.09±7.86	10.91±7.56	11.32±7.85	10.93±7.92	
	NRs(n=25)	10.40±8.45	8.48±8.77	10.36±8.53	9.44±8.12	
*p- value**	0.774					
Social	Rs (n=41)	12.12±8.46	12.20±8.67	12.90±7.89	13.05±7.30	0.757
	PRs(n=44)	12.86±8.07	12.68±8.48	13.36±8.46	13.77±8.72	
	NRs(n=25)	10.20±7.42	9.84±7.75	11.60±8.00	10.96±8.28	
*p- value**	0.956					
Functional	Rs (n=41)	7.07±6.59	7.24±6.71	8.22±6.72	8.73±6.67	0.846
	PRs(n=44)	7.55±7.17	7.64±6.93	7.68±6.63	7.70±6.63	
	NRs(n=25)	6.72±6.73	5.92±6.72	6.32±6.75	6.68±7.25	
*p- value**	0.609					
Physical	Rs (n=41)	8.15±7.18	8.37±6.97	9.54±7.24	9.90±6.94	0.425
	PRs(n=44)	8.43±8.01	8.32±8.00	8.95±7.58	9.52±7.86	
	NRs(n=25)	8.32±8.76	7.52±8.75	7.8±8.06	6.72±7.03	
*p- value**	0.347					
Additional concerns	Rs (n=41)	10.65±8.10	10.42±7.91	13.00±7.68	13.78±7.77	0.497
	PRs(n=44)	12.11±8.86	12.95±9.14	14.09±8.56	13.68±8.74	
	NRs(n=25)	11.92±8.70	9.88±9.69	12.50±10.24	12.46±10.29	
p- value*	0.763					

Responders (Rs); Partial Responders (PRs); Non-Responders (NRs); All values are expressed as numbers and mean ± Standard deviation; The QoL sections were non-significant within-subjects and multivariate analysis.

**Multivariate analysis (Wilk's Lambda)*

ǂ*Greenhouse-Geisser*

Table 4.10: Survival probability in relationship with QoL at different time points and chemotherapy

QoL Factors	Baseline (without treatment) HR (95% CI)	*p-value*	Chemotherapy	At 6 months after first-line chemotherapy HR (95%CI)	*p-value*
Spiritual Wellbeing	1.441(0.789-2.630)	0.554	Adjuvant	1.778 (0.519-6.090)	0.371
			Neo-adjuvant	0.990 (0.247-3.966)	
Physical Wellbeing (0-28)	**3.603** (1.306-9.941)	**0.008**	Adjuvant	1.714 (0.584-5.029)	0.320
			Neo-adjuvant	1.2 (0.49-4.789)	
Social Wellbeing (0-28)	1.013 (0.950-1.080)	0.349	Adjuvant	3.052(1.021-9.125)	**0.036**
			Neo-adjuvant	1.747 (1.010-3.021)	
Emotional Wellbeing (0-24)	0.945 (0.343-2.607)	0.913	Adjuvant	1.495 (0.172-1.420)*	0.994
			Neo-adjuvant		
Functional Wellbeing (0-28)	1.00 (0.614-1.134)	0.293	Adjuvant	0.031 (0.00-6.01) *	**0.034**
			Neo-adjuvant		

*HR was same for both the groups.

p-value represents the significance of Log-rank test.

4.4. Expression of the SAC components

4.4.1. Histology

The different histological subtypes found are presented in **Figure 4.7**. Due to health risk and physician's advice, molecular analyses was performed on 80 patients.

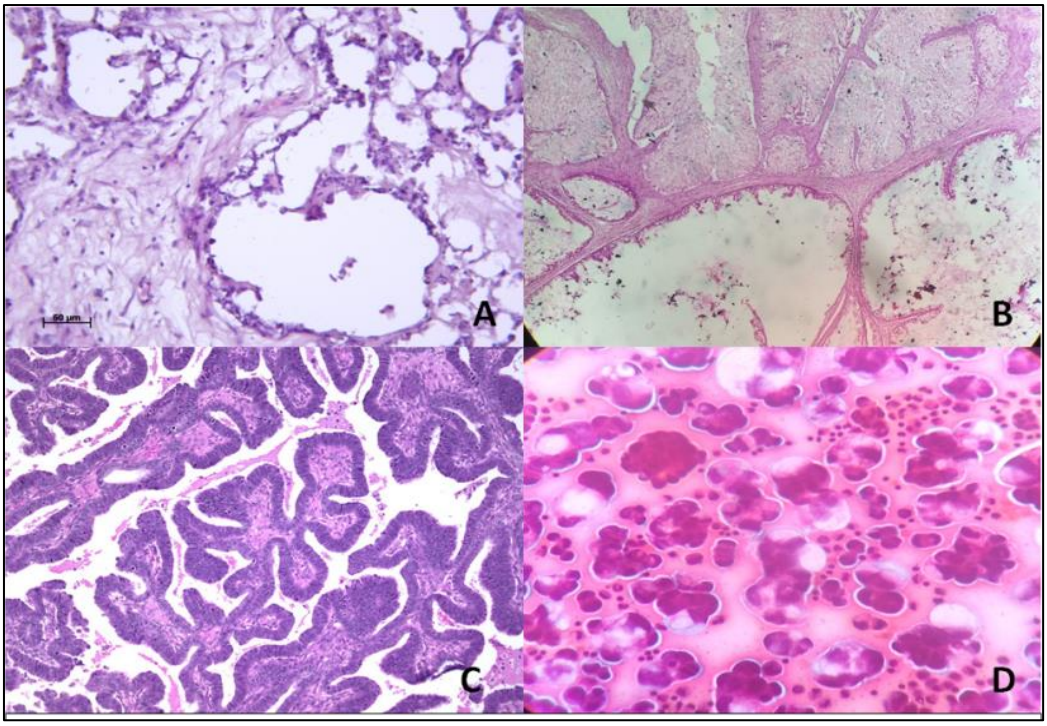


Figure 4.7: Histopathology of A, High grade serous papillary carcinoma (20X); B, Mucinous carcinoma (20X); C, Endometroid carcinoma of ovary; D, Ascitic fluid cytology showing adenocarcinoma deposits (40X)

4.4.2. Immunohistochemistry and mRNA expression

Moderate to high expression of MAD2 and BUB3 was seen in most of ovarian cancer tissues before chemotherapy and low/negative expression was seen for BUB1 and MAD1 in maximum cases indicating upregulation and downregulation of the SAC components (**Figures 4.8-4.11**).

MAD1 expression had significant association with CA125 levels (p=0.008). MAD2 and BUB1 expressions were found to be significantly different in primary OC tumors and tumors after interval debulking (NACT) (**Tables 4.11-4.14**). The analysis of IHC expressions with CA-125, gross type and chemotherapy were analyzed for only those proportions of patients whose complete data were available. Upon further analysis with clinical parameters, it was found that BUB3 expression was significantly correlated with complete or partial response (p=0.032), anxiety (p=0.012), and abdominal pain (p=0.003). MAD1 was significantly associated with weight loss (p=0.013), constipation (p=0.05), and nearly significant with indigestion (p=0.064). BUB1 and BUB3 (OR= 1.79, 95%CI= 0.16-19.77; OR= 1.93, 95%CI= 0.413-9.054) expression had higher risk

to poor survival outcome than MAD1 and MAD2 (OR= 0.792, 95%CI=0.19-3.24; OR=0.870, 95% CI= 0.187-4.036).

The mRNA analysis of the said markers through Real-Time PCR also showed similar results indicating minimal expression of MAD1 & BUB1 mRNA with comparatively much higher expression of MAD2 and BUB3 mRNA. The downstream target of SAC, CDC20 was upregulated when compared to BUB1 and MAD1. This result suggests that there is deficiency in SAC signaling components which may affect its activation and subsequent control over metaphase to anaphase transition. MAD2 mRNA was found to be inversely associated with survival outcome (p=0.001). miR-495 had negative correlation with BUB1 mRNA (r= -0.510, p=0.016). Log-rank test was found to be significant for both miR-143 (p=0.003) and miR-659 (p=0.025). But they did not have significant correlation with expression of any of the SAC proteins. miR-125b was significantly downregulated and had a directly proportional relationship with MAD1 mRNA expression.

Table 4.11: Immunohistochemical expression of MAD1 in proportion of OC tumors

Sl No.	Clinical parameters	Groups	MAD1			p-value
			High	Moderate	Low/ Negative	
1.	Clinical response (N=80)	Responders(N=29)	0	22.2%	16.6%	0.66
		Partial Responders(N=32)	10%	16.6%	22.2%	
		Non-Responders (N=19)	0	0	5%	
2.	CA-125(U/mL)	37-100	23.07%	7.6%	7.6%	**0.008**
		101-499	7.6%	7.6%	15.3%	
		500 and above	7.6%	0	30.7%	
3.	Gross type	Solid cystic	15.3%	15.3%	15.3%	0.105
		Cystic	7.6%	7.6%	15.3%	
		Solid	7.6%	7.6%	0	
4.	Chemotherapy	Adjuvant	30.7%	7.6%	23.07%	0.65
		Neoadjuvant	23.07%	7.6%	23.07%	

Table 4.12: Immunohistochemical expression of MAD2 in proportion of OC tumors

Sl No.	Clinical parameters	Groups	MAD2			p-value
			High	Moderate	Low/ Negative	
1.	Clinical response **(N=80)**	Responders (N=29)	27.7%	5%	5%	0.15
		Partial Responders(N=32)	22.2%	22.2%	5%	
		Non-Responders (N=19)	8%	5%	0	
2.	CA-125(U/mL)	37-100	11.7%	5.8%	0	0.521
		101-499	5.8%	5.8%	5.8%	
		500 and above	17.64%	0	0	
3.	Gross type	Solid cystic	17.64%	11.7%	5.8%	0.346
		Cystic	5.8%	11.7%	5.8%	
		Solid	5.8%	5.8%	0	
4.	Chemotherapy	Adjuvant	35.2%	11.7%	0	**0.014**
		Neoadjuvant	5.8%	17.64%	5.8%	

Table 4.13: Immunohistochemical expression of BUB3 in proportion of OC tumors

Sl No.	Clinical parameters	Groups	BUB1			p-value
			High	Moderate	Low/ Negative	
1.	Clinical response **(N=80)**	Responders(N=29)	5%	10%	33%	0.32
		Partial Responders(N=32)	0%	16.6%	33%	
		Non-Responders (N=19)	0%	3%	0%	
2.	CA-125(U/mL)	37-100	11.7%	5.8%	11.7%	0.521
		101-499	5.8%	11.7%	5.8%	
		500 and above	5.8%	5.8%	11.7%	
3.	Gross type	Solid cystic	0	17.64%	17.64%	0.346
		Cystic	5.8%	0	17.64%	
		Solid	0	5.8%	11.7%	
4.	Chemotherapy	Adjuvant	0	0	47.08%	**0.010**
		Neoadjuvant	5.8%	11.7%	5.8%	

Table 4.14: Immunohistochemical expression of BUB3 in proportion of OC tumors

Sl No.	Clinical parameters	Groups	BUB3			p-value
			High	Moderate	Low/ Negative	
1.	Clinical response **(N=80)**	Responders(N=29)	5%	10%	22.2%	**0.032**
		Partial Responders(N=32)	27.7%	22.2%	5%	
		Non-Responders (N=19)	0	5%	5%	
2.	CA-125(U/mL)	37-100	21.4%	21.4%	0	0.194
		101-499	0	21.4%	7.1%	
		500 and above	14.2%	0	21.4%	
3.	Gross type	Solid cystic	21.4%	21.4%	0	0.105
		Cystic	0	0	21.4%	
		Solid	7.1%	7.1%	21.4%	
4.	Chemotherapy	Adjuvant	21.4%	21.4%	21.4%	0.65
		Neoadjuvant	14.2%	14.2%	7.1%	

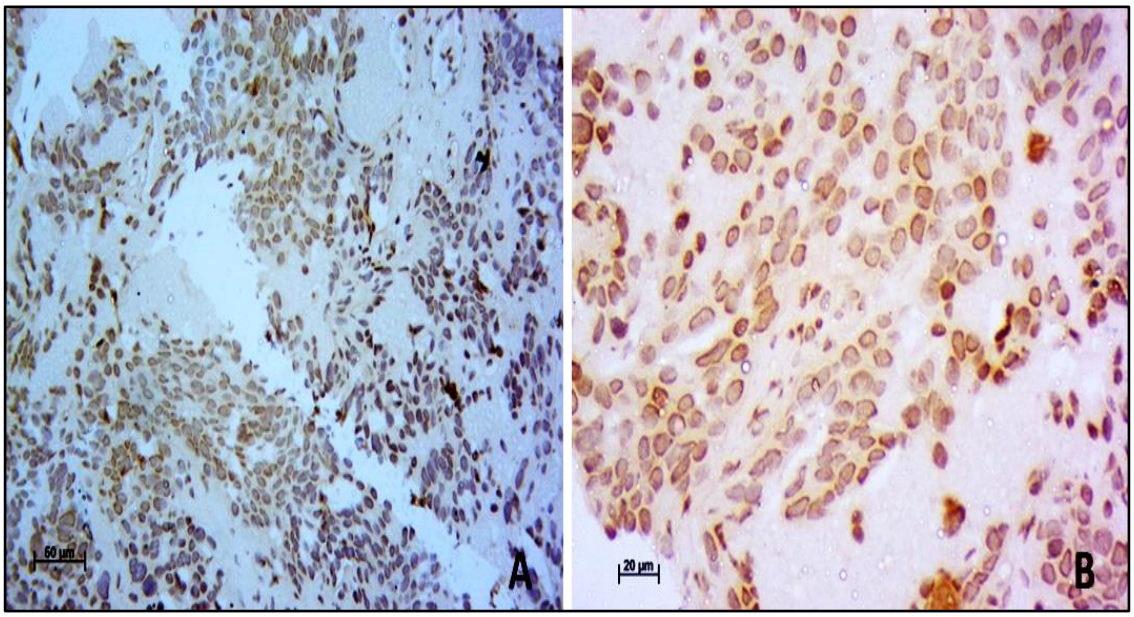


Figure 4.8: High grade serous papillary carcinoma stained with MAD2 antibody, A (20X), B (40X). The images show high nuclear immune-positivity of MAD2.

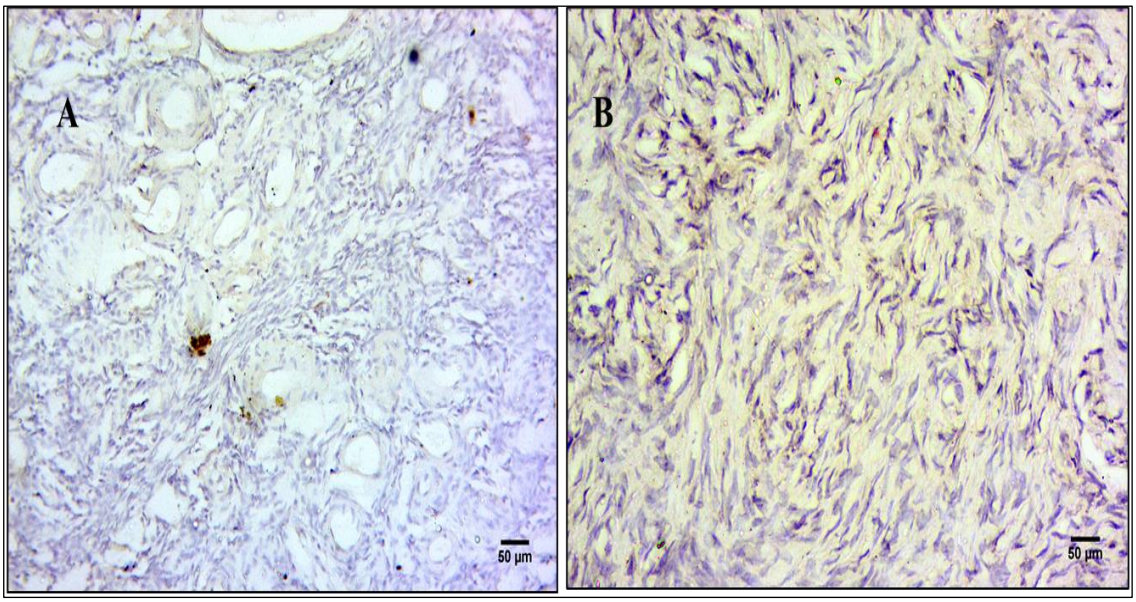


Figure 4.9: IHC expressions of MAD1 observed in advanced ovarian carcinoma tissues. Brown colour indicates very low to moderate intensity immunopositivity in both nucleus and cytoplasm, A (20X), B (40X).

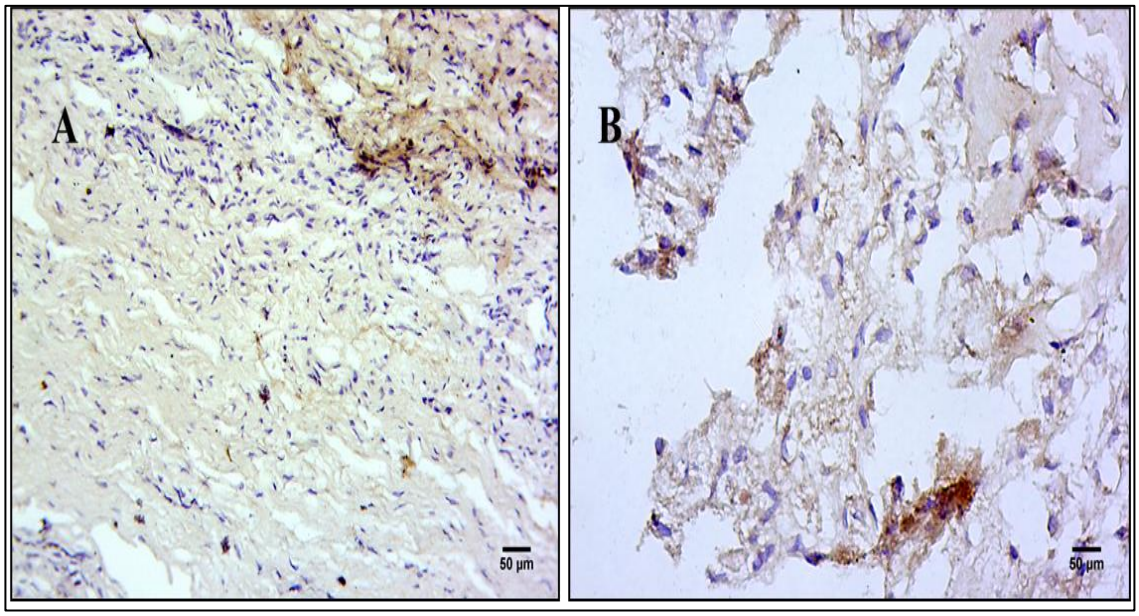


Figure 4.10: IHC expressions of BUB1 observed in ovarian cancer tissue, A (20X), B (40X).

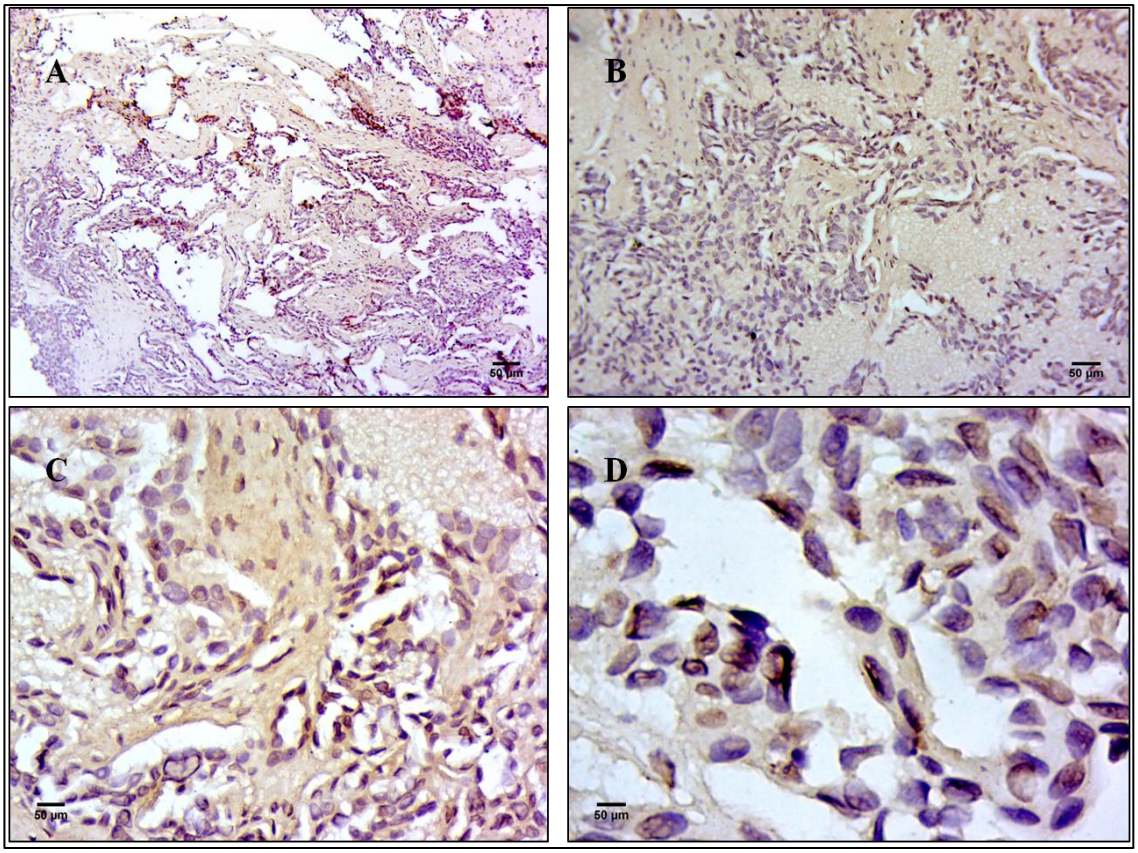


Figure 4.11: IHC expressions of BUB3 observed in ovarian cancer tissue, A (10X), B (20X), C (40X), D (100X).

Table 4.15: Ct median for the mRNA and miR

Sl No.	Markers	Ct median
1.	MAD1 mRNA	≥ 35
2.	MAD2 mRNA	23.96
3.	BUB1 mRNA	≥ 35
4.	BUB3 mRNA	33.63
5.	CDC20 mRNA	25.55
6.	miR-125b	≥ 35
7.	hsa-miR-495	27.06
8.	hsa-miR-143	25.30
9.	hsa-miR-695	33.15
10.	Gapdh	21

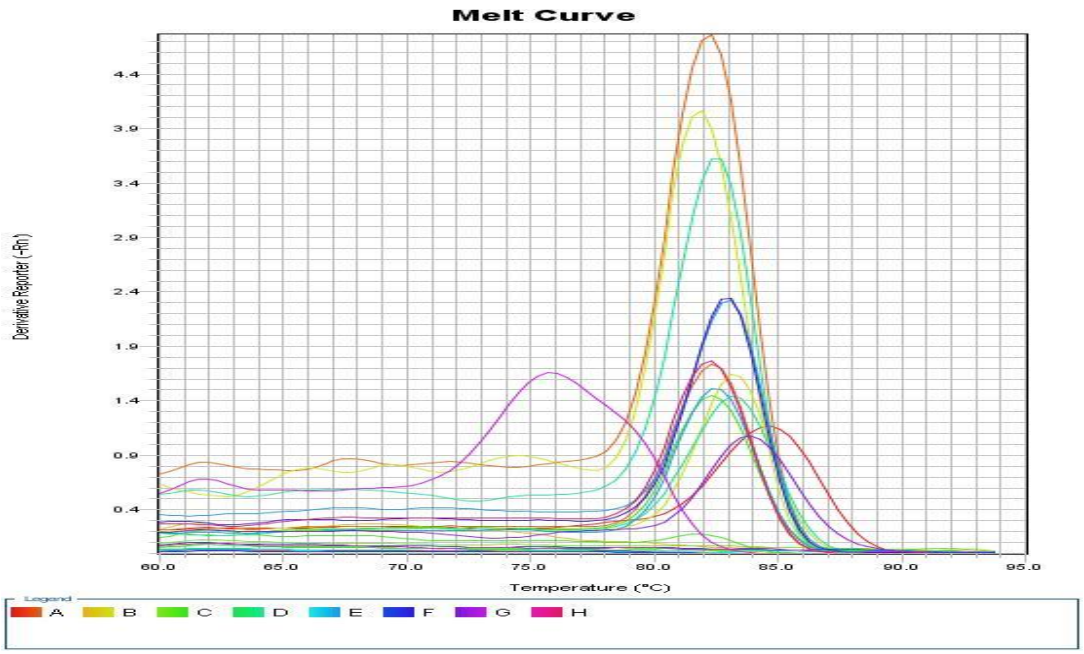


Figure 4.12: Melt curve of the qPCR

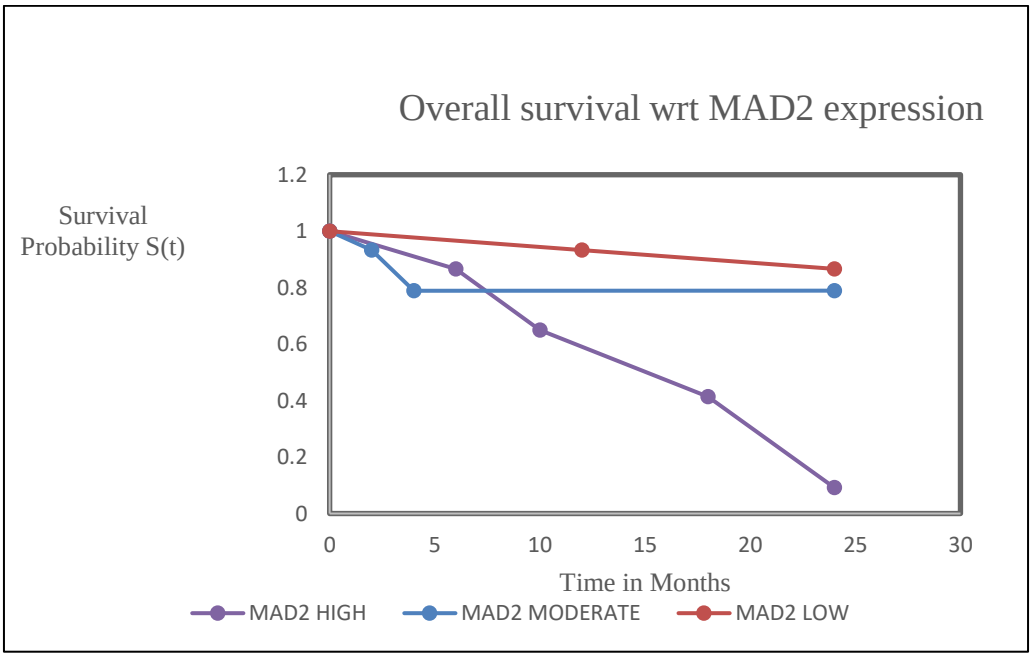


Figure 4.13: Survival curves with related to MAD2 expression (high, moderate, low) in ovarian cancer patients.

4.5. Translational relevance of the SNPs

The amplification, restriction digestion and allele frequencies are described in **Figures 4.14- 4.24** and **Tables 4.16** respectively.

Among all other SNPs, BUB1B (rs28989181) polymorphic homozygous genotypes (CC) and (TT) of 2530C>T were most prevalent in NRs & PRs respectively showing significant association in chemotherapy response (p=0.021). The allele frequencies were found to be C= 0.2215, and T= 0.7784. No significant relationship was observed with this SNP and toxicities. The survival outcome was nearly significant (p= 0.06) showing association of CC genotype with higher risk (HR=9.938, 95%CI= 1.19-82.9) when compared to CT (HR=0.885, 95%CI= 0.109-7.19) and TT (HR=0.409, 95%CI= 0.049-2.209). MAD2L1 (rs11972014, rs3752830) and MAD1L1 (rs121908981) had higher risk to survival i.e., HR= 2.9 (95%CI 0.00-7.59); HR= 1.2 (95%CI 0.451-3.426) and HR= 1.09 (95%CI 0.6-1.8) respectively. The remaining SNP did not possess risk to survival and were not associated with clinical response outcomes.

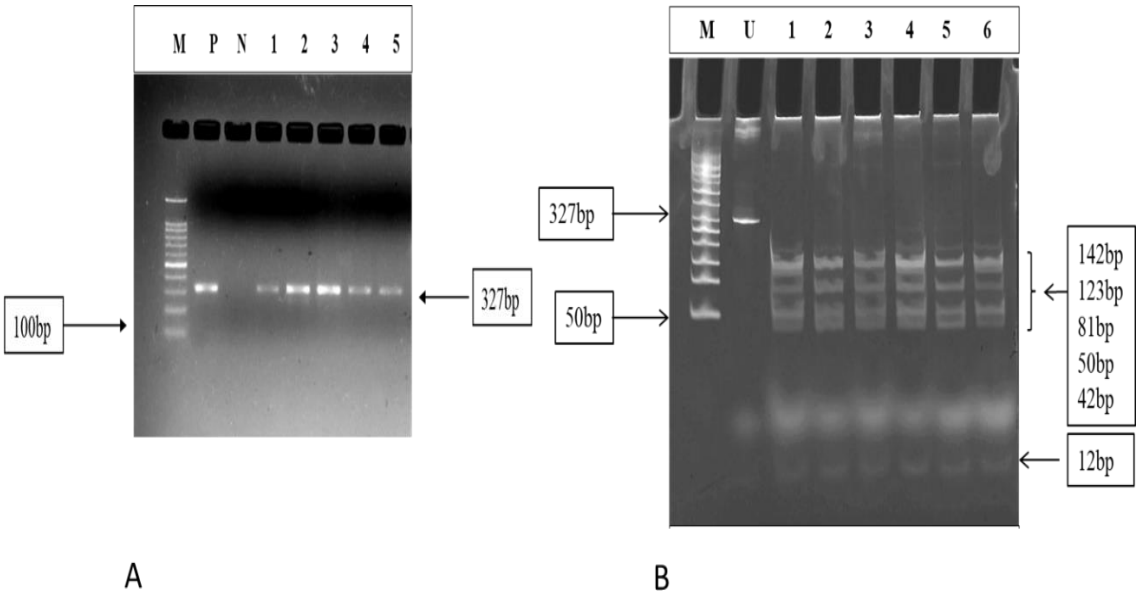


Figure 4.14: A: PCR amplification; B: RFLP of PCR products of MAD1L1(rs1801368); M= marker; P= positive control; N= negative control; U= undigested product.

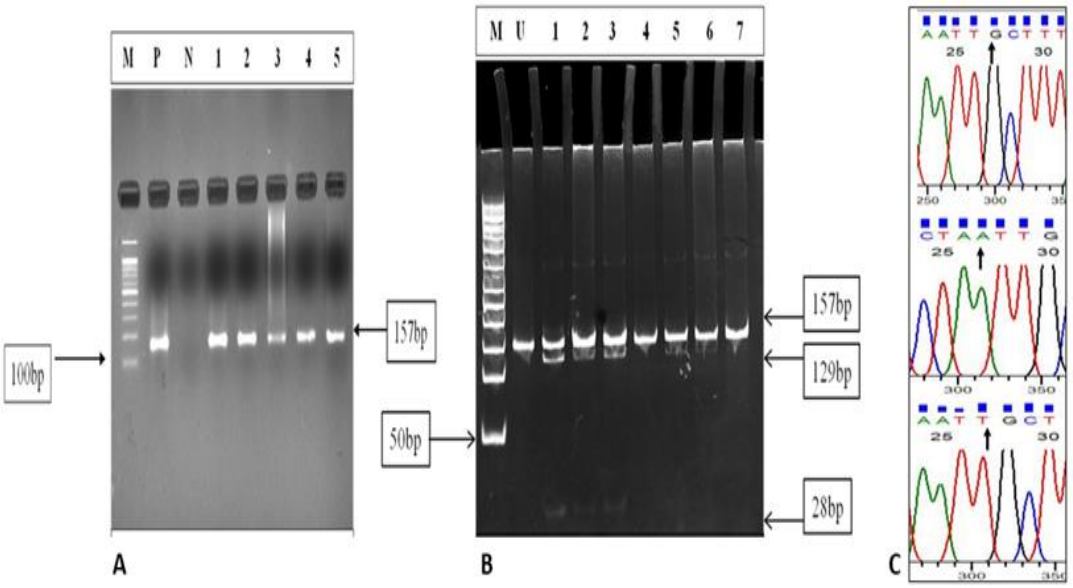


Figure 4.15: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs1972014); M= marker; P= positive control; N= negative control; U= undigested product.

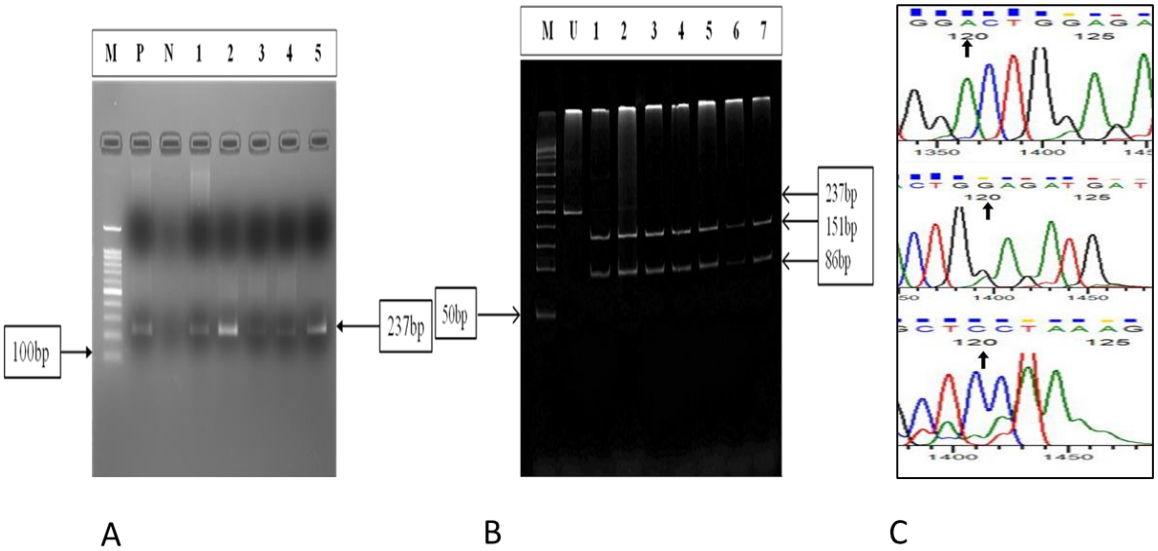


Figure 4.16: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs1546120); M= marker; P= positive control; N= negative control; U= undigested product.

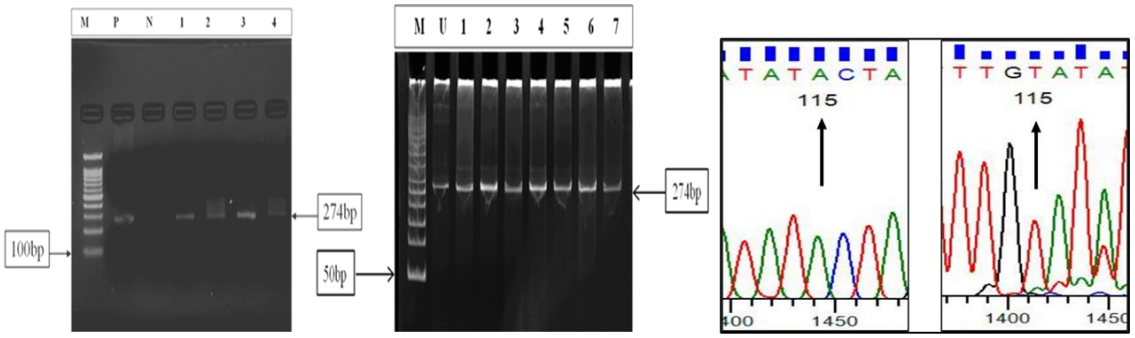


Figure 4.17: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs3752830); M= marker; P= positive control; N= negative control; U= undigested product.

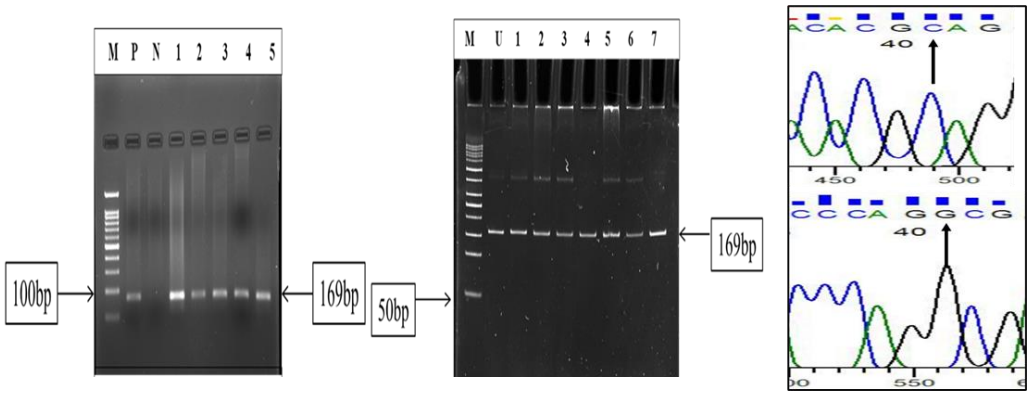


Figure 4.18: PCR amplification; B: RFLP of PCR products and C: Sequencing of MAD2L1 (rs121908981) M= marker; P= positive control; N= negative control; U= undigested product.

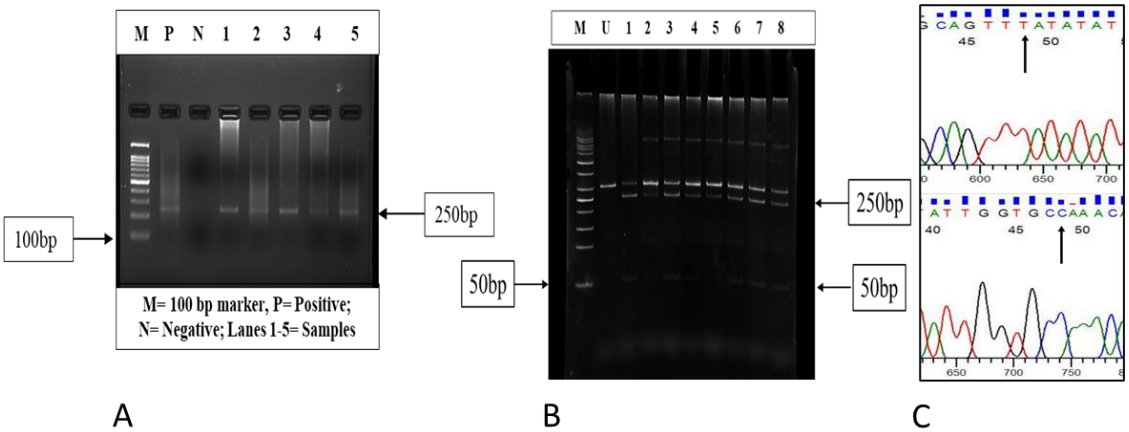


Figure 4.19: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1B (rs28989181) M= marker; P= positive control; N= negative control; U= undigested product.

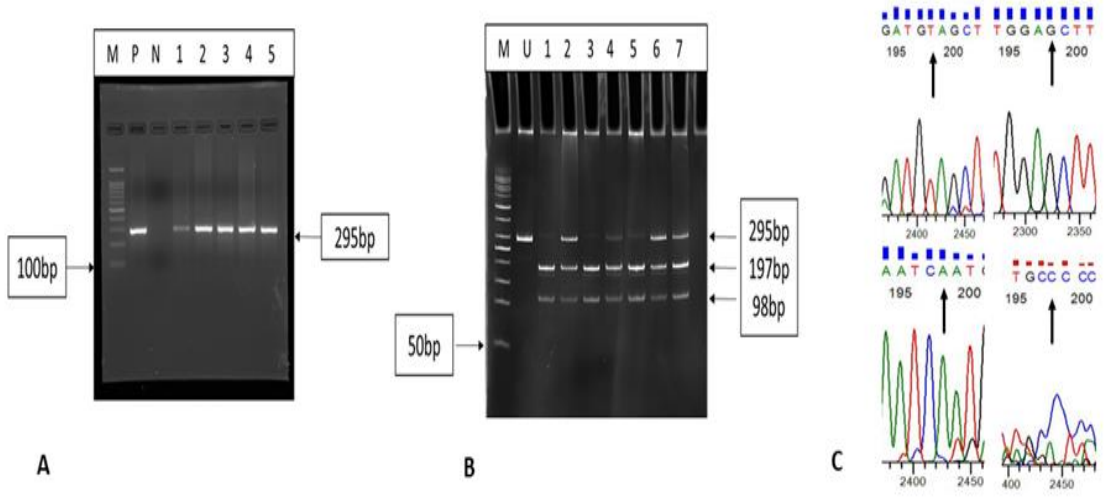


Figure 4.20: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1B (rs28989186); M= marker; P= positive control; N= negative control; U= undigested product

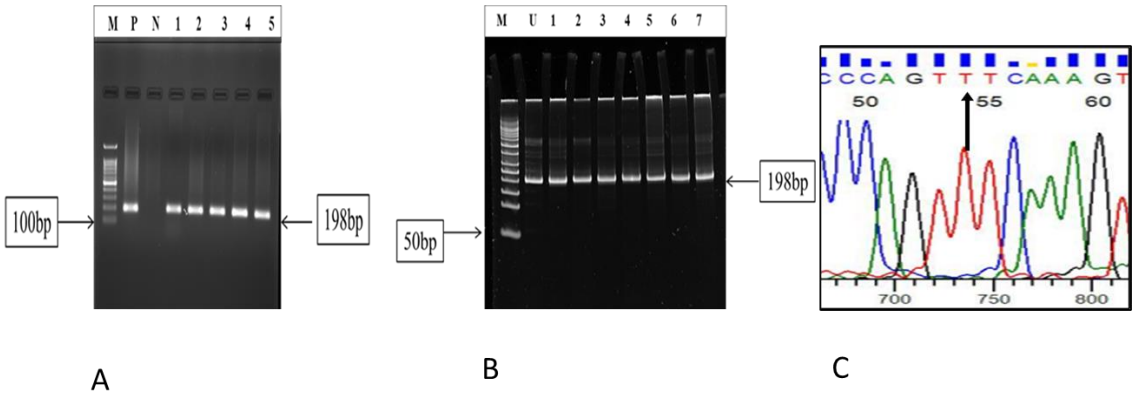


Figure 4.21: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB1 (rs121909055); M= marker; P= positive control; N= negative control; U= undigested product.

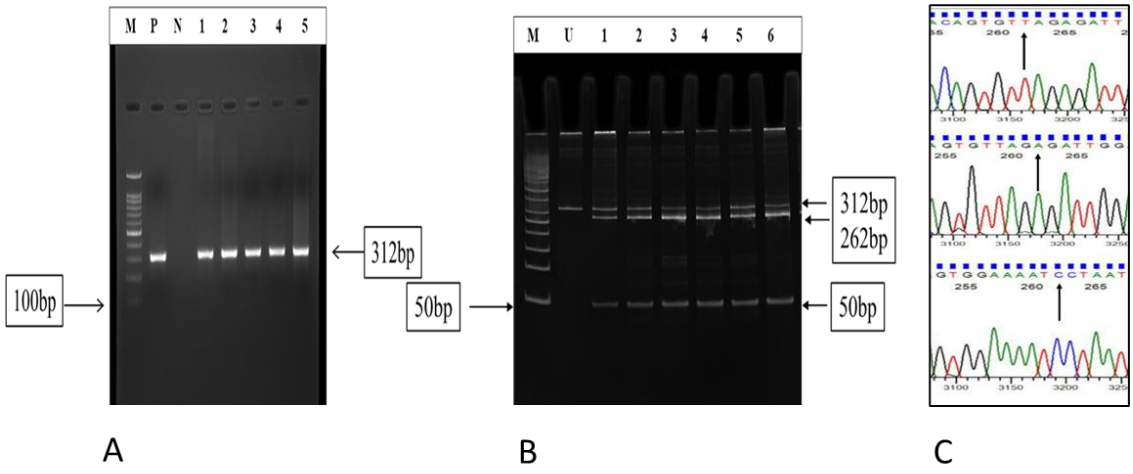


Figure 4.22: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs11248416); M= marker; P= positive control; N= negative control; U= undigested product.

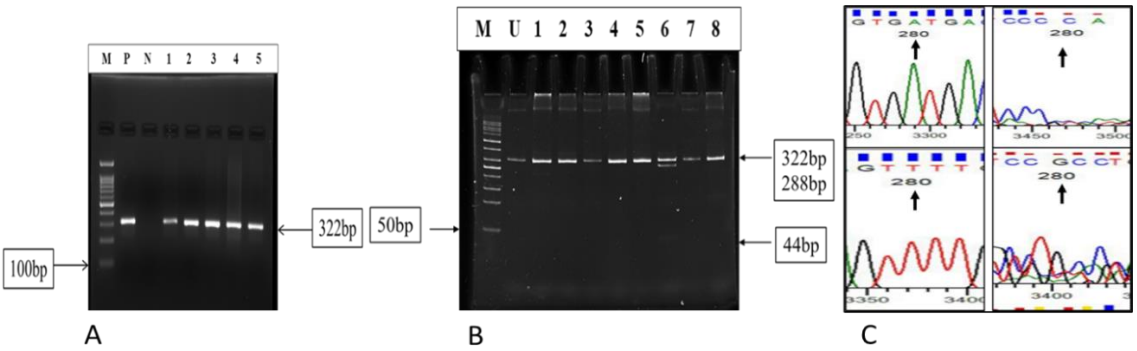


Figure 4.23: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs11248419); M= marker; P= positive control; N= negative control; U= undigested product.

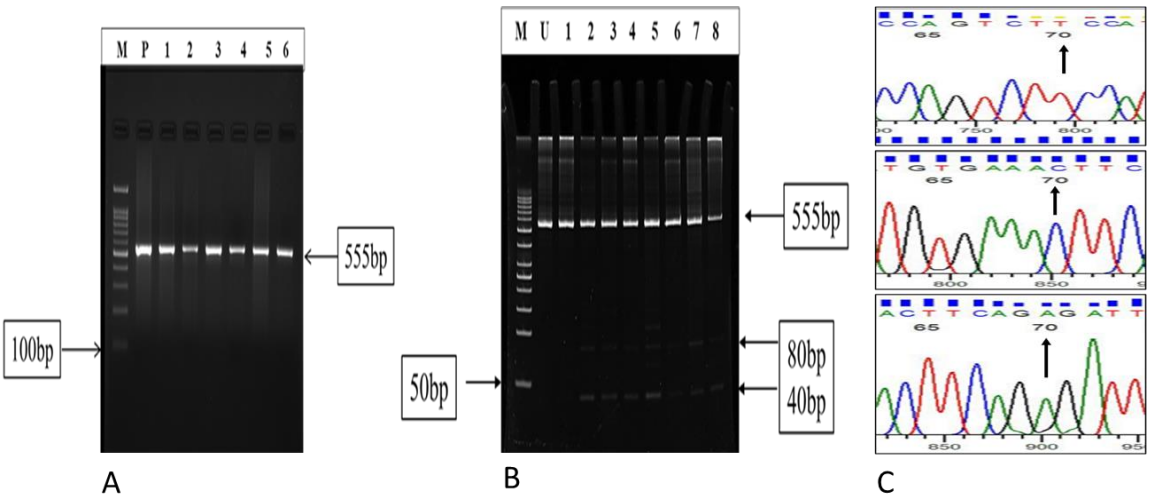


Figure 4.24: A: PCR amplification; B: RFLP of PCR products and C: Sequencing of BUB3 (rs6599657); M= marker; P= positive control; N= negative control; U= undigested product.

Table 4.16: Distribution of alleles in the ovarian cancer patients observed by RFLP

SNPs (N=80)	Variant	Percentage
MAD1L1(rs1801368)	GA or GC	0
	GG	39%
	AA or CC	61%
MAD2L1(rs1972014)	AG or AT	86.7%
	GG or TT	13.33%
	AA	0
MAD2L1(rs1546120)	AA or TT	0
	AC or AG	100%
	CC or GG	0
MAD2L1(rs3752830)	AG	0
	AT or TT	100%
	CT or CC	0
MAD1L1 (rs121908981)	GG	0
	CC	100%
	GC	0
BUB1 (rs121909055)	GG	0
	TT	100%
	GT	0
BUB1B (rs28989181)	CC	10.1%
	CT	24.05%
	TT	65.8%
BUB1B (rs28989186)	CC	0
	AC or AG	93.58%
	TT	6.4%
BUB3 (rs11248416)	AA	1.5%
	CT	50.7%
	GG or CG	3%
	AC	44.8%

SNPs (N=80)	Variant	Percentage
BUB3 (rs11248419)	GT or AG	2%
	GG	20%
	CC or AA	78%
BUB3 (rs6599657)	AG or GG	1.8%
	CT	24.6%
	AT or TT	73.7%

Table 4.17: Association of polymorphisms with clinical response, survival outcome and risk to survival

Polymorphisms	Association with Clinical response *	Log Rank test	Hazard Ratio (HR, 95% CI)
MAD1L1(rs1801368)	0.104	0.403	0.21 (0.00-2.91)
MAD2L1(rs1972014)	0.9	0.420	2.9 (0.00-7.59)
BUB1B(rs28989181)	0.021	0.065	9.9 (1.1-82.96)
BUB1B(rs28989186)	0.171	0.694	0.882 (0.063-7.325)
BUB3(rs11248416)	0.465	0.706	0.695 (0.315-1.534)
BUB3(rs11248419)	0.495	0.474	0.033 (0.0-2.09)
BUB3(rs6599657)	0.416	0.847	0.804 (0.178-3.808)
*Due to presence of only single type of polymorphism in all cases of MAD2L1(rs1546120), MAD2L1(rs3752830), MAD1L1 (rs121908981), and BUB1 (rs121909055) the statistics could not be computed.			

4.5.1. Association of MAD1 and MAD2 SNPs with toxicities

Anemia (p=0.004), vomiting (p=0.022), anxiety (p=0.009), neuropathy(p=0.020), weight loss (p=0.005), diarrhoea (p=0.003), constipation (p=0.012), indigestion (p=0.003), abdominal pain (p=0.009), and renal toxicity (p=0.013) was significantly associated with presence of MAD2L1 (rs1972014). No significant correlation was found to be associated with the polymorphism and clinical response (p=0.9).

4.5.2. Association of BUB1, BUB1b and BUB3 SNPs with toxicities

BUB1B (rs28989186) was significantly associated with anemia (p=0.014), anxiety/depression (p=0.015), and diarrhoea (p=0.000). BUB3 (rs11248416) was

associated with weight loss (p=0.000), diarrhoea (p=0.000) and constipation (p=0.05). BUB3 (rs11248419) was significantly associated with anemia (p=0.000), anxiety/depression (p=0.009), weight loss (p=0.056), constipation (p=0.000), indigestion (p=0.000) and abdominal pain (p=0.000).

Table 4.18: Association of MAD1L1(rs1801368) with toxicities

Parameters	**MAD1L1(rs1801368)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	AA or CC	7	0	0.568
		GG	4	1	
	Vomiting (n=26)	AA or CC	2	1	0.279
		GG	0	0	
	Anxiety/Depression(n=37)	AA or CC	1	1	0.677
		GG	1	0	
	Neuropathy(n=43)	AA or CC	2	1	0.635
		GG	1	0	
	Weight Loss(n=30)	AA or CC	0	1	0.343
		GG	1	0	
	Diarrhoea(n=35)	AA or CC	1	2	0.450
		GG	1	0	
	Constipation(n=40)	AA or CC	1	3	0.408
		GG	2	0	
	Indigestion(n=47)	AA or CC	3	0	0.863
		GG	2	0	
	Abdominal pain/swelling (n=33)	AA or CC	3	0	0.863
		GG	3	0	
	Renal Toxicity(n=25)	AA or CC	1	0	0.250
		GG	3	0	
*Association based using Goodman and Kruskal tau test					

Table 4.19: Association of MAD2L1(rs1972014) with toxicities

Parameters	**MAD2L1(rs1972014)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	AG or AT	17	6	**0.004**
		GG	6	3	
	Vomiting (n=26)	AG or AT	9	2	**0.022**
		GG	6	0	
	Anxiety/Depression(n=37)	AG or AT	13	3	**0.009**
		GG	0	0	
	Neuropathy(n=43)	AG or AT	17	1	**0.020**
		GG	1	0	
	Weight Loss(n=30)	AG or AT	12	6	**0.005**
		GG	4	0	
	Diarrhoea(n=35)	AG or AT	9	2	**0.003**
		GG	0	0	
	Constipation(n=40)	AG or AT	14	3	**0.012**
		GG	2	1	
	Indigestion(n=47)	AG or AT	12	6	**0.003**
		GG	4	0	
	Abdominal pain/swelling (n=33)	AG or AT	9	2	**0.009**
		GG	0	0	
	Renal Toxicity(n=25)	AG or AT	2	0	**0.013**
		GG	0	0	
*Association based using Chi-square approximation and Goodman and Kruskal tau test					

Table 4.20: Association of BUB1B (rs28989181) with toxicities

Parameters	**BUB1B (rs28989181)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	CC	6	0	0.67
		TT	27	3	
		CT or AT	9	2	
	Vomiting (n=26)	CC	2	0	0.432
		TT	7	1	
		CT or AT	4	0	
	Anxiety/Depression(n=37)	CC	3	0	0.240
		TT	10	6	
		CT or AT	9	0	

Parameters	**BUB1B (rs28989181)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
	Neuropathy(n=43)	CC	5	0	0.185
		TT	17	2	
		CT or AT	5	0	
	Weight Loss(n=30)	CC	3	0	0.741
		TT	14	0	
		CT or AT	6	0	
	Diarrhoea(n=35)	CC	1	1	0.682
		TT	12	5	
		CT or AT	4	0	
	Constipation(n=40)	CC	2	1	0.986
		TT	13	4	
		CT or AT	7	2	
	Indigestion(n=47)	CC	3	1	0.676
		TT	23	3	
		CT or AT	6	2	
	Abdominal pain/swelling (n=33)	CC	3	0	0.649
		TT	17	1	
		CT or AT	3	1	
	Renal Toxicity(n=25)	CC	1	1	0.100
		TT	14	0	
		CT or AT	5	0	
*Association based using Goodman and Kruskal tau test					

Table 4.21: Association of BUB1B (rs28989186) with toxicities

Parameters	**BUB1B (rs28989186)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	AC	39	4	0.014
		AG	0	0	
		TT	2	1	

Parameters	**BUB1B (rs28989186)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
	Vomiting (n=26)	AC	12	1	0.210
		AG	1	0	
		TT	1	0	
	Anxiety/Depression(n=37)	AC	20	4	0.015
		AG	1	0	
		TT	1	2	
	Neuropathy(n=43)	AC	26	1	0.151
		AG	1	0	
		TT	1	1	
	Weight Loss(n=30)	AC	23	0	0.350
		AG	0	0	
		TT	2	0	
	Diarrhoea(n=35)	AC	14	4	0.000
		AG	0	0	
		TT	2	1	
	Constipation(n=40)	AC	19	5	0.345
		AG	0	0	
		TT	1	2	
	Indigestion(n=47)	AC	28	4	0.138
		AG	0	0	
		TT	2	2	
	Abdominal pain/swelling (n=33)	AC	20	1	0.124
		AG	0	0	
		TT	2	1	
	Renal Toxicity(n=25)	AC	18	1	0.334
		AG	0	0	
		TT	3	0	
Based on Chi-Square approximation using Goodman and Kruskal tau test					

Table 4.22: Association of BUB3 (rs11248416) with toxicities

Parameters	**BUB3 (rs11248416)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	AA	1	0	0.455
		CT	17	2	
		GG or CG	1	0	
		AC	17	2	
	Vomiting (n=26)	AA	0	0	0.776
		CT	7	1	
		GG or CG	0	0	
		AC	6	0	
	Anxiety/Depression(n=37)	AA	0	0	0.402
		CT	10	5	
		GG or CG	0	0	
		AC	11	0	
	Neuropathy(n=43)	AA	0	0	.669
		CT	11	1	
		GG or CG	0	0	
		AC	12	0	
	Weight Loss(n=30)	AA	1	0	**0.000**
		CT	10	1	
		GG or CG	0	0	
		AC	7	0	
	Diarrhoea(n=35)	AA	1	0	**0.000**
		CT	10	2	
		GG or CG	0	0	
		AC	5	2	
	Constipation(n=40)	AA	1	**0**	**0.05**
		CT	13	2	
		GG or CG	0	0	
		AC	22	6	

Parameters	**BUB3 (rs11248416)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
	Indigestion(n=47)	AA	0	0	0.191
		CT	19	2	
		GG or CG	0	0	
		AC	10	2	
	Abdominal pain/swelling (n=33)	AA	0	0	0.662
		CT	12	1	
		GG or CG	0	0	
		AC	8	1	
	Renal Toxicity(n=25)	AA	0	0	0.608
		CT	9	0	
		GG or CG	0	0	
		AC	8	1	
*Association based using Goodman and Kruskal tau test					

Table 4.23: Association of BUB3 (rs11248419) with toxicities

Parameters	**BUB3 (rs11248419)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	GT or AG	0	0	**0.000**
		GG	5	0	
		CC or AA	21	4	
	Vomiting (n=26)	GT or AG	0	0	0.359
		GG	2	1	
		CC or AA	8	0	
	Anxiety/Depression(n=37)	GT or AG	0	1	**0.009**
		GG	4	0	
		CC or AA	12	2	
	Neuropathy(n=43)	GT or AG	1	0	0.763
		GG	3	0	
		CC or AA	14	1	

Parameters	**BUB3 (rs11248419)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
	Weight Loss(n=30)	GT or AG	1	0	**0.056**
		GG	6	0	
		CC or AA	11	0	
	Diarrhoea(n=35)	GT or AG	1	0	0.620
		GG	3	0	
		CC or AA	7	4	
	Constipation(n=40)	GT or AG	0	1	**0.000**
		GG	3	0	
		CC or AA	13	3	
	Indigestion(n=47)	GT or AG	0	1	**0.000**
		GG	4	0	
		CC or AA	15	0	
	Abdominal pain/swelling (n=33)	GT or AG	0	1	**0.000**
		GG	0	0	
		CC or AA	11	0	
	Renal Toxicity(n=25)	GT or AG	1	0	0.635
		GG	10	0	
		CC or AA	8	0	
*Association based using Goodman and Kruskal tau test					

Table 4.24: Association of BUB3 (rs6599657) with toxicities

Parameters	**BUB3 (rs6599657)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
Toxicities	Anemia (n= 65)	AG or GG	0	0	0.801
		CT	7	1	
		AT or TT	21	4	
	Vomiting (n=26)	AG or GG	0	0	0.809
		CT	3	0	
		AT or TT	7	2	

Parameters	**BUB3 (rs6599657)**		**Grades of toxicities**		***p-value****
			1-2	**3-4**	
	Anxiety/Depression(n=37)	AG or GG	0	0	0.563
		CT	4	1	
		AT or TT	15	4	
	Neuropathy(n=43)	AG or GG	0	0	0.181
		CT	4	0	
		AT or TT	17	2	
	Weight Loss(n=30)	AG or GG	0	0	0.482
		CT	4	0	
		AT or TT	14	0	
	Diarrhoea(n=35)	AG or GG	0	0	0.660
		CT	3	1	
		AT or TT	10	3	
	Constipation(n=40)	AG or GG	0	0	0.334
		CT	7	0	
		AT or TT	9	5	
	Indigestion(n=47)	AG or GG	0	0	0.239
		CT	7	0	
		AT or TT	13	0	
	Abdominal pain/swelling (n=33)	AG or GG	0	0	0.762
		CT	4	0	
		AT or TT	10	0	
	Renal Toxicity(n=25)	AG or GG	1	0	0.520
		CT	2	0	
		AT or TT	8	0	
*Association based using Goodman and Kruskal tau test					

CHAPTER 5

DISCUSSION

In the management of primary cases of advanced ovarian cancer, carboplatin and paclitaxel chemotherapy, along with cytoreductive surgery, are undisputed throughout the world. This study describes the sociodemographic, gynaecological background and clinical characteristics of the Indian patient population and reports the clinical outcomes of their advanced stage ovarian malignancy with respect to spindle assembly checkpoint genes/proteins.

The data showed a mean age of 49·15±10·8 years with majority of serous histological subtype (81%) in the OC patients. Similar mean age of 50-53 years was observed in Delhi population of OC (Khandakar et al., 2015, Dash et al., 2016). However, the age range of 53-61·5 years was seen in different studies from USA, Israel and Germany (Aghajanian et al., 2012, Brotto et al., 2016, Bian et al., 2016, Bruchim et al., 2016) and serous histological subtype was reported in tumors ranging from 65 % to 92% (Brotto et al., 2016, Bruchim et al., 2016, Dash et al., 2016).

As per our observation, mean menopausal age was 44·27 years that poses a risk to survival (HR=1·076) and is less than the average menopausal age (46 years) of Indian (Prasad et al., 2021; Ahuja, 2016) and western population (51 years) (Tao et al., 2015, Women's Health, 2020). One patient of age 60 years had primary amenorrhea with only secondary sexual characteristics expressed. She was diagnosed with Stage IIIC serous papillary carcinoma with a baseline CA125 > 5000 U/ml were noted. The lowest age of first pregnancy was observed to be 15 years and highest was 37 years. The highest parity observed was 8 in one patient. Even though pregnancy had been found to have protective roles in developing ovarian carcinoma (Del et al., 2018; Han et al., 2013), 59·09% women in this study had more than one pregnancy.

There were 3 cases of primary peritoneal cancers who were all partial responders. In the present study the ORR is reported to be 77.27% in contrast to another single institutional experience with Carboplatin and nab-paclitaxel where the ORR was 100% (Parisi et al., 2019). The current study did not reach the median overall survival with 2 years. The median progression free survival was 6 months in this study however, phase 3 trials

NCT01802749 and EudraCT 2012-004362-17 reported median PFS of standard chemotherapy was 8.8 months (Pignata et al., 2021). The MITO-7 trial reports a median PFS of 17.5 months. This variation is may be due the inclusion of Stage IC-IV patients, whereas in the present study only advanced stages (III-IV) were included for the analysis (Pignata et al. 2014). Even with several comparative trials combining targeted therapy and standard tri-weekly regimen, dose-dense regimen or single-agent treatment, the standard chemotherapy was still found superior or at par with other combinational chemotherapies even in vulnerable geriatric patients (Falandry et al., 2021). The most common causes of deaths were cardiac arrest, deteriorating health owing to several toxicities.

The standard regimen precipitated Grade 3/4 toxicities in anemia (8·1%), anxiety/depression (5·4%), diarrhoea (5·4%) and constipation (8·1%). In contrast the study by Huang et al., 2020 reported higher frequencies of grade 3/4 anemia (22·7%), neutropenia (77·3%), thrombocytopenia (13·6%). The lesser frequencies in the present study may be due to the use of prophylactic GCSF (Granulocyte colony stimulating factor) which prevented the grade 3/4 toxicities. Peripheral neuropathy was observed in 39·09% patients in the present study in comparison to 36·4% and 25·7% observed in the recent studies from Taiwan and UK (Huang et al., 2020; Blagden et al., 2020). In comparison with ICON8 trial to our study the occurrence of adverse drug reactions are as follows: anemia (66% vs 59%), leukopenia (45% vs 14%), nausea (63% vs 16·3%), vomiting (33% vs 23·6%), weight loss (13% vs 27·2%), diarrhoea (35% vs 31·7%), constipation (69% vs 36·2%), renal toxicity (12% vs 22·7%) (Clamp et al., 2019) and in comparison with TRINOVA-3/ENGOT-ov2/GOG3001 trial the toxicities are anemia [44% vs 59%], leucopenia [24% vs 14%], thrombocytopenia [24% vs 5·45%], nausea [63% vs 16·3%], vomiting [35% vs 23·6%], peripheral neuropathy [34% vs 39·06], diarrhoea[36% vs 31·7%], constipation [42% vs 36·2%] and abdominal pain [36% vs 29·6%] (Vergote et al., 2019). From ICON8 similar observations were noted in cases of anemia and diarrhoea; but contrasting evidences were noted in nausea, vomiting, weight loss, constipation and renal toxicity. However, in TRINOVA-3/ENGOT-ov2/GOG3001 nearer values were noted in peripheral neuropathy, diarrhoea, constipation, abdominal pain and contrasting evidences were noted for anemia, leucopenia, thrombocytopenia, nausea and vomiting.

Pain is one of the most distressing and persistent symptoms in the ovarian cancer patients who were assessed for different kinds of nociception throughout the first six months of their treatment and the summary of results demonstrate that that there was no improvement of pain from diagnosis and after completion of 6 cycles of chemotherapy.

Consistent with the study by Ferell et al., 2003, the persistent abdominal pain (60, 54.54%) and swelling was one of the most prevalent and discomforting symptoms that lead the patients in the present study to seek medical attention. In this study, 57 (51.8%), 49 (44.5%) and 44 (40%) patients reported resting, movement, and sleep-associated pain at the time of diagnosis and study entry. Upon follow up, after six cycles of chemotherapy the frequencies of resting and sleep-associated pain remained same but movement associated pain increased by 6.4% to 56 (50.9%) patients. Similar results were observed in the study by Portenoy et al, 1994 that reported 60% advanced stage ovarian cancer patients experiencing pain at disease onset. There are strong reports of sleep disturbances linked to depression/anxiety in ovarian cancer patients (Clevenger 2013; Donovan et al., 2016). In the present study, we were unable to assess the pain associated depression/ anxiety but the population represented significant percentages of sleep-associated pain (40%) and depression/anxiety (33.6%) that needed interventions to manage.

The movement associated pain was significantly associated with deteriorating physical and functional wellbeing of the patients. This observation was similar to the study by Nho et al., 2017 that reports about pain and CIPN symptom clusters to negatively impact general quality of life. The neuropathic pain was reported by patients after receiving chemotherapy and was mostly felt in the extremities (fingers, toes and legs). Burning, pressing, cold sensation, pins/needles and tingling were the most reported CIPN in the patient population. These symptoms were persistent till throughout the study and did not have any significant correlation with the treatment outcomes. There was lack of quality-of-life improvement observed in the study that is like the outcome reported by Magnowoska et al., 2018. In contrast, they reported gabapentin to provide benefit for CIPN that was not observed in our study even with gabapentin, or other drugs.

The quality of life of Indian OC patients receiving front-line chemotherapy and debulking surgeries has been assessed in this study.

The increment of spiritual factor score is indicative of better spiritual well-being. This section had questions about mental peacefulness, productivity, purpose, comfort, harmony, faith and spiritual faith/beliefs of life. The mean spiritual well-being scores of ovarian cancer patients in this study improved slightly (non-significant) except in the non-responder's category. On a scale of 0-40, the mean scores were between 12-21. In this study the patients had low to moderate spirituality as seen in another study by Davis, that life events paired with a decrease in peace experienced the worst psychological outcomes at one-year (Daviz LZ et al., 2018).

Spirituality is associated with emotions, and the mean scores of emotional well-being were seen to decline (non-significantly) in the partial responders and non-responders except responders. Another study by Ferell et al., 2003, analyzed natural correspondence of ovarian cancer patients where they reported the positive and negative effects of disease and shared their coping mechanisms with their loved ones. In our study the patients responded to the questionnaire with a positive attitude and some of them expressed their acceptance of the disease and its outcome with realistic expectations. Overall, the emotional well-being was on the better side, considering the mean score in the range of 8-11out the maximum score of 24. The increment in emotional factor scores is indicative of QoL's detriment.

Emotional and social well-being is essential for overall health. In this study, on a scale of 0-20, the mean scores ranged from approx 10 – 13 indicating moderate social well-being of the patients with family and friends. Most of them had their husbands or children or both being the caregivers and the rest depended on family/friends/others. Majority were unemployed, mainly home-makers. There were several instances reported of husbands abandoning them for various reasons including financial and social burden/stigma. These incidents left the patients with sadness and socially burdened. 3 women were dependent on crowd-funding to afford the treatment. The patients were never fully satisfied with their communication with caregivers/family and sometimes preferred not to answer the questionnaire. Hill et al., 2016 reported that social support seeking was a coping mechanism and therefore, is an important consideration in QoL and mental health of ovarian cancer patients (Hill et al., 2016). Meraner et al., 2012 found a statistically significant decrease in anxiety and depressive symptoms and improved social life (Meraner et al., 2012).

Functional well-being comprises the patients' ability to work, enjoy work and life, accept illness, sleep, and impression of current quality of life. Higher mean scores indicate higher QoL and vice versa. On a scale of 0-28, the mean scores range from approx. 6-8 that indicates poor functional well-being. There was no change observed in the QoL scores from baseline to 6 months. The poor functional ability can be caused by high tumor burden at baseline and side effects of chemotherapy during the 6 cycles. Only 20 patients reported improvement of their QoL on asking and others reported no change.

The ovarian cancer and its treatment can cause significant physical and psychological morbidity Lowe et al., 2007. At baseline 38 patients reported no lack of energy, no nausea, no trouble meeting family needs and no pain in the physical well-being questionnaire and after 6 months there were 21 similar patients. The overall mean score of physical well-being indicates a moderate to good performance throughout the 6 months. Contradictorily, von Gruenigen et al.2010, reported post operative physical well-being to improve after 6 months.

Disease-specific concerns also play a crucial role in determining the overall well-being of the patients. The additional concerns included query about stomach swelling, weight loss/gain, control of bowels, vomiting, alopecia, appetite, appearance of body, cramps, sexual interests and concerns about child-bearing. Most patients in the study reported belly swelling after treatment, moderate control of bowels, and less vomiting. However, 100% of them were bothered by the hair loss and were concerned about future hair growth, did not fully accept their appearance, had less appetite and fewer libidos. The younger patients (25-35 years) were extremely worried about their sexual life and womanhood after undergoing TAH+BSO.

The drug-related toxicities are also important determinants of QoL. In our study, anemia was the most observed toxicity related to fatigue, dyspnea, dizziness, activity intolerance, headaches, concentration difficulties, and sleep disturbance (Holzner et al., 2002). The high incidence of diarrhoea and constipation seen in this study negatively affects the patients' daily lives and can be attributed to the poor functional well-being. It is very common for patients with advanced ovarian cancer to go through repeated chemotherapeutic treatments due to disease relapse. Sun et al., 2007 reported a good quality life for complete responders compared to partial responders and progressive disease which was contradictory to our study.

Despite the similarity in the financial background the patient population had high heterogeneity in the socio-cultural/ spiritual factors that may have a hidden confounding factor affecting the survival outcome. Clinically, it is seen the survival benefit of patients in the adjuvant chemotherapy group was inferior to the neoadjuvant chemotherapy group. This can be attributed to the complications arising from the sudden removal of bulky tumors (≥ 20cm) and immediate start of chemotherapy, toxicity and pre-existing comorbidity which were not taken into consideration and is a limitation to our study.

After molecular evaluation the study reported very low MAD1 protein and mRNA expression in ovarian cancer tissues which does not carry any significance to treatment outcome or survival. Han et al., 2000 also reported reduced expression of MAD1 in poorly differentiated ductal invasive breast carcinoma that was a significant factor in predicting recurrence but not overall survival. As we correlated some miRs to associate the expression of the SAC proteins, we analyzed the miR-125b in MAD1 deficient tissues given their established negative correlation in human head and neck cancer tissues (Bhattacharya et al., 2013). But, in this present research we were unable to establish this correlation as miR-125b was also very poorly expressed. Luo et al., 2015 reported similar results where they found that miR-125b had significantly ($p<0.01$) reduced expression in human ovarian neoplastic tissue than normal ovarian tissues. Also, they reported that miR-125b may have tumor suppressive function in ovarian cancer (Lin et al., 2011; Liu et al., 2011). Similarly high MAD1 expression was correlated with high proliferative index in primary gastric cancer tissues (Han et al., 1999). These results bring us to the inference that most of the ovarian cancers in our study had lost the ability to suppress the tumor proliferation through MAD1 and miR-125b mediated pathways and they have different relationship/functions in different cancers despite the epithelial origins. MAD1 positivity was significantly associated with some drug related toxicities that include weight loss ($p=0.013$), constipation ($p=0.05$), and nearly significant with indigestion ($p=0.064$).

The next SAC component analyzed was MAD2, which has been the most researched protein of this pathway. From several studies it is known that MAD2 is a protein of contradiction. MAD2 has been associated with poor survival and highly proliferative cancers (Bargiela-Iparraguirre et al., 2016; Mcgrogan et al., 2014). Not only tumor aggressiveness, MAD2 is extensively studied and reported to impact in neoplastic

transformation from preneoplastic lesions like leukoplakia (Rizzardi et al., 2014; Monteiro et al., 2021). In the present study, majority of the advanced tumors were MAD2 positive. MAD2 mRNA expression was significantly associated with overall survival (p=0.001). High MAD2 was associated with shorter overall survival among patients but greater PFS (r= 0.399, p>0.05). Similar prognosis of positive correlation with PFS was reported by (Park et al., 2013). High expression of MAD2 is also correlated with poor prognosis in urothelial bladder cancer (Choi et al., 2013). However, the risk of death was relatively low with MAD2 i.e., HR=0.870, (95% CI= 0.187-4.036) which is also implied in the study by Byrne et al., 2017 where the risk of all-cause mortality was decreased in ovarian cancer patients (pooled HR = 0.50, 95% CI, 0.25-0.97; p = 0.04, n = 3). With innumerable reports of MAD2 being a prognostic biomarker, research on its association with chemotherapy outcomes remains modest. However, the present study fails to show such significant association and neither the expression was correlated with drug induced side effects. Again, in order to understand the SAC signaling status CDC20 mRNA expression was analyzed. MAD2 and CDC20 had significant and strong positive correlation, r=0.755; p=0.001 in the cancer tissues. It has previously been reported that MAD2 and CDC20 alone can activate a SAC signal (London and Biggins, 2014) in cells however it was outside the scope of our objective to have conducted that experiment. So far, we can only assume that in absence of MAD1 and BUB1, MAD2 and CDC20 were able to activate the mitotic checkpoint in presence of paclitaxel and carboplatin. miR-143 and MAD2 had a positive correlation and independently miR-143 was associated with good prognosis of survival (p=0.003).

Budding Uninhibited by Benzimidazoles (BUB) are a group of genes that are of central importance in mitotic checkpoint activation. BUB1 recruitment at the kinetochore is an important prerequisite step in the formation of APC/C inhibitor, tetrameric mitotic checkpoint complex (MCC). In the present study, we found absence of this protein in most of the primary tumor tissues and due to such downregulation of BUB1 statistical correlation with treatment response did not attain significance. But presence of low BUB1 had greater risk to survival i.e. OR= 1.79 (95%CI= 0.16-19.77). Suppression of BUB1 expression was an indicator of poor prognosis in gastric adenocarcinoma with relation to tumor, and extent of metastases (Stahl et al., 2017). Contradictory to these results, *in silico* analysis of early stage (I and II) ovarian cancer samples reported BUB1 positive expression had poor prognosis (Ocana et al., 2016).

The meagre amount of BUB1 immunopositive cases had very low diffused expression in the cytoplasms, hardly any case was found with nuclear expression. There was significant difference of expression (p=0.010) in tumors of the interval debulking surgery that received 3 cycles of chemotherapy. This result may be plausible because interval debulking surgery is performed in those whose cancer cell burden was reduced (i.e. response) after the first 3-4 cycles of chemotherapy, so much so that the ovaries have attained their normal sizes and those ovarian tissue starts expressing BUB1 to reverse the pathological characteristics. Obviously, this finding must be further validated in a larger cohort. Similar observation of MAD2 expression significantly lower in neoadjuvant chemotherapy samples can be explained as the pathological features are responding to the treatment and high expression of MAD2 is coming to the desired amount required for the physiological activities. miR-495 was found to have a strong negative correlation with BUB1 expression (r= -0.551, p=0.16). This data was supported by the evidence that reported increased upregulation of BUB1 with remarkably low expression of miR-495-3p in retinoblastoma tissues (Zhou et al., 2021). Independently, miR-495 was significantly associated with OS (p=0.007) as increased expression prolonged survival time. Previously, Zou et al., 2017 reported miR-495 increased the sensitivity of Taxol in resistant ovarian and gastric cancer cells. The nexus of BUB1 and miR-495 may be further elucidated *in vitro.*

BUB3, another humble character in the mitotic checkpoint complex which has many other related activities being explored. Inhibition of BUB3 was found to be associated with increased chemosensitivity in oral cancer (Silva et al, 2019). Even though the present study did not have significant difference with BUB3 expression in the responders, partial and non-responders; it poses significant risk to death, i.e., OR= 1.93, 95%CI= 0.413-9.054. Presence and absence of BUB3 expression in cytoplasm and nucleus within a tissue shows intra tumor heterogeneity. Along with expression of the proteins, presence of single nucleotide proteins was also assessed. MAD1L1(rs1801368; G → A) is a missense variant, coding sequence variant in the nucleotide 1673 that results in a replacement of Arg by a His at codon 558 that modifies the amino acid sequence in the second leucine zipper of MAD1. It was previously reported to affect SAC efficiency and worse outcome to induction chemotherapy in ovarian cancer (Santibanez et al., 2013). However, no such association was found in our study as it was not significantly associated with clinical response (p=0.104) and overall survival (p=0.403) with lower

risk of death (HR=0.21). From the sequencing data polymorphic AA or CC was found in 61% cases whereas wild-type GG was found in 39% cases, but polymorphic heterozygous GA was not observed in the Indian patient cohort. Presence of the C polymorphic type is not reported till date. However, clinical utility of this SNP was not clearly understood as it was also not related to any chemo-induced toxicity. MAD1L1 (rs121908981; G → C) another stop gained mutation that leads to a premature polypeptide was found to have pathogenic role in lymphoma. In the ovarian cancer cohort however, the RFLP results found that CC genotype was most prevalent which may imply its significance in disease predisposition but not in treatment outcomes. MAD2L1(rs1972014; A/G) is an intronic SNP however, it was significantly associated with all the chemo-induced toxicities ($p<0.05$). The polymorphic heterozygous (AG) allele of MAD2 (rs1972014) was most frequent (83%) and homozygous GG (17%) were observed but homozygous AA was not found in the patient cohort. Clinical response was not associated but carrier of the polymorphic genotype had significant risk to death HR=2.9 (95% CI 0.00-7.59). Previously no other study reported about this SNP. MAD2L1(rs1546120) is also an intron variant that was not found mutated in the patient cohort. Only the non-polymorphic AA genotype was visible. MAD2L1 (rs3752830) is also an intron variant with no polymorphic changes found throughout the patient cohort and does not carry any risk to treatment outcomes. The homozygous polymorphic TT genotype was found in BUB1 (rs121909055) in all the patient cohort further implicating its role in tumorigenesis as is previously reported in colorectal cancer (Cahill et al., 1998) however, it did not have clinical significance with treatment outcome and statistical correlation could not be computed with presence of only one genotype. BUB1B (rs28989181) is previously reported to precipitate constitutional aneuploidy and cancer development by Hanks et al., 2004 and highly deleterious (Akhoundi et al., 2016). In our study, this variant with the heterozygous and homozygous CT and TT polymorphic genotypes were found to be associated with nearly significant survival outcome among the responder categories ($p=0.06$) but poses high risk to survival [HR= 9.9 (95% CI-1.1-82.96)] and poor clinical response. ($p=0.021$). The SNP is a missense variant that replaces a Leucine to Phenylalanine in BUBR1 protein. It remains to be further validated whether the mutant protein creates any changes to the activities; as of now polymorphic genotypes exhibited more toxicity burden but did not have statistically significant value. Similarly, the novel BUB1B (rs28989186) identified and previously reported to be pathogenic variant (Hanks et al., 2004). In a different collaborative work, we have found it to be

highly deleterious (manuscript communicated). This stop-gained variant translates a smaller protein. The produces lesser toxicities probably because the shorter protein has lesser crosstalk with other signalling pathway. To support the hypothesis, we have observed lesser burden of toxicities in mutation carrier. Significant toxicities observed with this SNP were anemia, anxiety/depression, and diarrhoea.

BUB3 (rs11248416) associated with weight loss, diarrhoea, and constipation and BUB3 (rs11248419) was significantly associated with weight loss, anxiety/depression, constipation, indigestion, and abdominal pain ($p<0.05$). Such data is previously not reported and indicates crosstalk of BUB3 with different pathways. Another study used FACS analysis to show that paclitaxel treatment after BUB3 siRNA transfection did not cause cell cycle arrest (G2/M). Therefore, we suggest that G2/M arrest by paclitaxel treatment may relate with increased BUB3 level, which is required for cell cycle arrest. Furthermore, BUB3 was required for cell cycle arrest in response to loss of microtubule function (Lee et al., 2005). The study did not find association with BUB3 and miR-659 expression.

The spindle assembly checkpoint (SAC) signaling cascade has long been dismissed as a straightforward process with unidirectional effects. However, it was discovered from the variety of study findings that mitotic checkpoint components have roles both inside and outside of mitosis. Additionally, it has been shown that an ineffective SAC might result in undesirable outcomes such cancer, drug resistance, aneuploidy, and developmental abnormalities. The molecular mechanism of SAC and how it interacts with other pathways are still not fully understood. A defective SAC does not appear to be a strong enough indicator of the development of cancer, but the impact of mutations and protein expression on the treatment outcomes cannot be completely ruled out. The limitation of our study is the small sample size, single centre, non-randomized study and hence it needs to be further validated with a larger population. The study also failed to analyze the effect on mitotic arrest with the absence and presence of the SAC proteins and associated single nucleotide polymorphisms.

CHAPTER 6

CONCLUSION

Incidences of ovarian malignancies are on the rise and with the lack of effective screening methods or policy, the disease is diagnosed at the widely metastatic stages. Treatment at the advanced stages comes with a burden of multiple surgeries, several cycles of chemotherapy and with the constant risk of disease recurrence. Hence, it is of utmost importance to understand the heterogeneous tumor biology and identify biomarkers for treatment success in order to enhance the therapy experience thereby improving the quality of life.

The conclusions drawn from the present study are as follows,

1. Indian women diagnosed with ovarian cancer have an overall response rate of 77.27% to first-line paclitaxel-carboplatin chemotherapy. The responders, partial and non-responders have significantly different overall survival outcome ($p<0.05$) and PFS of 6 months.

2. Even though the chemotherapy and cytoreductive surgeries reduces the cancer burden initially, it precipitates several Grade 3 & 4 toxicities that adds to the distress and lowers the QOL of the patients. Anemia, leukopenia, granulocytopenia, weight loss, nausea, indigestion, anxiety, diarrhoea, and constipation are significantly associated with lowered functional, physical, and emotional wellbeing.

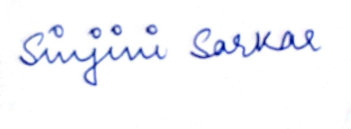
3. Several types of pain are associated with the disease as well as the treatment which was found to be persistent throughout the follow up. Rescue analgesia provided temporary relief. Significant association of movement-related pain with non-responders also implies deterioration of functional and physical wellbeing.

4. Along with the clinical evidences we have analyzed the spindle assembly checkpoint proteins MAD1, MAD2, BUB1 and BUB3. It was found that they were downregulated in the advanced ovarian cancer tissues. High MAD2 was found to be associated with lower survival time and can be established as a predictive biomarker. Absence of MAD1 is also a marker for poor prognosis.

BUB1 and BUB3 is associated with much higher risk for death than MAD1 and MAD2. miR-495 and miR-125b may be involved in regulation of BUB1 and MAD1 downregulation. miR-143 and miR-659 expression were not significantly correlated with the MAD2 or BUB3 but were independently found to be associated with better survival outcomes.

5. When deciding on the optimum course of treatment for cancer patients, predicting each patient's risk of chemotherapy-induced serious adverse reactions is crucial. SNPs in genes related to the pharmacodynamics of Paclitaxel and Carboplatin were identified and analyzed. BUB1B (rs28989181) was found to increase the risk of survival and implicated with worse outcome. MAD2 (rs1972014), BUB1b (rs28989181, rs28989186) and BUB3 (rs11248416, rs11248419) were found to be significantly associated with toxicities like anemia, neuropathy, weight loss, diarrhoea, constipation, and renal toxicity as recorded during the first 6 cycles of chemotherapy.

From the present study we can conclude that there is a scope for improvement in the management of Indian ovarian cancer patients in the aspect of mitigation of toxicities and enhancement of QoL. Spindle assembly checkpoint is dysregulated in the fully advanced tumor and how paclitaxel will generate a SAC signal remains a mystery but the pathological features are a strong indication of poorer outcome. Previously SAC has not been investigated as a marker for treatment success/failure. In future we intend to find the crosstalk of SAC components and evaluate their therapeutic potential in cancer treatment.



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ANNEXURE I

INSTITUTIONAL
ETHICS
COMMITTEE

Chittaranjan National Cancer Institute
An autonomous body under the Ministry of
Health & Family Welfare, Govt. Of India
37, S.P.Mukherjee Road, Kolkata-700026, WB, India
Tel: 2475-9313/8057, Fax: +91-33-2475-7606
Web: www.cnci.org.in

IEC Ref: A-4.311/VN/27/06/2018-10 Date: 12.07.2018

Dr. Vilas Nasare, CNCI

Sub.: IEC decision on review of the project, sponsored by Department of Health Research (DHR), New Delhi

Protocol Title: **Study on MAD and BUB1 genes of spindle assembly checkpoint with response to primary adjuvant chemotherapy in advanced epithelial ovarian cancer**

Study Site: Chittaranjan National Cancer Institute, Kolkata

Dear Dr. Nasare,

In its meeting held on 27th June 2018, the members of IEC, reviewed and discussed project submitted by Ms. Sinjini Sarkar under your mentorship, sponsored by DHR, New Delhi and other related documents for the proposed study entitled "Study on MAD and BUB1 genes of spindle assembly checkpoint with response to primary adjuvant chemotherapy in advanced epithelial ovarian cancer"

The following documents were reviewed:

1. Model form to be filled by the Principal Investigator (PI) for submission to Institutional Ethics Committee (IEC)
2. Project with Summary
3. Informed Consent Sheet in 3 languages
4. Administrative Approval
5. Reply to IEC quaries

The following members of the committee were present:

Prof. (Dr.) Shyamal Sarkar (Chairman)
Prof. (Dr.) Santanu Tripathi (Member)
Prof. (Dr.) Ranajit Kumar Mandal (Member)
Prof. (Dr.) Kalyan Kusum Mukherjee (Member)
Dr. Chinmay Kumar Panda (Member)
Dr. Madhumita Roy (Member)
Dr. Smarajit Pal (Member)
Ms. Sutapa Biswas (Member)
Mr. Himadrisekhar Chakraborty (Member)
Mrs. Sonali Dasgupta (Member)
Dr. Rathindranath Baral (Member-Secretary)

The IEC approves the DHR project as proposed and presented in the IEC meeting. Out of 11 members present in the meeting, 11 voted in favour and none voted against.

INSTITUTIONAL
ETHICS
COMMITTEE

Chittaranjan National Cancer Institute
An autonomous body under the Ministry of
Health & Family Welfare, Govt. Of India
37, S.P.Mukherjee Road, Kolkata-700026, WB, India
Tel: 2475-9313/8057, Fax: +91-33-2475-7606
Web: www.cnci.org.in

Ms. Sutapa Biswas,
CFI, Kolkata

Mr. Himadrisekhar
Chakraborty, LLB

Mrs. Sonali Dasgupta
'Hitaishini' (NGO)

The committee should be informed:

I. About the progress of the study annually
II. Any changes in the protocol and patient information/ informed consent documents, prior to their implementation

The Project was approved by IEC in this meeting

Final report of the study shall have to be submitted to the IEC in all cases, even when the study is abandoned for any reason(s).

Yours Sincerely,

Member Secretary 12/07/2018
IEC, CNCI

Member Secretary
Institutional Ethical Committee
Chittaranjan National Cancer Institute
Kolkata

S Sarkar 16/07/18
Chairman
IEC, CNCI

Chairman
Institutional Ethics Committee
Chittaranjan National Cancer Institute
37, S.P. Mukherjee Road, Kolkata

ANNEXURE II

Chittaranjan National Cancer Institute

37, S.P. Mukherjee Road, Kolkata -700026

Study on MAD and BUB1 genes of Spindle Assembly Checkpoint with response to Primary Adjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer

(Project funded by DHR, Young scientist Fellowship Category B)

Subject Information Sheet

Introduction:

You are being invited to take part in a research study being conducted at Chittaranjan National Cancer Institute, 37 S. P. Mukherjee Road, and Kolkata 700026. Your participation in this study is voluntary, which means you can decide whether or not you want to participate in the study. If you don't want to be a part of the study, you will not be prevented from receiving any medical care or other benefits that you are entitled to.

Before you agree to volunteer for this study, it is important that you read the following information completely and ask as many questions as necessary to the study doctor or nurse to be sure that you understand what you will be asked to do. This subject information sheet provides you with detailed information about the study.

Prior to being enrolled in the study, you will be required to personally sign and date the Subject Information Sheet and Informed Consent Form provided at the end of this document. A copy of the signed Informed Consent Form will be provided to you.

Ask your study doctors Dr. Manisha Vernekar and Dr. K.K. Mukherjee, Dr. Partha Nath of the project, if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

- To assess the clinical efficacy of cisplatin/carboplatin and paclitaxel chemotherapy in advanced epithelial ovarian cancer patients.
- To assess the immunohistochemical expressions of Mad1, Mad2, Bub1 and Bub3 in ovarian cancer patients.
- To investigate the effects of single nucleotide polymorphisms of Mad1, Mad2, Bub1 and Bub3 and their allele frequencies in response to combinational chemotherapy in ovarian carcinoma.
- Analysis of data for prediction and clinical management of epithelial ovarian carcinoma patients.

What will be happen during the study?

Before you join this study, you will be assessed against a few criteria for selection as a study participant. You will be considered for the study, if you fulfill these criteria. You may be asked questions regarding drugs treatment and quality of life. Questions may also be asked regarding adverse event using drug treatment. Once the clinician/scientist or the staff has received all the answers you will be asked to read, sign this consent form given along with date. Below is a detailed description of what will happen to you during the study.

Study Procedure:

For this study, about 500mg - 1gm of tumor will be taken from the surgical specimen of ovarian cancer and 5ml of blood will be taken from the respective patients after taking proper consent in patient consent form.

Why should I participate in the study?

You have been chosen to participate in the study because of the following reasons:

You have been diagnosed with epithelial ovarian cancer and you will be given primary adjuvant therapy consisting of Cisplatin/ Carboplatin and Paclitaxel. This chemotherapy has precipitated many adverse drug reactions. This study will focus on the quality of life of the patients undergoing the therapy. In cases of recurrent ovarian cancer, patient does not respond to primary adjuvant therapy as resistance occurs. Potential mutations in mitotic checkpoint genes may be responsible for this chemoresistance phenomenon. So this research will evaluate single nucleotide polymorphisms (SNP) and expressions of Mad1, Mad2, Bub1 and Bub3 genes by taking patient samples.

How many other people like me will be participating in the study?

The total number of ovarian cancer patients who will be participating in the study will be 77.

What are the possible risks / discomforts?

There may be a minimal risk while giving blood and biopsy and in case of any emergency the attending clinician will take care.

What are the potential benefits?

This study will help to estimate the clinical efficacy and quality of life of patients receiving primary adjuvant therapy for epithelial ovarian cancer and frequency of Mad1, Mad2, Bub1 and Bub3 gene polymorphisms and their response to paclitaxel and cisplatin/carboplatin treatment which will be helpful in determining the effective dose with lesser adverse drug reactions in ovarian cancer patients.

Compensation: You will not be charged for any tests of the study.

If I take part what are my responsibilities?

Your primary responsibilities include visiting the hospital as per treatment schedule, follow study staff instructions. You will not be allowed to take certain medications and treatments while you are participant in this study. Please check with your doctor for drugs prohibited for usage during the study. The usage of other chemotherapy agents that do not fall under standard regimen also will not be permitted during the treatment period.

Will the participation in the study be kept confidential?

Confidentiality of your medical records will be maintained to the extent permitted by law. Independent auditors and / or regulatory bodies and monitors will be able to have access to your original medical records for the verification of clinical study procedures. No information will be disclosed to anyone, other than may be required by law. Your identity will not be revealed.

What will happen to the results of the research study?

All information collected during the research study will be kept in a written report, where you will not be mentioned by name but only by your assigned code and date of birth. Your confidentiality will be protected.

Contact Details:

If you have any questions about this study or you experience a side effect, illness or injury that you believe results from this study you may contact the following study doctors:

Dr. Manisha Vernekar,
MBBS, MS(OBGY)
Dept. of Gynecologic Oncology

Dr. K. K. Mukherjee,
MBBS, MD (RT), FCCM, ECMO
Associate Prof and Head, Medical Oncology

Dr. Partha Nath,
MBBS
Chief Medical Officer
Medical Oncology

Chittaranjan National Cancer Institute
37 S. P. Mukherjee Road, Kolkata 700 026
Tel No.: +91-33-2476 5101/02/04
Fax: +91-33-2475 7606

Informed Consent Form IC No.

PROJECT TITLE**: Study on MAD and BUB1 genes of Spindle Assembly Checkpoint with response to Primary Adjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer**

Name of the participant: Sinjini Sarkar

Mentor 1: Dr. Vilas D. Nasare, Senior Scientific Officer Grade-II, Dept. of Pathology and Cancer Screening, Chittaranjan National Cancer Institute (CNCI). Kolkata

Mentor 2: Dr. Kalyan Kusum Mukherjee, MBBS, MD, FCCM, ECMO**,** Head, Medical Oncology, CNCI, Kolkata

Co-Investigator: Dr. Manisha Vernekar, MBBS, MD**,** Dept. Of Gynecologic Oncology, CNCI, Kolkata

Co-Investigator: Dr. Partha Nath, MBBS, Chief Medical Officer, Medical Oncology, CNCI, Kolkata

Name of Institution: Chittaranjan National Cancer Institute, Kolkata 700026

Name and address of the Sponsoring/ Funding Agency: Department of Health Research, Ministry of Health and Family Welfare, 2nd Floor, Indian red Cross Society Building, 1, Red Cross Road, New Delhi-110001.

Documentation of the Informed Consent:

I, _____, have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am 18 years or above of age and exercising my free power of choice, hereby give my consent to be included as a participant in the project entitled " Study on MAD and BUB1 genes of Spindle Assembly Checkpoint with response to Primary Adjuvant Chemotherapy in advanced Epithelial Ovarian Cancer**".**

I have read and understood this consent form and the information provided to me.

I have had the consent document explained to me.

I have been explained the nature of study.

My rights and responsibilities have been explained to me by the investigator.

I have informed the investigator of all the treatments I am currently taking or have been taking for the past ___ months which also includes any 'desi' (alternative) treatments.

I hereby give permission to the investigator to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Government agencies and ethics committee. I understand that they may inspect my original records.

My identity will be kept confidential if my data are publicly presented.

I have had my questions answered to my satisfaction.

I have decided to part in the research study.

I am aware that if I have any questions during the study, I should contact the Mentor/ Principal Investigators/ Co- investigators of the project. By signing this consent form, I attest the information in this document.

I will be given a copy of this consent document.

_____

Participant's (or legal representative if participant is incompetent) signature/ Thumb impression

_____

Name and Signature of impartial witness (required for illiterate patients)

Date: Time:

Place:

Address and Contact number of impartial witness:

_____

_____

_____

Name and signature of the Mentor/PI/ Co-PI:

Name:

Signature: Date:

Demographic Information

Serial No.	Characteristics	Details of participant
1.	ID Number	
2.	Date of registration (DD/MM/YY)	
3.	Name	
4.	Contact/Mobile No.	
5.	Address	
6.	Setup	1. Urban 2. Rural
7.	Education	1 Illiterate ; 2 Primary/Secondary ; 3 Graduate and above
8.	Occupation	1.Student;2 Housewife/ Unemployed;3 Self-employed/ Business; 4 Professional/ Desk job ;5 Laborer; 6 Farmer; 7.Retired
9.	Age	Years:
10.	Gender	FEMALE
11.	Religion	1.Hindu ; 2. Muslim; 3. Christian; 4. Other
12.	Marital Status	1.Unmarried; 2. Married; 3. Widowed; 4. Divorced
13.	Family history of cancer	1.No 2. Yes. Specify: 1. Breast Cancer 2. Ovarian Cancer 3. Other Family member: Mother / Sister / Grandmother / Other
14.	Personal history of cancer	1. No; 2. Yes Specify:
15.	**Gynaecologic History**	
	i. Menstrual Cycle	1.Regular; 2. Irregular
	ii. No. of periods per year	1.12; 2. 6-9; 3. < 6
	iii. Menstrual Hygiene	1.Good 2. Poor
	iv. Contraceptive Usage	1.Yes; 2. No
	v. Menopause	1.Yes, _____ Years 2. No
	vi. Hormone Replacement Therapy	No Yes If Yes, Duration:
	vii. Diagnosed with underlying gynecological disease before (if any)	1.No; 2. PCOD; 3.PCOS; 4. Endometriosis 5.Other Specify:
16.	**Obstetric History**	
	Age at first pregnancy:	Parity: / Breast feeding history:

ANNEXURE III

Current, worst and average pain numeric rating scale.

Numerical Rating Scale (NRS) - Current Pain/Resting Pain

Please rate your pain by making an 'X' in the appropriate box to indicate your pain level right now.

0	1	2	3	4	5	6	7	8	9	10
No pain					Moderate pain					Worst pain

Numerical Rating Scale (NRS) – Worst Pain

Please rate your pain by making an 'X' in the appropriate box that best describes your pain at its worst in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No pain					Moderate pain					Worst pain

Numerical Rating Scale – Average Pain

Please rate your pain by making an 'X' in the appropriate box that best describes your pain on the average in the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain					Moderate Pain					Worst Pain

Pain interference with sleep – Numerical Rating Scale (NRS)

Please rate your pain by making an 'X' in the appropriate box that describes how your pain interfered with sleep during the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain					Moderate Pain					Worst Pain

Numerical Rating Scale – Average Pain

Please rate your pain by making an 'X' in the appropriate box that describes how your pain interfered with sleep on an average during the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No Pain					Moderate Pain					Worst Pain

Ref:- Jacox, A., Carr, D.B., Payne, R., et al. (1994). Management of Cancer Pain. Clinical Practice Guideline No. 9. AHCPR Publication No. 94- 0592. Rockville, MD: Agency for Health Care Policy and Research, U.S. Department of Health and human Services.

Neuropathic Pain Symptom Inventory (NPSI)

We wish to know if you feel spontaneous pain that is pain without stimulation. Please rate your pain by making an 'X' or a circle in the appropriate boxes that describe best, your average spontaneous pain severity in the past 24 hours. (Indicate any one number in the scale.)

i. Does your pain feel like burning?

0	1	2	3	4	5	6	7	8	9	10
No burning pain					Moderate burning pain					Worst burning pain imaginable

ii. Does your pain feel like squeezing?

0	1	2	3	4	5	6	7	8	9	10
No Squeezing Pain					Moderate Squeezing Pain					Worst Squeezing Pain imaginable

iii. Does your pain feel like pressure?

0	1	2	3	4	5	6	7	8	9	10
No Pressure					Moderate Pressure					Worst Pressure imaginable

- During the past 24 hours your spontaneous pain was present: Yes / No.
  If Yes, then tick the response that best describes your case.

  Continuously…… [ ](1)
  Between 8-12 hours…… [ ] (2)
  Between 4-7 hours……... [ ] (3)
  Between 1-3 hours……... [ ] (4)
  Less than 1 hour……... [ ] (5

We wish to know if you have brief attack of pain. Please rate your pain by making an 'X' or a circle in the appropriate boxes that describe best, your average pain attack severity in the past 24 hours. (Indicate any one number in the scale.)

i. Does your pain feel like electric shock?

0	1	2	3	4	5	6	7	8	9	10
No electric shocks					Moderate					Worst shocks imaginable

ii. Does your pain feel like stabbing?

0	1	2	3	4	5	6	7	8	9	10
No Stabbing					Moderate					Worst Stabbing imaginable

- During the past 24 hours how many of these pain attacks have you had?

20 or more [ ] (1)
Between 10-19 [ ] (2)
Between 6-9 [ ] (3)
Between 1-5 [ ] (4)
No pain attacks [ ] (5)

We wish to know if you feel your pain is provoked by any external stimuli. Please rate your pain by making an 'X' or a circle in the appropriate boxes that describe best, your average provoked pain severity in the past 24 hours. (Indicate any one number in the scale.)

i. Is your pain feel provoked or increased by light touching the painful area?

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst pain imaginable

ii. Is your pain feel provoked or increased by pressure on the painful area?

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst pain imaginable

iii. Does your pain feel provoked or increased by cold sensation on the painful area?

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst pain imaginable

We wish to know if you feel abnormal sensations on the painful area. Please rate your pain by making an 'X' or a circle in the appropriate boxes that describe best, your average abnormal pain severity in the past 24 hours. (Indicate any one number in the scale.)

i. Do you feel pins and needles?

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst of pins and needles imaginable

ii. Do you feel tingling?

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst tingling imaginable

Daily Sleep Interference Rating Scale

We wish to know if your pain interfere your sleep. Please rate your pain by making an 'X' in the appropriate box that describes how your neuropathic pain interfered with sleep on an average during the past 24 hours.

0	1	2	3	4	5	6	7	8	9	10
No					Moderately					Worst Pain (Could not sleep at all)

ANNEXURE IV

Quality of Life

Serial No.	SPIRITUAL FACTOR	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Do you feel peaceful?	0	1	2	3	4
2.	Do you have any reason for living?	0	1	2	3	4
3.	Has your life been productive?	0	1	2	3	4
4.	Do you have trouble feeling peace of mind?	0	1	2	3	4
5.	Do you feel sense of purpose in your life?	0	1	2	3	4
6.	Are you able to reach deep down into yourself for comfort?	0	1	2	3	4
7.	Do you feel a sense of harmony in yourself?	0	1	2	3	4
8.	Does your life lack meaning and purpose?	0	1	2	3	4
9.	Do you find comfort in your faith and spiritual beliefs?	0	1	2	3	4
10.	Do you find strength in your faith and spiritual beliefs?	0	1	2	3	4
11.	Does your illness strengthen your faith or spiritual belief?	0	1	2	3	4
12.	Do you think whatever happens to your illness, things will be okay?	0	1	2	3	4

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Do you have a lack of energy?	0	1	2	3	4
2.	Do you experience nausea?	0	1	2	3	4
3.	Because of your physical condition, do you have trouble meeting the needs of my family?	0	1	2	3	4
4.	Do you have pain?	0	1	2	3	4
5.	Are you bothered by side effects of treatment?	0	1	2	3	4
6.	Do you feel ill?	0	1	2	3	4
7.	Are you forced to spend time in bed?	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Do you feel close to your friends?	0	1	2	3	4
2.	Do you get emotional support from your family?	0	1	2	3	4
3.	Do you get support from friends?	0	1	2	3	4
4.	Has your family accepted your illness?	0	1	2	3	4
5.	Are you satisfied with family communication about your illness?	0	1	2	3	4
6.	Do you feel close to your partner (or the personwho is your main support)?	0	1	2	3	4
7.	Are you satisfied with your sex life?	0	1	2	3	4

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Do you feel sad?	0	1	2	3	4
2.	Are you satisfied with how you are coping withyour illness?	0	1	2	3	4
3.	Are you losing hope in the fight against yourillness?	0	1	2	3	4
4.	Do you feel nervous?	0	1	2	3	4
5.	Do you worry about dying?	0	1	2	3	4
6.	Do you worry that your condition will getworse?	0	1	2	3	4
	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Are you able to work (include work at home)?	0	1	2	3	4
2.	Your work (include work at home) is fulfilling?	0	1	2	3	4
3.	Are you able to enjoy life?	0	1	2	3	4
4.	Have you accepted your illness?	0	1	2	3	4
5.	Are you sleeping well?	0	1	2	3	4
6.	Are you enjoying the things you usually do forfun?	0	1	2	3	4
7.	Are you content with the quality of your liferight now?	0	1	2	3	4

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
1.	Do you have swelling in your stomach area?	0	1	2	3	4
2.	Are you losing weight?	0	1	2	3	4
3.	Do you have control of your bowels?	0	1	2	3	4
4.	Have you been vomiting?	0	1	2	3	4
5.	Are you bothered by hair loss?	0	1	2	3	4
6.	Do you have a good appetite?	0	1	2	3	4
7.	Do you like the appearance of your body?	0	1	2	3	4
8.	Are you able to get around by yourself?	0	1	2	3	4
9.	Are you able to feel like a woman?	0	1	2	3	4
10	Do you have cramps in your stomach area?	0	1	2	3	4
11	Are you interested in sex?	0	1	2	3	4
12	Do you have concerns about your ability tohave children?	0	1	2	3	4

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ORIGINAL ARTICLE

Assessment of quality of life among advanced ovarian cancer patients in a tertiary care hospital in India

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Abstract

Purpose The study aims to record the quality of life (Qol) and its changes while ovarian cancer (OC) patients undergo debulking surgeries and chemotherapy in a tertiary care hospital of Eastern India.

Methods Patients with advanced epithelial OC (FIGO stages III–IV) were recruited. They underwent primary/interval debulking surgeries with classical chemotherapy (adjuvant/neoadjuvant) of intravenous tri-weekly doses of paclitaxel + carboplatin. QoL was assessed using Fact- O + FACIT-Sp-12 questionnaire with a set of 51 questions in different domains (spiritual, physical, social, emotional, and functional factors) and a special set for OC patients under the heading "Additional concerns." The responses from patients were recorded at baseline (diagnosis/study entry), 2, 4, and 6 months during the treatment visits. Overall survival (OS) was assessed using Kaplan Meier curve.

Results A majority of patients were 49.15±10.8 years of age, school-educated (54%), unemployed/homemakers (73.5%), belonging from rural setup (64.6%) with a monthly income of Rs. 2000/- to Rs. 5000/-. There was no statistically significant ($p>0.05$) improvement found in Qol from the baseline till the end of the study, neither overall nor in subsets (responders (Rs)/partial responders (PRs)/non-responder (NRs) groups or the adjuvant and neoadjuvant chemotherapy groups). The common toxicities like anemia, constipation, and weight loss were significantly ($p<0.05$) correlated with the patients' physical, functional, emotional, and social well-being.

Conclusion Ovarian cancer patients represent a poor functional, social, and disease-specific quality of life that needs to be addressed, identified, and improved by the growing nexus of healthcare providers and researchers.

Keywords Ovarian cancer · Quality of life · Neoadjuvant therapy · Adjuvant chemotherapy · Paclitaxel · Carboplatin · Toxicity · Survival

Introduction

The incidence of ovarian cancer cases (OC) was 313959, with 207252 mortalities worldwide and 45701 new cases with 32077 deaths in India [1]. OC is the most lethal gynecological cancer diagnosed at advanced stages (FIGO III & IV) due to asymptomatic, secret growth of tumors and lack of effective screening methods [2]. The common disease-related symptoms like fatigue, abdominal pain, swelling, backache, gastrointestinal issues, and irregular menstrual cycle can unfavorably affect patients' quality of life (QoL) by diminishing physical, sexual, and psychological well-being [3]. The first-line approach of ovarian cancer treatment aims to aggressively eradicate the disease with surgery and chemotherapy of platinum-taxane combination [4]. However, advanced cases do not always respond well

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to chemotherapy. They may result in cumulative adverse effects like anemia, neurotoxicity, gastrointestinal disorders, fatigue, and alopecia of varying severities [5, 6] that negatively affects a patient's social, functional, emotional, and economic well-being. Decision-making for the anti-cancer treatment often involves balancing efficacy with safety/benefit and risk/harm. While certain clinical benefits can harm the patients' quality of life, others can provide a parallel benefit that increases the merit of the treatment/therapy beyond the clinical measures of response and survival. In recent times, research analyzing important patient-centered outcomes focuses on maximizing outcomes and Gynecologic Oncology Group (GOG) and National Cancer Institute (NCI) trials have recommended QoL measurement as an emerging primary endpoint along with survival analysis [7, 8]. QoL is an important predictor of the success of healthcare services and hence has become a research priority nowadays.

So far, there has been much research published on the QoL of ovarian cancer patients from France [9], the USA [10], Canada [11], and others that are reflective of the Western population perspective. However, illness is perceived as an integral part of life; cultural and social predeterminism play crucial roles in a patient's belief system, thus creating multiethnic differences in well-being [12]. To the best of our knowledge, there is sparse data available on the Indian scenario, so our study aims to assess the QoL of Indian OC patients undergoing first-line treatment in a high-volume tertiary care hospital.

Patients and methods

The study was conducted between July, 2018 till January 2021 with patients >18 years who had histologically confirmed diagnosis of advanced epithelial ovarian cancer (FIGO stages III–IV), primary peritoneal cancer and co-existing fallopian tube cancer *in situ* with adequate bone marrow, hepatic, neurologic, cardiologic function and coagulation parameters. The exclusion criteria included patients under the age of 20 years; patients with recurrent disease who have received prior radiotherapy, chemotherapy and had undergone complete oophorectomy; pregnant or nursing women; patients with other specific and invasive malignancies; acute hepatitis; active infection, uncontrolled diabetes, serious non-healing wound, bleeding disorder, coagulopathy, bone fracture; significant proteinuria; clinically significant cardiovascular complications and significant autoimmune disease uncontrolled with treatment.

Patients received the tri-weekly (21 days) chemotherapy consisting of intravenous doses of 175mg/m2 paclitaxel on Day 1 (3 h) + carboplatin AUC 5-6mg.min/mL (over 1 h) on Day 2. Primary debulking surgery [total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), omentectomy, bilateral pelvic and para-aortic lymphadenectomy with cytoreduction] was performed before adjuvant chemotherapy. The inoperable cases had to undergo neoadjuvant chemotherapy with interval debulking surgery after 3 cycles [4]. The clinical response was evaluated by monitoring blood biomarker CA125 and radiographic images (USG and CECT) at baseline, after the third and sixth cycle. Maximum grades of toxicities were noted as per National Cancer Institute (NCI) common toxicity criteria (CTC) (CTCAE v4) [13]. Informed consent (English/ Hindi/ Bengali) was obtained from every patient before inclusion to the study. A thumb impression with a signature from a concerned literate representative of the illiterate patients was obtained on the consent forms. QoL was assessed using Fact- O questionnaire (FACT-O: For patients with ovarian cancer) including FACIT-Sp-12 for spiritual well-being [14]. The questionnaire set had a total of 51 questions in different domains that include spiritual, physical, social, emotional and functional factors and a special set of questions designed for ovarian cancer patients under the heading "Additional concerns." Patients were interacted/ interviewed on a one-to-one basis in the presence of a physician during every visit to the hospital while they answered the questionnaires. The illiterate patients were asked the questions in their local language (Bengali/Hindi) and the answers were filled by investigators/physicians in the presence of a literate representative. The filled answers were then cross-checked by the representative and submitted to the investigators. Quality of life (QoL) was assessed at diagnosis, 2 months, 4 months, and 6 months. The study was planned and conducted following Good Clinical Practice guidelines and the Declaration of Helsinki and was approved by Institutional Ethics Committee, Chittaranjan National Cancer Institute [A-4.311/VN/27/06/2018-10].

Descriptive statistics were used to analyze the frequencies of patient characteristics. Two-way repeated measure analysis of variance (ANOVA) was used for tests of within-subject effect for QoL domains vs groups (Rs, PRs and NRs/ adjuvant and neoadjuvant chemotherapy). Greenhouse-Geisser and Wilk's Lambda significance values ($p< 0.05$) were taken into consideration. Cross-tabulation was applied to find out association (chi-square, $\chi 2$) of different toxicities with QoL domains. Kaplan Meier survival analysis was performed after monitoring survival for 24 months. Cox regression analysis was used to determine the risk (Hazard Ratio) of death associated with the Qol domains at baseline and after first-line chemotherapeutic treatment between the adjuvant and neoadjuvant chemotherapy groups.

Results

Participants were 49.15±10.8 (mean±SD) years of age, had a mean body weight of 46.18 ± 9.39 kg, mostly school-educated (54%), unemployed/homemakers (73.5%), belonging

from rural location (64.6%) with a monthly income of Rs. 2000/- to Rs. 5000/-. The majority of them were diagnosed with stage III ovarian cancer (82.02%) of the serous histological subtype (81%) (Table 1). The mean and standard deviation scores of the FACT-O questionnaire domains are represented in Table 2. There were no statistically significant differences found (p>0.05) in baseline Qol and in all the QoL domains (spiritual, emotional, social, functional, physical and additional concern) throughout the 6 months. There were also no significant differences between the Qol of the responders, partial responders and non-responders or between adjuvant and neoadjuvant chemotherapy arms, as per Table 3. Combined Qol mean scores are plotted in Figure 1. Even though there was no significant improvement in the Qol, there was no significant decline noted as well**.** The commonly observed toxicities included anemia, leucopenia, thrombocytopenia, granulocytopenia, nausea, vomiting, anxiety/depression/neurologic conditions, neuropathy, weight loss/gain, diarrhea, constipation, indigestion/bloating/hyperacidity, abdominal pain, renal toxicity and mucositis are documented in Table 4. Among the adversities, anemia and constipation were found to be significantly associated with the physical (p=0.003 & p=0.004), emotional (p=0.016 & p=0.000), functional (p=0.006 & p=0.001) wellbeing, and additional concerns (p=0.000 & p=0.000). Additional concerns specific to ovarian cancer

Table 1 Patient characteristics (demographics and clinical characteristics)

Characteristics (n=110)		Frequency (%)
Age (Years)	20–40	24 (21.8)
	41–60	76 (69.09)
	61–80	10 (9.09)
Education	Illiterate	39 (35.4)
	School education	61 (55.4)
	Graduates and above	10 (9.09)
Religion	Hindu	90 (81.81)
	Muslim	20 (18.19)
Marital status	Unmarried	7 (6.36)
	Married	86 (78.18)
	Widowed/Divorced	17 (15.45)
Occupation	Unemployed/Homemaker	83 (73.5)
	Self employed/business	8 (7.1)
	Professional/Desk job	7 (6.36)
	Laborer	7 (6.36)
	Farmer	5 (4.54)
Location	Urban	37 (33.6)
	Rural	73 (66.36)
Caregiver	Husband	53 (48.18)
	Children	35 (31.81)
	Family and Friends	6 (5.45)
	Other	26 (23.63)
Monthly Income	< Rs 2000/-	26 (23.63)
	Rs 2001 to Rs 5000/-	75 (68.18)
	Rs 5001 and above	9 (8.18)
FIGO	III	91 (82.72)
	IV	19 (17.27)
Tumor histology	Serous	80 (72.72)
	Other	30 (27.27)
Size of tumor mass (pre-treatment)	>5 cm	80 (72.72
	<5 cm	30 (27.27)
Surgeries	Primary debulking (Adjuvant Chemotherapy)	55
	Interval debulking (Neoadjuvant Chemotherapy)	55
Clinical Response	Complete Resonders	41 (37.3)
	Partial Responders	44 (40)
	Non- Responders	25 (22.7)

Table 2 FACT-O scores at various time intervals among responders, partial responders and non-responders

Qol Domains	Groups	Baseline	2nd month	4th month	6th month	*p-value≠ (within groups)*
Spriritual	Rs (*n*=41)	20.22±13.37	20.10±13.39	21.20±12.27	21.49±11.68	0.662
	PRs (*n*=44)	16.23±14.00	16.30±13.95	17.73±13.80	17.39±13.25	
	NRs (*n*=25)	14.32±14.14	11.56±14.02	11.80±14.26	12.44±14.06	
p-value(between groups)*	0.635					
Emotional	Rs (*n*=41)	10.22±7.51	9.71±7.35	10.78±7.64	11.10±7.54	0.472
	PRs(*n*=44)	11.09±7.86	10.91±7.56	11.32±7.85	10.93±7.92	
	NRs (*n*=25)	10.40±8.45	8.48±8.77	10.36±8.53	9.44±8.12	
p-value(between groups)*	0.774					
Social	Rs (*n*=41)	12.12±8.46	12.20±8.67	12.90±7.89	13.05±7.30	0.757
	PRs (*n*=44)	12.86±8.07	12.68±8.48	13.36±8.46	13.77±8.72	
	NRs (*n*=25)	10.20±7.42	9.84±7.75	11.60±8.00	10.96±8.28	
p-value(between groups)*	0.956					
Functional	Rs (*n*=41)	7.07±6.59	7.24±6.71	8.22±6.72	8.73±6.67	0.846
	PRs (*n*=44)	7.55±7.17	7.64±6.93	7.68±6.63	7.70±6.63	
	NRs (*n*=25)	6.72±6.73	5.92±6.72	6.32±6.75	6.68±7.25	
p-value(between groups)*	0.609					
Physical	Rs (*n*=41)	8.15±7.18	8.37±6.97	9.54±7.24	9.90±6.94	0.425
	PRs (*n*=44)	8.43±8.01	8.32±8.00	8.95±7.58	9.52±7.86	
	NRs (*n*=25)	8.32±8.76	7.52±8.75	7.8±8.06	6.72±7.03	
p-value(between groups)*	0.347					
Additional concerns	Rs (*n*=41)	10.65±8.10	10.42±7.91	13.00±7.68	13.78±7.77	0.497
	PRs (*n*=44)	12.11±8.86	12.95±9.14	14.09±8.56	13.68±8.74	
	NRs (*n*=25)	11.92±8.70	9.88±9.69	12.50±10.24	12.46±10.29	
p-value(between groups)*	0.763					

Responders (Rs); Partial Responders (PRs); Non Responders (NRs). All values are expressed as mean±SD. The mean scores of QoL domains were non-significant within-subject effect and multivariate analysis.

**Multivariate analysis (Wilk's Lambda)*

≠Greenhouse-Geisser

Mauchly's sphericity was significant (p<0.05).

was associated (p=0.000) with leucopenia, granulocytopenia, neuropathy, weight loss, indigestion and abdominal pain. Functional well-being was associated with weight loss (p=0.014) and diarrhea (p=0.008). Emotional well-being was significantly associated with granulocytopenia (p=0.000), nausea (p=0.008), anxiety (p=0.006), weight loss (p=0.000), indigestion (p=0.040), and abdominal pain (p=0.000). Social well-being was associated with thrombocytopenia (p=0.017) and weight loss (p=0.005).

The overall survival curve is depicted in Figure 2. The log-rank test of the survival among responders, partial responders and non-responders was significant (***p*=0.019**), indicating survival distributions of different groups are not equal in the patient population (data not shown). The survival among adjuvant and neoadjuvant chemotherapy arms was not significant (p=0.373). The survival probability and hazard ratios (HR with 95% confidence interval) in relationship with Qol at baseline and after chemotherapy are documented in Table 5. It is evident from the table that poor physical well-being has the highest risk of death at baseline, but adjuvant chemotherapy arm has a higher risk of death than the neoadjuvant group at 6 months.

Discussion

In managing primary cases of advanced ovarian cancer, carboplatin and paclitaxel chemotherapy, along with cytoreductive surgery, are undisputed throughout the world. The quality of life of Indian OC patients receiving front-line chemotherapy and debulking surgeries has been assessed in this study.

The increment of spiritual factor score is indicative of better spiritual well-being. This section had questions

Table 3 FACT-O scores at various time intervals among adjuvant and neoadjuvant chemotherapy group

Qol Domains	Groups	Baseline	2nd month	4th month	6th month	*p-value≠ (within groups)*
Spiritual	Adjuvant	18.69±13.56	17.75±13.76	18.04±13.70	18.38±12.99	0.753
	Neoadjuvant	15.87±14.18	15.53±14.31	17.31±13.70	17.20±13.53	
p-value(between groups*	0.547					
Physical	Adjuvant	8.27±7.35	8.45±7.164	9.00±7.024	8.84±6.63	0.729
	Neoadjuvant	8.33±8.33	7.85±8.36	8.82±8.06	9.22±8.13	
p-value(between groups*	0.725					
Social	Adjuvant	13.51±7.662	13.36±7.97	13.98±7.84	13.89±7.64	0.847
	Neoadjuvant	10.45±8.25	10.35±8.60	11.60±8.20	11.84±8.51	
p-value(between groups*	0.657					
Emotional	Adjuvant	10.25±7.409	9.45±7.32	10.35±7.35	10.38±7.11	0.89
	Neoadjuvant	10.96±8.24	10.36±8.22	11.45±8.38	10.93±8.46	
p-value(between groups*	0.908					
Functional	Adjuvant	8.56±6.56	7.96±7.02	8.71±7.24	9,05±7.25	0.577
	Neoadjuvant	5.8±6.4	6.24±6.46	6.44±5.89	6.65±6.14	
p-value(between groups*	0.647					
Additional concerns	Adjuvant	12.54±7.92	11.89±8.22	13.56±8.39	13.74±8.50	0.696
	Neoadjuvant	10.52±9.02	10.78±9.50	13.11±8.87	13.15±8.97	
p-value(between groups*	0.483					

All values are expressed as mean±SD. The mean scores of QoL domains were non-significant within-subject effect and multivariate analysis.

**Multivariate analysis (Wilk's Lambda)*

≠Greenhouse-Geisser

Mauchly's sphericity was significant (p<0.05).

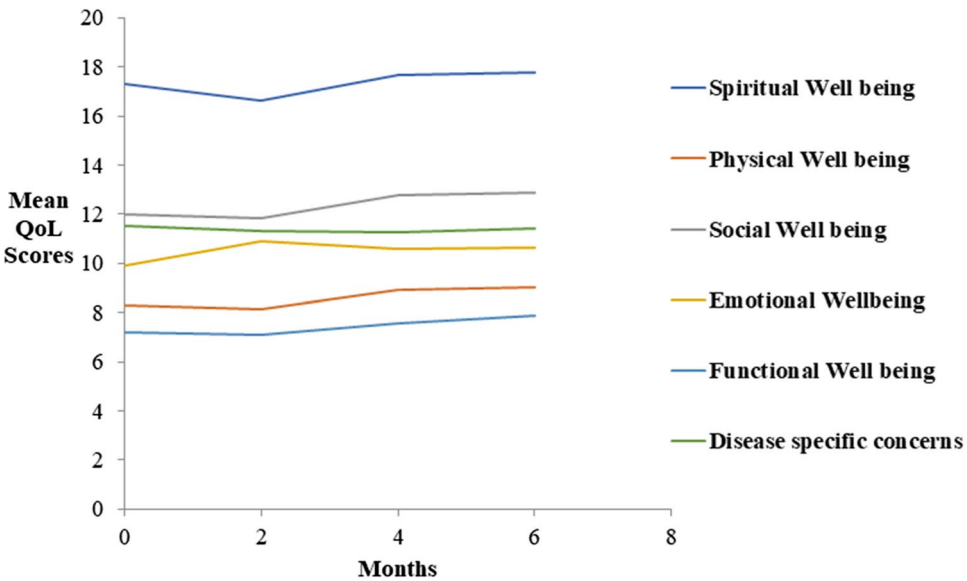

Fig. 1 Overall QoL mean scores for the advanced ovarian cancer patients

about mental peacefulness, productivity, purpose, comfort, harmony, faith and spiritual faith/beliefs of life. The mean spiritual well-being scores of ovarian cancer patients in this study improved slightly (non-significant) except in the non-responders category. On a scale of 0–40, the mean scores were between 12 and 21. In this study, the patients had low to moderate spirituality as seen in another study by Davis, that life events paired with a decrease in peace experienced the worst psychological outcomes at 1 year [15].

Table 4 Grades of common Side effects experienced by the patients (*N*=110)

Sl No.	Adverse Effects	Grades 1–2	Grades 3–4
1.	Anemia (*n*= 65)	56 (50.9)	9 (8.1)
2.	Leukopenia (*n*=16)	15 (13.6)	1 (0.9)
3.	Thrombocytopenia (*n*=6)	6 (5.45)	0 (0)
4.	Granulocytopenia (*n*=3)	3 (2.7)	0 (0)
5.	Nausea (*n*=18)	16 (14.5)	2 (1.8)
6.	Vomiting (*n*=26)	22 (20)	4 (3.6)
7.	Anxiety/Depression (*n*=37)	31 (28.1)	6 (5.4)
8.	Neuropathy (*n*=43)	40 (36.36)	3 (2.7)
9.	Weight Loss (*n*=30)	30 (27.2)	0 (0)
10.	Diarrhoea (*n*=35)	29 (26.3)	6 (5.4)
11.	Constipation (*n*=40)	31 (28.1)	9 (8.1)
12.	Indigestion (*n*=47)	40 (36.36)	7 (6.3)
13.	Abdominal pain/swelling (*n*=33)	31 (28.1)	2 (1.8)
14.	Renal Toxicity (*n*=25)	24 (21.8)	1 (0.9)

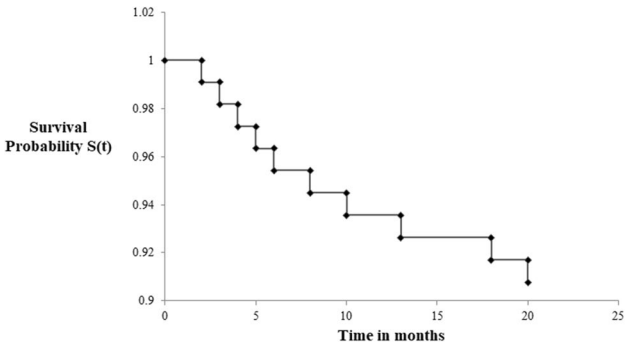

Fig. 2 Two-year overall survival curve of the advanced ovarian cancer patients

Spirituality is associated with emotions, and the mean scores of emotional well-being were seen to decline (non-significantly) in the partial responders and non-responders except responders. Another study by Ferell et al., 2003 [16], analyzed natural correspondence of ovarian cancer patients where they reported the positive and negative effects of disease and shared their coping mechanisms with their loved ones. In our study the patients responded to the questionnaire with a positive attitude and some of them expressed their acceptance of the disease and its outcome with realistic expectations. Overall, the emotional well-being was on the better side, considering the mean score in the range of 8–11 out the maximum score of 24. The increment in emotional factor scores is indicative of Qol's detriment.

Emotional and social well-being is essential for overall health. In this study, on a scale of 0-20, the mean scores ranged from approx 10–13 indicating moderate social well-being of the patients with family and friends. Most of them had their husbands or children or both caregivers, and the rest depended on family/friends/others. Majority were unemployed, mainly homemakers. Several instances reported of husbands abandoning them for various reasons including financial and social burden/stigma. These incidents left the patients with sadness and socially burdened. 3 women were dependent on crowd-funding to afford the treatment. The patients were never fully satisfied with their communication with caregivers/family and sometimes preferred not to answer the questionnaire. Hill et al., 2016 reported that social support seeking was a coping mechanism and therefore, is an essential consideration in QoL and mental health of ovarian cancer patients [17]. Meraner et al., 2012 found a statistically significant decrease in anxiety and depressive symptoms and improved social life [6].

Functional well-being comprises the patients' ability to work, enjoy work and life, accept illness, sleep, and

Table 5 Survival probability in relationship with QoL at different time points

QoL Factors	Baseline (without treatment) HR (95% CI)	*p-value*	Chemotherapy	At 6 months after first- line chemotherapy HR (95%CI)	*p-value*
Spiritual Wellbeing	1.441 (0.789–2.630)	0.554	Adjuvant	1.778 (0.519–6.090)	0.371
			Neo-adjuvant	0.990 (0.247–3.966)	
Physical Wellbeing (0-28)	3.603 (1.306–9.941)	**0.008**	Adjuvant	1.714 (0.584–5.029)	0.320
			Neo-adjuvant	1.2 (0.49–4.789)	
Social Wellbeing (0-28)	1.013 (0.950–1.080)	0.349	Adjuvant	3.052 (1.021–9.125)	**0.036**
			Neo-adjuvant	1.747 (1.010–3.021)	
Emotional Wellbeing (0-24)	0.945 (0.343–2.607)	0.913	Adjuvant	1.495 (0.172–1.420)*	0.994
			Neo-adjuvant		
Functional Wellbeing(0-28)	1.00 (0.614–1.134)	0.293	Adjuvant	0.031 (0.00–6.01)*	**0.034**
			Neo-adjuvant		

*HR was same for both the groups.

p-value represents the significance of Log-rank test.

impression of current quality of life. Higher mean scores indicate higher Qol and vice versa. On a scale of 0-28, the mean scores range from approx. 6–8 that indicates poor functional well-being. There was no change observed in the Qol scores from baseline to 6 months. The poor functional ability can be caused by high tumor burden at baseline and side effects of chemotherapy during the 6 cycles. Only 20 (18.1%) patients reported improvement of their Qol on asking and others reported no change.

The ovarian cancer and its treatment can cause significant physical and psychological morbidity Lowe et al., 2007 [18]. At baseline 38 patients reported no lack of energy, no nausea, no trouble meeting family needs and no pain in the physical well-being questionnaire and after 6 months there were 21 similar patients. The overall mean score of physical well-being indicates a moderate to good performance throughout the 6 months. Contradictorily, von Gruenigen et al.2010 reported that postoperative physical well-being improved after 6 months [19].

Disease-specific concerns also play a crucial role in determining the overall well-being of the patients. The additional concerns included query about stomach swelling, weight loss/gain, control of bowels, vomiting, alopecia, appetite, appearance of body, cramps, sexual interests and concerns about child-bearing. The majority of patients in the study reported belly swelling after treatment, moderate control of bowels, and less vomiting. However, 100% of them were bothered by hair loss, concerned about future hair growth, did not fully accept their appearance, and had less appetite and fewer libidos. The younger patients (25-35 years) were apprehensive about their sexual life and womanhood after undergoing TAH+BSO.

Drug-related toxicities are also important determinants of QoL. In our study, anemia was the most commonly observed toxicity related to fatigue, dyspnea, dizziness, activity intolerance, headaches, concentration difficulties, and sleep disturbance [20]. The high incidence of diarrhea and constipation are seen in this study negatively affects the patients' daily lives and can be attributed to poor functional well-being. It is very common for patients with advanced ovarian cancer to go through repeated chemotherapeutic treatments due to disease relapse. Sun CC et al., 2007 reported a good quality life for complete responders compared to partial responders and progressive disease, which contradict our study [21].

It is evident from the results of Table 5 that even though adjuvant chemotherapy group had better Qol scores they had poorer survival outcome. Despite the similarity in the financial background the patient population had high heterogeneity in the socio-cultural/ spiritual factors that may have a hidden confounding factor affecting the survival outcome. However, clinically, the survival benefit of patients in the adjuvant chemotherapy group was seen as inferior to the neoadjuvant chemotherapy group. This can be attributed to the complications arising from the sudden removal of bulky tumors (≥ 20cm) and immediate start of chemotherapy, toxicity, and pre-existing comorbidity, which were not considered and are a limitation to our study. The study focuses and reports the factors according to FACT-O questionnaires and has the potential to impact clinical oncology practice in low and middle-income countries.

Conclusion

There was no significant improvement in the Qol domains at baseline till 6 months of the treatment. In this study, the Indian ovarian cancer patients report a poor functional, social and disease-specific quality of life that needs to be addressed, identified and improved by the growing nexus of healthcare providers and researchers.

Author contribution SS and VDN conceptualized and wrote the main manuscript. SS, PKS, TM, and PC collected the data. SS, RP, SM, TB, and MV analyzed the data. All the authors contributed in re-writing and editing the final manuscript. All authors read and approved the final manuscript.

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Data availability N/A

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Declarations

Ethics approval The present study was approved by Institutional Ethics Committee, Chittaranjan National Cancer Institute [A-4.311/VN/27/06/2018-10].

Consent to participate Informed consents were obtained from all the participants in their preferred language, i.e. English, Bengali or Hindi.

Consent for publication N/A

Conflict of interest The authors declare no competing interests.

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REVIEW

Mitotic checkpoint defects: en route to cancer and drug resistance

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Abstract Loss of mitosis regulation is a common feature of malignant cells that leads to aberrant cell division with inaccurate chromosome segregation. The mitotic checkpoint is responsible for faithful transmission of genetic material to the progeny. Defects in this checkpoint, such as mutations and changes in gene expression, lead to abnormal chromosome content or aneuploidy that may facilitate cancer development. Furthermore, a defective checkpoint response is indicated in the development of drug resistance to microtubule poisons that are used in treatment of various blood and solid cancers for several decades. Mitotic slippage and senescence are important cell fates that occur even with an active mitotic checkpoint and are held responsible for the resistance. However, contradictory findings in both the scenarios of carcinogenesis and drug resistance have aroused questions on whether mitotic checkpoint defects are truly responsible for these dismal outcomes. Here, we discuss the possible contribution of the faulty checkpoint signaling in cancer development and drug resistance, followed by the latest research on this pathway for better outcomes in cancer treatment.

Keywords Mitotic checkpoint · Spindle assembly checkpoint · Microtubule targeting agents · Carcinogenesis · Drug resistance

Abbreviations

MC	Mitotic checkpoint
SAC	Spindle assembly checkpoint
MCC	Mitotic checkpoint complex
APC/C	Anaphase-promoting complex/cyclosome
MAD	Mitotic arrest deficiency protein
BUB	Budding uninhibited by benzimidazole
BUBR1	Budding uninhibited by benzimidazole-related 1
CIN	Chromosomal instabiliy
MTA	Microtubule targeting agents
CDC20	Cell division control protein 20
CycB-Cdk1	Cyclin B and CDk1 complex
AOM	Azoxymethane
DMBA	7,12-Dimethylbenz[a]anthracene
ER-beta	Estrogen receptor-beta
RZZ	Rod-Zw10-Zwilch
KMN	Knl1-Mis12-Ndc80 complex
MPS1	Monopolar spindle protein 1
SASP	Senescence-associated secretory phenotype

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Introduction

The purpose of mitosis is equal distribution of duplicated genome in each daughter cell through appropriate chromosome segregation. The mitotic checkpoint (MC) or the spindle assembly checkpoint (SAC) is a quality control mechanism that functions to preserve the genome from chromosome imbalances during mitosis by delaying the anaphase onset until all the chromosomes are properly attached to the mitotic spindle (London and Biggins 2014). This machinery was first discovered in a genetic experiment on budding yeast, which identified some of the major components of SAC: the budding uninhibited by benzimidazole genes (BUB1, BUB2, and BUB3) and the mitotic arrest-deficient genes (MAD1, MAD2, and MAD3) (Hoyt et al. 1991; Li and Murray 1991). The downstream target of SAC is the anaphase-promoting complex (APC/C) that is inhibited by a diffusible inhibitor, named mitotic checkpoint complex (MCC). In the event of improperly attached or unattached kinetochores, the MCC is assembled with MAD2, BUB3, and BUBR1 (MAD3, in yeast) at the kinetochore and acts as the main effector of SAC. Reider and colleagues observed that in one cell type, a single unattached kinetochore can delay the mitotic progression for at least 3 h, which showed the excellent sensitivity of this checkpoint (Rieder et al. 1994). The MCC production is stopped as soon as the sister chromatids are captured by the mitotic spindle and cells progress towards anaphase.

An accurate mitotic checkpoint is important for cell survival but a compromised checkpoint predisposes aneuploidy (Kops et al. 2005; Nicholson and Cimini, 2011). Several studies have reported the prevalence of germline and somatic mutations of SAC genes in different types of cancers (Hahn et al. 2016; Guo et al. 2010) that are responsible for weakened SAC response. Altered expressions of the checkpoint proteins were associated with increased aneuploidy and chromosomal instability (CIN) (Holland and Cleveland 2012). Thus, these defects in SAC are thought to contribute in the process of carcinogenesis. Similarly, the overexpression or downregulation of the SAC genes in cancer cells fails to arrest the mitosis even with an active checkpoint, ultimately leading to mitotic slippage, malignant cell survival and resistance to microtubule targeting agents (MTAs) (Henriques et al. 2019; London and Biggins 2014).

In this review, we are aiming to describe the SAC signaling cascade and address the impact of a faulty checkpoint on carcinogenesis and drug resistance, with a discussion on recent developments on this pathway as a potential therapeutic target.

The metaphase to anaphase transition

Anaphase-promoting machinery

During mitosis, the transition from metaphase to anaphase takes place when the attachments that hold sister chromatids together, are dissolved. This is a highly regulated mechanism involving activities of the anaphase-promoting complex (APC/C, also known as cyclosome) and its co-activator cell division control protein 20 (CDC20). Prior to anaphase the sister chromatids are connected by a proteinaceous bridge, that is a multi-protein complex called cohesion or the "molecular glue." Separase cleaves the cohesin subunit SccI when Securin is ubiquitinated and destroyed by APC/C. Separase is inhibited by Securin before anaphase (Nasmyth 2002). The APC/C-CDC20 complex activates anaphase onset and controls mitotic exit by proteasome-dependent degradation of securin and cyclin B (Oliveira et al. 2010). The timely entry and exit from mitosis are regulated by CycB-Cdk1 activity which promotes a burst of protein phosphorylation (He´garat et al. 2016).

The function of the spindle assembly checkpoint

The SAC is a complex signaling cascade essential for human cell survival that negatively regulates APC/C activity (Kops et al. 2004; Michel et al. 2004a, b). This pathway inspects microtubule attachment or tension of each kinetochore on the mitotic spindle and prevents anaphase onset in the presence of unattached or incorrectly attached kinetochores (Musacchio 2011, 2015). Thus, it ensures proper transmission of genetic materials into the daughter cells and preserves the genetic stability which is essential for cellular fitness (Santaguida and Amon 2015; Vitale et al. 2016; Gordon et al. 2012.)

Signal generators of SAC

The signal generators of the mitotic checkpoint (SAC) are unattached or inappropriately attached kinetochores

(Fig. 1). Lack of chromosome attachments are communicated to the cytoplasm and components of a kinetochore-localized signaling starts to accumulate. Merotely is a state of kinetochore attachment, which is particularly dangerous and a major source of aneuploidy because it is not detected by the error correction system. When a kinetochore is not attached to microtubules, it changes its shape and size to a large, crescent-shaped structure called the fibrous corona. The complete functions of corona are still elusive but it can create harmony between accuracy and the speed of spindle assembly (Kops and Gassmann 2020).

Formation of the MCC

The potent inhibitor of anaphase-promoting machinery comprises of MAD2, CDC20, BUBR1/MAD3, and BUB3. Kinetochore serves as a hub for the hierarchical recruitment of SAC proteins (London and Biggins 2014) required for the production of MCC. In early mitosis, MPS1 is accumulated in the kinetochore and subsequently activated by autophosphorylation (Kang et al. 2007; Mattison et al. 2007). MPS1 associates with the NDC80-C subcomplex of the KMN network (Nijenhuis et al. 2013) controlled by the activity of Aurora B kinase (Kemmler et al. 2009). Aurora B is a serine/threonine protein kinase that orchestrates the correction of improper kinetochore-microtubule attachments by setting off changes in the microtubule dynamics and weakening the affinity of kinetochore for microtubules (Carmena et al. 2012). The cascade then hierarchically recruits the BUB1-BUB3 complex, followed by the BUBR1-BUB3 complex. The KNL1 is a component of the KMN network also known as CASC5 or Blinkin, has an array of large repeat motifs called the MELT motifs. These MELT motifs are phosphorylated by MPS1 and form phospho-docking sites for BUB3. BUB3 carries either BUB1 or BUBR1 with it and bridges KNL1 to the BUB paralogs. BUBR1 recruitment mechanism is more complex because BUBR1 requires a correct localization of BUB1 to be recruited at the kinetochore but not vice versa (London et al. 2012; Shepperd et al. 2012; Yamagishi et al. 2012). Finally, the recruitment of heterotetramer of MAD1-MAD2 takes place. Two distinct conformations are adopted by MAD2; the unbound/ open conformation (O-MAD2) and upon binding with MAD1 or CDC20, the bound/ closed conformation(C-MAD2). Upon mitotic entry, the MAD1-C-MAD2 is localized at the kinetochore. Further, the O-MAD2 in the cytosol is then recruited to the kinetochore-bound MAD1-C-MAD2 (Luo and Yu 2008). This O-MAD2 bound with MAD1-C-MAD2 then binds with the CDC20 (Fig. 2). The conformational activation of MAD2 from a free "open" state (O-MAD2) to the CDC20 bound closed form (C-MAD2) is the key step in the formation of the APC/C inhibitor (Skinner et al. 2008).

Inhibition of APC/C-CDC20

Finally, the MCC is composed of CDC20 in complex with the BUB1-related protein (BUBR1), BUB3, and MAD2. This complex inhibits the APC/C-CDC20 activity and triggers the "wait anaphase" signal until proper chromosome attachment and alignment are achieved. The silencing of the SAC signaling is achieved by reversing mitotic phosphorylation with phosphatase activity and proteolysis of the checkpoint proteins at mitotic exit (Lesage et al. 2011, Musacchio & Salmon, 2007). Allan et al. (2020) studied that the kinetochore corona-MAD1 generates a robust SAC signal, and the key mitotic kinase, cyclin B1:CDK1 has a scaffolding role, which leads to inhibit its own degradation (Allan et al. 2020). The number of 'MELT' motifs in the kinetochore protein Spc105/KNL1 and their BUB3-BUB1 binding affinities determine the SAC strength and responsiveness (Roy et al. 2020).

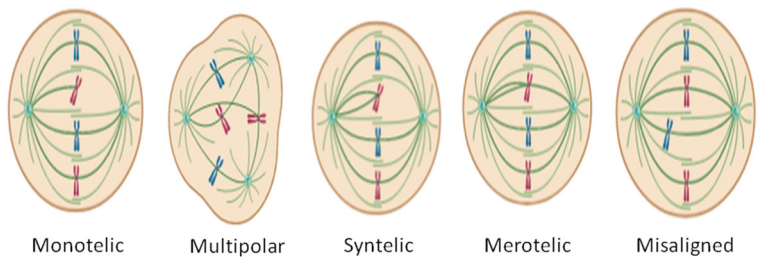

Fig. 1 Different states of kinetochore attachments that generate the SAC activation signal

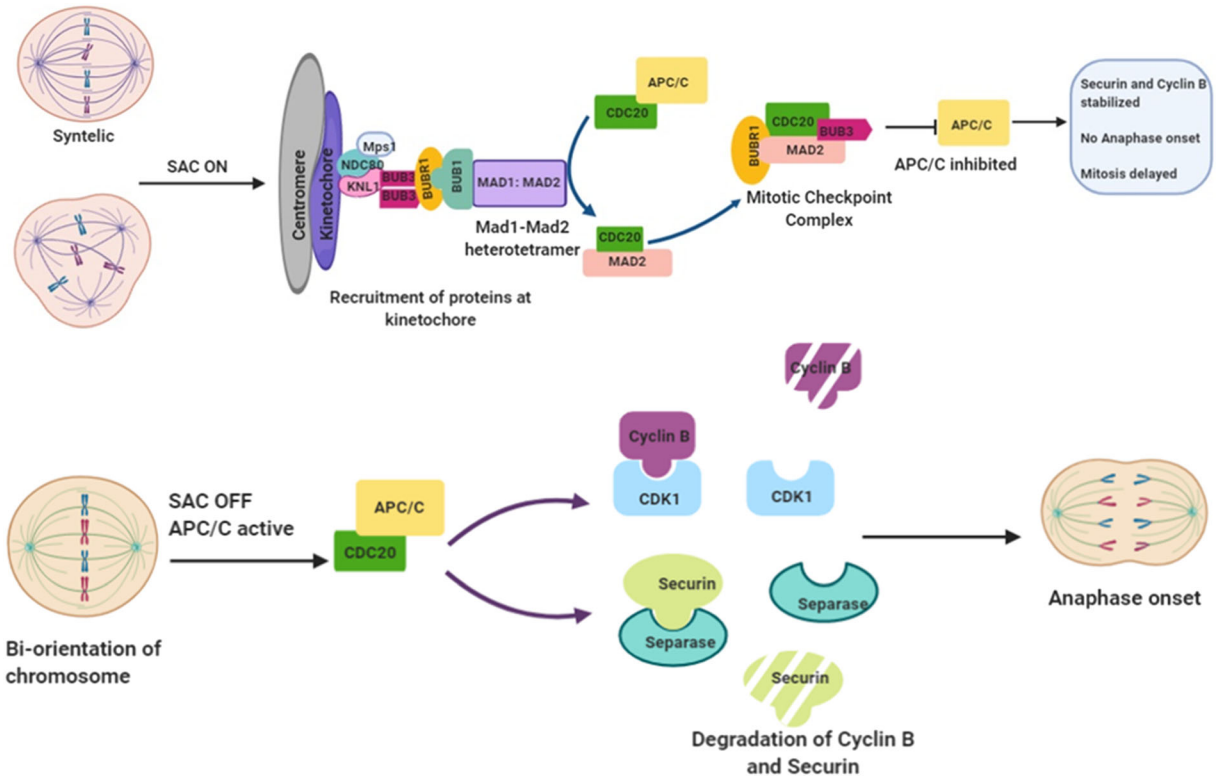

Fig. 2 Schematic diagram of SAC ON and SAC OFF states. SAC spindle assembly checkpoint

The role of mitotic checkpoint defects in carcinogenesis

Mutations in SAC genes

The mitotic checkpoint signaling is not an all-or-none response because the strength of the checkpoint response varies with the number of unattached kinetochores (Collin et al. 2013). Cahill et al. (1998) first reported that inactivating BUB1 mutations generated a weakened checkpoint response and caused chromosomal instability CIN in a subset of colon cancer cell lines. Lee et al. (1999) reported about acquired mutations in BUB1 and p53 genes and loss of spindle assembly checkpoint in tumors with BRCA2 deficiency from animals. They concluded that these inactivating mutations of the checkpoint genes cooperate with BRCA2 deficiency, thus contributing to tumorigenesis in inherited breast cancer. The somatic missense mutation in the mitotic checkpoint gene hBUB1, a polymorphism in codon 93 of exon 4, a substitution of guanine-to-thymine, was first reported in a lung cancer cell line and a primary lung tumor (Gemma et al. 2000). On the other hand, Hernando et al. (2001) screened for various potential mutations in hsMAD2, hBUB1, and hBUB3 genes in bladder cancer, soft tissue sarcomas, and hepatocellular cancer tissues as well as cell lines. They found some rare point mutations in the respective diseases which may not be responsible for cancer development. These findings were similar to the other study that showed mutational inactivation of hsMAD2 was less frequent in sporadic digestive tract cancers (Imai et al. 1999). Tsukasaki (2001), examined a total of 44 cell lines (hematopoietic, prostate, osteosarcoma, breast, glioblastoma, and lung) and 133 fresh cancer cells (hematopoietic, prostate, breast, and glioblastoma) for alterations of MAD1L1 and found eight heterozygous mutations with high mutation frequency in prostate cancer. They placed a truncated MAD1L1 in three different cell lines and observed less inhibitory effect of cell proliferation when compared to the wild type. Thus, they concluded that MAD1L1 has a potentially pathogenic role in carcinogenesis. Only one clinical study observed hBUB1 missense mutation (Ala130Ser) was associated with lymph node metastasis in colon cancer and thus, it is an indicator of disease progression

(Shichiri et al. 2002). Guo et al. (2010) reported missense variations of MAD1L1 and MAD2L1 confers susceptibility to lung cancer and weakens the SAC function. Zhong et al. (2015) identified two genetic variations, MAD1L1 Arg558His and MAD2L1 Leu84Met, to be associated with increased risk of colorectal carcinoma in the Chinese population. In our departmental clinical study, we have found prevalence of MAD1 and MAD2 mutations in Indian ovarian cancer patients who may carry the risk of developing the ovarian carcinoma (Sarkar. et al., unpublished observations).

Altered protein expressions of SAC machinery are common in cancer cells

Apart from mutational defects, the checkpoint-induced aneuploidy is often associated with deregulation of mRNA and changes in the protein levels of the primary SAC components. Dai et al. (2004) reported that RNA interference mediated downregulation of BubR1, significantly reducing the levels of securin. They also observed rapid development of lung and intestinal adenocarcinomas in BubR1 (+/−) haploinsuffient mice as compared to their wild-type littermates, when challenged with carcinogens. Significant increase in number of aneuploid fibroblasts was observed in mice with reduced levels of MAD2 and BUB1B. Tumor incidence increased up to 6% in mice with severely reduced BUBR1 and mice heterozygous for functional BUBR1 had a tendency of developing colon and lung carcinomas after treatment with AOM and DMBA, respectively. Twenty-eight percent of mice developed papillary lung adenocarcinomas (Dai et al. 2004; Michel et al. 2001). Furthermore, overexpression of CDC20 was observed in oral cancer cell lines and primary tumors that were associated with premature anaphase onset and chromosomal abnormalities in oral squamous cell carcinomas (Mondal et al. 2007). It is known that along with Mad2, other factors like Bub3/BubR1 also act as more potent inhibitors of Cdc20 function during SAC arrest. Thus, during SAC arrest, the cellular Cdc20 levels are decreased via degradation by the APC complex (Pan and Chen 2004; Michel et al. 2004a, b). So, the observation of Mondal et al. support the prediction that the mitotic spindle checkpoint is one of the important points of failure in chromosomal instability and altered expression of genes involved in this pathway would be expected to be a contributor to human cancer (Mondal et al. 2007). High CDC20 expression was correlated with high tumor grade in ovarian cancer (Gayyed et al. 2016). The cytokinesis and mitotic exit are the final stages of mitosis. Cytokinesis is inhibited when MAD2 is overexpressed which stabilizes securin and cyclin B. Hence, tumors with overexpressed MAD2 contribute to cytokinesis failure leading to both aneuploid and tetraploid cells (Hernando et al., 2004; Sotillo et al. 2007). The protumorigenic effect can be observed here as overexpression of this SAC component deregulates the timing of APC/C and induces aneuploidy, but the oncogenic process seems to be unclear. MAD2 overexpression specifically can increase the susceptibility to hepatoma, hepatocellular carcinoma, lung adenoma, fibosarcoma, and lymphoma as established (Sotillo et al. 2007). Their results suggest that deregulation of mitotic checkpoint pathways by Rb inactivation or other mechanisms may be an early and transient event in the initiation and evolution of a wide variety of common cancers.

MAD2 is overexpressed in several other tumors of different origins like osteosarcoma, endometrial, gastric, mucinous ovarian, nasopharyngeal, soft tissue sarcoma, testicular germ cell, hepatocellular, prostate, breast, and lung that are highly proliferative, and its expression level typically correlates with ki67 labelling indices in patients (Murray et al. 2012; McGrogan et al. 2014; Bargiela-Iparraguirre et al. 2016). Contradictorily, Wang et al. (2019) found that MAD2 was downregulated in cervical cancer and HSIL followed by LSIL and chronic cervicitis. Recently, Huang et al. (2020) reported that TTK or MPS1 expression was significantly higher in gastric cancer cell line than that of immortalized gastric epithelial cells and it was essential for malignant cell survival and proliferation. It was also reported that TTK regulates the apoptosis and proliferation of tumor cells through Akt-mTOR pathway.

Are different pathways involved?

Furthermore, there are evidence of involvement of different pathways in the regulation of expression of SAC components. The transcription of CDC20 and BUB1B is controlled by tumor suppressor gene p53. Banerjee et al. (2009) reported that both ectopically expressed and DNA damage induced endogenous p53 downregulates CDC20 by binding the consensus p53 binding site on CDC20 promoter and through chromatin remodeling. Similarly, MAD1 expression is repressed by direct recruitment of p53 on promoter consensus element (Chun

and Jin 2003). Upregulated MAD1 has an interphase role in tumor promotion via the p53 destabilization by displacing MDM2 from Promyelocytic leukemia (PML) nuclear bodies in breast cancer and cultured cells (Wan et al. 2019). Li and Zhang (2003) reported overexpression of MAD2 associated with mutated p53 in colorectal carcinogenesis. BRCA1 is a positive regulator of MAD2 transcription, and BRCA1 mutations contribute to MAD2 downregulation. MAD2 and BRCA1 low co-expression has been established as a significant prognostic marker for overall survival of high-grade serous ovarian carcinoma (Byrne et al. 2018). Inactivating RB mutations deregulates the E2F family of transcription factors resulting in MAD2 overexpression and chromosomal instability (Schvartzman et al. 2011). Sotillo et al. (2010) investigated the tumorigenesis pathway after MAD2 overexpression but concluded that this does not affect the regression of Kras-driven lung tumors upon Kras inhibition. MAD2 also interacts with DNA repair protein XPD, FAT10, ER-beta (Bates et al. 2020). Increased MAD2 and BUBR1 expression in advanced stage ovarian tumors are correlated with increased cellular proliferation. Reduced nuclear intensity of MAD2 identified patients with poorer time to recurrence irrespective of their tumor histologic subtype or treatment received (McGrogan et al. 2014). BUB1 is identified as histone H2A kinase and BUB1-KD has generated multinucleated cells and disrupted cancer cell growth in vivo and in vitro (Maeda et al. 2018). The cellular H2A serine 121 is replaced by alanine in histone H2A S121A mutant, which mimics the Bub1 kinase-dead mutant (Bub1-KD) in disturbing the centromeric localization of Shugoshin protein, which plays multiple roles in ensuring the accuracy of chromosome segregation during both mitosis and meiosis. Thus, these observations strengthen the case for the importance of histone H2A T120 phosphorylation by BUB1 in chromosome segregation during mitosis (Maeda et al. 2018).

Data needed!

The role of malfunctioning spindle assembly checkpoint in tumorigenesis remains an exciting challenge. The mutations in mitotic checkpoint are rare and can be mostly found in colon, gastric, lung, and breast cancers. However, their presence and functions cannot be ignored. In addition, the variations of gene expressions that are found in different types of cancer correlate with tumor progression, grade, and chemotherapy outcomes and precipitate the CIN phenotype. In the MAD2 overexpressed tumors, SAC has failed to restrain the cell cycle progression which leads to chromosomal missegregation. Cancer-associated defects in the tumor suppressor genes also contribute to the abnormal expression of SAC proteins. The large amount of studies on the SAC components suggest that they could be useful biomarkers for tumor proliferation as well as aneuploidy and tetraploidy, depending on the cancer type. Overexpression of MAD1, MAD2, CDC20, BUB1, and BUBR1 is correlated with poor overall survival in cancer patients (Sun et al. 2013). The MPS1 has potential as a diagnostic biomarker for cancer (Xie et al. 2017). The exact mechanism of tumorigenesis driver involving SAC is still under investigation. Both increased and decreased SAC gene expression can create a favorable environment for neoplastic transformation, depending on their regulation of gene expression and the gene functions. However, the topic is still under speculation whether these mutations/altered gene expression are the cause or the result of carcinogenesis. Hence there is need to fill the gap between other players involved in triggering carcinogenesis after mitotic checkpoint-mediated aneuploidy.

The role of mitotic checkpoint defects in development of drug resistance

The spindle poisons

Spindle poisons or the microtubule targeting agents (MTAs) either stabilize (taxol) or destabilize (Vinca alkaloid, nocodazole) the microtubule polymerization disrupting the microtubule dynamics and activating the mitotic checkpoint. Upon activation of mitotic checkpoint, the cells undergo a prolonged mitotic arrest. Paclitaxel and Vinka alkaloids are prototypes of MTAs, which are used in the first-line treatment of several cancers of breast, ovarian, non-small cell lung, and head and neck (Rowinsky and Donehower 1995). Mitosis-arrested cells eventually die by the activation of intrinsic apoptosis pathway as accumulation of proapoptotic signals has been observed by many groups (Hashchka et al. 2018; Shi et al. 2011; Gascoigne and Taylor, 2008).

Mitotic slippage: unsolved mystery

Mitotic slippage is a process that occurs after mitotic arrest with an active checkpoint when APC/C-CDC20-mediated degradation of cyclin B exceeds a certain threshold before apoptosis is initiated resulting in chromosome missegregation and generation of tetraploid cells. Again, depending on the status of the genes, TP53, Rb, P38 and the degree of DNA damage caused by MTAs, the slipped cells may arrest in interphase and undergo senescence or post-mitotic death (Rieder and Maiato 2004). The G1 tetraploid cells are generally unfit to proceed to mitosis, but the tetraploid DNA content and supernumerary centrosome can further duplicate and induce genome instability (Storchova and Kuffer 2008). A few studies have also demonstrated a correlation between increased proportion of mitotic slippage and drug resistance in vitro. Cells overexpressing p31comet displayed increased tendencies to enter mitotic slippage and cell survival following exposure to antimicrotubule drug treatment in cancer cell lines (Ma et al. 2012, Habu and Matsumoto 2013). Similarly, weakening of SAC was exploited in studies that used si-RNA and target-specific miRNA against MAD2 or inhibitors of MPS1, and it was found that mitotic slippage limits the efficacy of antimicrotubule drugs while promoting resistance (Prencipe et al. 2009; Tambe et al. 2016; Haschka et al. 2018). Contradictory results were observed when other studies promoted mitotic slippage by using CDK1 inhibitor (Xiao et al. 2014; Sakurikar et al. 2014; Chan et al. 2008), Aurora kinase inhibitor (Salmela et al. 2013), hyperthermia (Giovinazzi et al. 2013), DNA damage (Bukowska et al. 2016), siRNAs against BUBR1 (Lee et al. 2004), and found increased efficacy of the drugs. Cheng and Krasta (2017) reviewed that mitotic slippage can induce senescence that potentially acquire chemoresistance by a SASP (senescence-associated secretory phenotype) mediated pathway. The SASP phenotype in cells can also exhibit pro-tumorigenic effects like proliferation, migration, invasion and angiogenesis (Fig. 3).

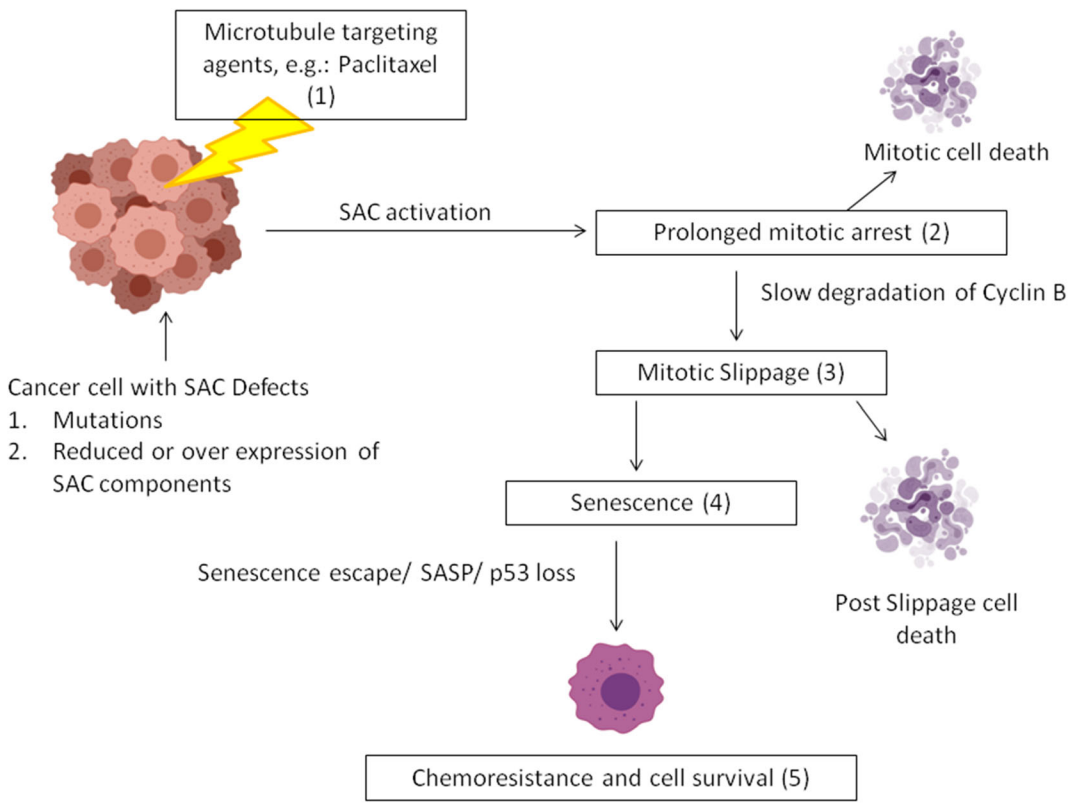

Fig. 3 Different outcomes after administration of microtubule targeting drugs in cancer cells with defective spindle assembly checkpoint (SAC). SASP senescence-associated secretory phenotype

Upregulation and downregulation of protein: good or bad?

Drug sensitivity or resistance has been associated with altered expression of various SAC components. The first evidence of paclitaxel sensitivity being dependent on a functional SAC showed that downregulation of MAD2 and BubR1 increased cell survival (Sudo et al. 2004). Overexpression of cyclins E and A are linked with adverse outcomes (Takahashi et al. 2005). These cyclins are important regulators of CDK1 activity, which is required for cells to enter mitosis and SAC functionality. Thus, the cyclins have associations with taxane sensitivity. A nexus between a weakened SAC and antimicrotubule drug resistance in vitro has become apparent (Weaver and Cleveland 2006; Yamada and Gorbsky 2006). Paclitaxel-resistant ovarian cancer cell line demonstrated weakened SAC with reduced BUBR1 expression (Fu et al. 2007). Zhou et al. (2010) showed that the upregulation of Akt2 mediates paclitaxel resistance in A2780 ovarian cancer cells by inhibiting Bub1 expression. In another study, MAD2 was knocked down by MAD2-specific siRNA in paclitaxel-sensitive A2780 cells and recombinant eukaryotic expression plasmid pEGFP-MAD2 was transfected into paclitaxel-resistant SKOV3 cells. Results of paclitaxel sensitivity assay revealed that paclitaxel sensitivity reversed in both the cell lines after transfection in terms of cells arrested at G2/M phase and Bcl-2 expression significantly changed. They suggested that weakened SAC with reduced MAD2 expression was associated with paclitaxel resistance in ovarian cancer cells with involvement of Bcl-2 in the process (Hao et al. 2010). Increased expression of cyclin G1 (CCNG1), an atypical cyclin, leads to paclitaxel-induced, SAC-mediated mitotic arrest, independent of p53 integrity or signaling through the SAC component, BUBR1. CCNG1 overexpression aids to cell survival after paclitaxel exposure and conversely, cyclin G1 depletion by RNA interference delays mitotic slippage and taxane-induced apoptosis (Russell et al. 2012). Santibanez et al. (2013) showed that a MAD1 1673 G → A polymorphism identifies worse response to chemotherapeutic agents. This polymorphism also alters the dynamics of microtubules and affects SAC functionality in ovarian cancer patients (Santibanez et al. 2013). Similar results are observed in our departmental study which shows majority of ovarian cancer patients having a dominant allele of MAD1 and MAD2 SNPs are non-responders to the first-line platinum and taxane chemotherapy (Sarkar eta al, unpublished observations).

The miR-493-3p confers resistance to microtubule drugs in cancer cells and high level of this particular miR-493-3p is associated with reduced survival of ovarian and breast cancer patients undergoing paclitaxel therapy with aggressive tumors. This microRNA targets the 3′UTR of MAD2 mRNA, thereby preventing translation of MAD2 protein and in cancer cells, and its overexpression induces premature mitotic exit leading to increased frequency of aneuploidy and cellular senescence. Tambe et al. (2016) suggests that intratumoral profiling of miR-493-3p and MAD2 can have diagnostic value in predicting efficacy of taxane drugs. Direct targeting MAD2 by siRNA using chitosan nanoparticles induced apoptosis and restored cisplatin sensitivity in a cisplatin-resistant lung cancer model. High-grade serous ovarian cancer with low expression of MAD2 correlates with shorter time to recurrence and a paclitaxel-resistant senescent cell phenotype (Nascimento et al. 2016). Syneuclein gamma is a breast cancer-specific gene which interacts with checkpoint proteins CDC20 and BUBR1 and compromises SAC function thus promoting resistance to chemotherapy-induced apoptosis (Miao et al. 2014). Contradictorily, Schukken et al. (2017) reported that a defective SAC is particularly sensitive to microtubule poisons. Furlong et al. (2012) performed in vitro analyses of five ovarian cancer cell lines and demonstrated that cells with low MAD2 expression were less sensitive to paclitaxel and paclitaxel-induced activation of the SAC and apoptosis were abrogated in cells transfected with MAD2 siRNA. They also reported that miR-433 has a binding domain in the Mad2 3′UTR and Mad2 expression was down-regulated in pre-miR-433 transfected A2780 cells. They further concluded that reduced MAD2 protein expression in pre-miR-433-transfected A2780 cells rendered the cells less sensitive to paclitaxel. MAD2 expression is also correlated with risk for recurrence in ovarian serous adenocarcinoma. The expression was significantly lower in recurrent group than in the relapse-free group. The overall survival was also significantly shorter in the low-expression group than the high-expression group (Nakano et al. 2012).

BRCA1 and p53 regulates the BUBR1 expression. BRCA1 is also a co-activator of p53 and positively regulates MAD2 thus influences APC inhibition. Therefore, in cells with BRCA1 deficiency, premature onset of anaphase onset is observed that is linked to paclitaxel resistance (Chabalier et al. 2006; Egawa et al. 2001). It is known that tumor cells become resistant to both

chemo- and radiotherapy in a hypoxic microenvironment, and it is an interesting finding that MAD2 is also downregulated in the hypoxic environment of different cancers (McEvoy et al. 2015; Mcdermott et al. 2016; Yasuda 2008). ULK1, a key protein in autophagy, phosphorylates MAD1and strengthens the interaction between MAD1 and RZZ complex. The deletion of ULK1 increases CIN and cytotoxicity of paclitaxel, resulting in impairment of cancer cell growth. These findings suggests that ULK1 as a protein kinase controls the fidelity of chromosome segregation (Yuan et al. 2019). Thus, the spindle assembly checkpoint is critical for clinical efficacy of taxane drugs. There is a crosstalk between various other signaling pathways with these checkpoint components that influence the drug sensitivity which needs to be extensively researched.

Drug development exploiting the mitotic checkpoint

The enginery that delays the anaphase onset upon incorrect kinetochore attachments has been and is being exploited for developing successful drugs in order to improve cancer treatment strategy. Theoretically, as per Taylor et al. (2018), if death signals accumulation is accelerated and/or mitotic slippage is retarded, then it should be possible to have a control over cell fate and influence the efficacy of antimitotics. The strategy to accelerate apoptosis would lead to cell death before mitotic slippage while the strategy to delay mitotic slippage will increase the time cells spent in mitosis allowing more time to apoptosis signals to accumulate (Gascoigne and Taylor 2008; Bennett et al. 2016). Both the strategies might improve the sensitivity of apoptosis resistant, slippage-prone or SAC defective cancer cells to the antimitotics. Henriques et al. (2019) targeted the SAC silencing pathway in order to increase the duration of mitosis and promote cell death. The role of transcription factors of the SAC proteins seems to be crucial in determining the cell fate, tumorigenesis, and chemotherapy outcomes. This is a huge research area that needs to be focused immediately to understand the threshold levels of the proteins required for an accurate functioning of the SAC. Cheng and Krasta (2017) propose to develop targeted therapies that eliminate senescent cells and may act synergistically with the MTAs.

Some of the recent studies are biomarker-driven and development of novel therapeutic targets exploring the mitotic checkpoint and auxiliary pathways for better chemotherapy outcomes. MLN4924, which inhibits mitotic slippage in human cells, has recently been proposed as a beneficial combination therapy (Balachandran et al. 2016). Aurora kinase A synergistically enhances cytotoxicity in ovarian clear cell carcinoma cell lines when treated with cisplatin and ENMD-2076. Aurora kinase A can also be a promising biomarker for predicting patient outcomes (Chiba et al. 2017). A study on 263 ovarian cancer patients (stages I/II) revealed the association of bad prognosis with high Polo-like kinase (PLK) 1 expression. Strong mitotic arrest in ovarian cancer cell lines was induced by triple treatment with paclitaxel, BI6727 and proTAME which targets the microtubules, PLK1 and anaphase-promoting complex, respectively. This triple treatment activated apoptosis in cell lines and primary ovarian cancer cells derived from patients. BI6727/paclitaxel/proTAME stabilizes cyclin B1 and leads to mitotic arrest, which initiates mitochondrial apoptosis by inactivating antiapoptotic Bcl-2 family proteins and is followed by caspase-dependent effector pathways. The triple treatment has prevented endoreduplication and reduced CIN, the two mechanisms involved in drug resistance, and thus, it has important implications for developing paclitaxel-based combinatorial treatment in ovarian carcinoma (Raab et al. 2019). Ubiquitin-associated and SH3 domain containing B (UBASH3B) have been found to control SAC silencing and faithful chromosome segregation and loss and gain of function of UBASH3B have strong effects on mitotic progression. The downregulation of UBASH3B prevents SAC satisfaction and leads to inhibition of chromosome segregation, mitotic arrest, and cell death. Data mining approaches identified a correlation between mRNA levels of UBASH3B and SAC components in a set of primary patient tumors including kidney and liver carcinomas. Thus, inhibition of UBASH3B offers an attractive therapeutic perspective for cancers (Krupina et al. 2017). Bhattacharjya et al. (2013) reported miR125b suppresses MAD1 and thereby shifts the cells towards apoptosis. Millepachine is a novel compound found to regulate and influence SAC (Wu et al. 2018). Silva et al. (2019) reported BUB3 and Spindly to be potential biomarkers of proliferation in oral cancer and highlighted the therapeutic benefit of inhibiting these two markers with cisplatin (Silva et al. 2019). BOS172722 is a potent and orally bioavailable inhibitor of spindle assembly checkpoint kinase MPS1, indicated for triple-negative breast cancer (Anderhub et al. 2019). Apcin is a small molecule inhibitor that causes either net APC/C inhibition, prolonging mitosis when SAC activity is lower, or net APC/C activation,

shortening mitosis when SAC activity is higher, demonstrating that it can produce opposing effects depending on regulatory context (Richeson et al. 2020). Crocin, a saffron spice constituent, exerted antiproliferative effects by activating spindle assembly checkpoint through BUBR1 and MAD2. They synergistically improved the effectiveness of cisplatin, doxorubicin, and combrestatin-A-4 in HeLa cells (Sawant et al. 2020).

Conclusion and future perspective

The signaling cascade of spindle assembly checkpoint (SAC) has long been underestimated as a simple mechanism, and its effects were thought to be one-directional. However, from the plethora of research results, it was found that components of the mitotic checkpoint have roles within mitosis and outside mitosis. It is also observed that an inefficient SAC can produce unwanted effects such as developmental defects, aneuploidy, cancer, and drug resistance. A true understanding of the molecular pathway of SAC and its crosstalk with other pathways is yet to be unraveled. The events that occur at the kinetochore and the cytosol to generate a strong signal and a failed signal are yet to be understood. Faulty SAC-mediated aneuploidy does not seem to be a strong enough signal for the cancer development, but the effects of mutations and protein expressions cannot be ruled out entirely. Similarly, the SAC-mediated drug resistance is a debatable topic with contradictory results from different studies. Majority of the observations have been made based on results from transformed cell lines and require strong validation on clinical settings. The mitotic checkpoint has been explored in various ways for various research objectives focusing on the drug development but extracting a collective conclusion and connecting the dots between these research outcomes remains to be a huge task for future.

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Compliance with ethical standards

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ORIGINAL ARTICLE

Evaluation of numerical rating scale and neuropathic pain symptom inventory pain scores in advanced ovarian carcinoma patients undergoing surgery and first-line chemotherapy

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ABSTRACT

Background and Aim: Advanced epithelial ovarian cancer (OC) has a high disease manifestation with difficult-to-manage symptoms that limit the patients' functionality. Abdominal pain, persistent back pain, and neuropathic pain are among the common discomforts associated with OC and its treatment. Our study aims to determine pain scores in advanced OC patients undergoing surgery and chemotherapeutic treatment with carboplatin and paclitaxel.

Methods: One hundred and ten patients with advanced epithelial OC were enrolled and treated with surgery and an adjuvant/neoadjuvant chemotherapy regimen of carboplatin-paclitaxel for six cycles (triweekly). Pain intensity was analyzed using the validated numerical rating scale for resting, movement, sleep interference-associated pain, and neuropathic pain scores were evaluated using the neuropathic pain symptom inventory scale. Pain was correlated with Qol according to Fact-O questionnaires. Chemo-response was evaluated using the CA125 blood biomarker and CT scan of the abdomen and thorax. Data were recorded at baseline, 2, 4, and 6 months of the six chemotherapy cycles.

Results: Of the 110 patients, no statistically significant differences were found in pain at baseline and after treatment ($P > 0.05$) and between the responder and non-responder categories ($P > 0.05$). However, movement-associated pain had a significant correlation with chemo-response and a strong positive correlation with the patients' physical and functional wellbeing. There were more chemo-induced neuropathy occurrences ($P = 0.001$) in the neoadjuvant chemotherapy group.

Conclusion: Patients in the neoadjuvant chemotherapy arm experienced more chemo-induced neuropathy that was persistent and did not improve with the treatment.

Relevance for Patients: Peripheral neuropathy is a common adverse effect of platinum and taxane chemotherapeutic drugs that persists throughout cancer treatment and in survivorship. This research provides evidence that chemotherapy-associated neuropathy affects Qol of patients and it will be helpful to improve pain and palliative care management policies.

1. Introduction

Carcinoma of the ovaries ranks fifth in cancer deaths among women, with an estimated 295,414 new cases and 184,799 deaths worldwide and India records 36170 new cases and 24,015 deaths per year with a 5-year survival rate of 48% [1,2]. The most relevant clinical symptoms of OC include persistent abdominal swelling, pain, bloating, vaginal bleeding, altered bowel habits, indigestion, and loss of appetite [3,4]. Pelvic and abdominal

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pain is expected in the advanced stages before diagnosis due to adnexal mass and accumulation of ascitic fluid leading to increased abdominal girth [5]. The standard chemotherapy comprising carboplatin and paclitaxel is the most important cause of neurotoxicity and neuropathic pain (painful paresthesia, diminished vibratory sense, and numbness among patients) [6]. Chemo-induced sensory neuropathy is reported as burning, pins and needles sensation, tingling, shooting, cramping, and deep aching [7,8]. About 60-85% of patients with ovarian cancer (OC) experience cancer or chemotherapy-related pain during their treatment or even afterward [9]. Persistent pain in cancer patients is associated with decreased quality of life (QoL), mostly lower levels of physical well-being, and an increase in dependency on healthcare services [10,11]. Advances in cancer diagnosis and treatment have dramatically increased the survival probability of patients with several cancers. For most cases pain is the first sign of cancer and the majority will experience low, and moderate to severe pain and/or neuropathy during their disease, chemotherapy, and survivorship [12].

To the best of our knowledge, there are limited reports of pain assessment among OC patients during their first-line treatment, and thus, we aim to evaluate the pain experienced and its impact on the physical and functional well-being of the OC patients at different time points.

2. Methods

2.1. Study design

This is a non-randomized and prospective study on advanced epithelial OC (FIGO stages III-IV) patients recruited after obtaining written informed consents, who underwent surgery from the Dept. of Gynecological Oncology and received their respective chemotherapy at Medical Oncology. The study was conducted between July 2018 and January 2021 and included patients older than 18 years, with histopathologically confirmed epithelial OC, adequate bone marrow, hepatic, neurologic, and cardiologic functions, adequate coagulation parameters, and ECOG performance status ≤3. Patients under the age of 18 years; with recurrent disease who have received prior radiotherapy, chemotherapy and; pregnant or nursing women; patients with other malignancies; acute hepatitis; active infection, uncontrolled diabetes, serious non-healing wound, bleeding disorder, coagulopathy, bone fracture; significant proteinuria; clinically significant cardiovascular complications and significant autoimmune disease uncontrolled with treatment, were ineligible and excluded from the study.

According to operability, patients underwent either primary debulking surgery with adjuvant chemotherapy or interval debulking surgery and neoadjuvant chemotherapy. The chemotherapy regimen consisted of intravenous doses of 175 mg/m^2 paclitaxel on Day 1 (3 h) + carboplatin AUC 5-6mg. min/mL (over 1 h) on Day 2 [13]. The regimen was repeated every 3 weeks for six cycles. The clinical pain intensity scores were recorded at hospital visits and the Palliative Care Unit for the admitted patients. Patient follow-up and data analysis were performed at Pathology and Cancer Screening, Chittaranjan National Cancer Institute (CNCI). The core dataset was compiled with demographic profile, clinical features, and post-chemo clinical response analyzed by blood biomarker CA125 and CT- scan of abdomen and thorax to categorize the responders, partial responders, and non-responders (NRs) [14-16].

This study was approved by Institutional Ethical Committee, CNCI (A-4.311/VN/27/06/2018-10).

Table 1. Demographic characteristics of ovarian cancer patients

Characteristics (*n*=110)	Frequency (%)
Age (years)	
20-40	24 (21.8)
41-60	76 (69.09)
61-80	10 (9.09)
Education	
Illiterate	39 (34.5)
School education	61 (54.0)
Graduates and above	10 (8.8)
Religion	
Hindu	90 (79.6)
Muslim	20 (10.7)
Marital status	
Unmarried	7 (6.2)
Married	86 (76.1)
Widowed/Divorced	17 (15.0)
Occupation	
Unemployed/Housewife	83 (73.5)
Self employed/business	8 (7.1)
Professional/Desk job	7 (6.2)
Laborer	7 (6.2)
Farmer	5 (4.4)
Setup	
Urban	37 (32.7)
Rural	73 (64.6)
Monthly income	
<Rs 2000/-	26 (23.6)
Rs 2001 to Rs 5000/-	75 (68.2)
Rs 5001 and above	9 (8.2)

n=numbers of the patients; percentage (%) and all values represent in frequency

2.2. Pain intensity and QoL measurement

The evaluation of pain intensity scores of patients was conducted using the numerical rating scale (0-10) for spontaneous resting, movement, sleep interference-associated pain, and neuropathic pain (neuropathic pain symptom inventory scale) [17-19]. Patients reported pain score 0, as no pain; and pain score of 10, as the worst pain imaginable [20,21]. QoL was assessed using FACT-O (Version 4) questionnaire (FACT-O) [22] and common adverse effects were also recorded and graded as per CTCAE [23]. The analysis was done at baseline, 2-, 4- and 6-months during hospital visits. Most of the patients were prescribed paracetamol, diclofenac (topical), tramadol, and rarely morphine for rescue

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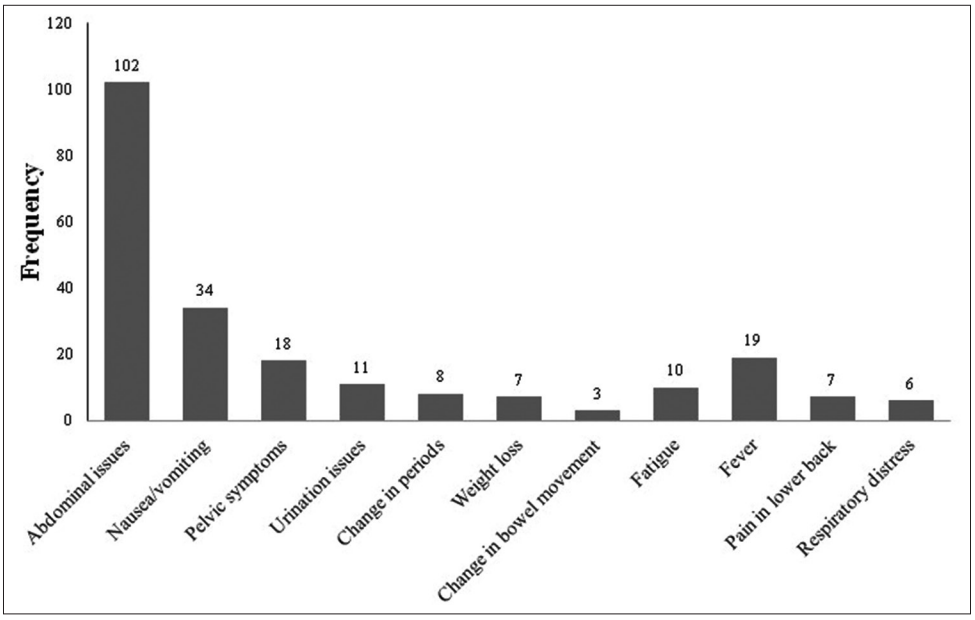

Figure 1. Distributions of symptom in patients with advanced ovarian cancer. Abdominal issues include pain, swelling, and bloating.

Table 2. Clinical characteristics of ovarian cancer patients

Characteristics (*n*=110)	Group	Frequency (%)
ECOG performance status	0	20 (18.9)
	1	68 (62.2)
	2	15 (13.5)
	3	7 (5.4)
FIGO Stage	III	91 (82.02)
	IV	19 (17.98)
Tumor histology	Serous/papillary	80.41 (81.0)
	Other	29.59 (26.9)
Gross type	Solid Cystic	65 (58.8)
	Cystic	32 (29.4)
	Solid	13 (11.8)
Size of tumor mass (pre-treatment)	>5 cm	80 (73.2)
	<5 cm	30 (26.8)
Clinical response	Responders	41 (37.3)
	Partial responders	44 (40)
	Non responders	25 (22.7)

n=numbers of the patients; percentage (%) and all values represent in frequency. Non Responders include Stable disease, Progressive, Time to treatment, palliative care and Not evaluable

analgesia. Gabapentin and Vitamin B12 were advised to them as a supportive treatment of chemo-induced neuropathy.

2.3. Statistical analysis

Descriptive statistics were used to analyze the frequencies of symptoms, clinical characteristics, and commonly observed toxicities among patients. Cross-tabulation was applied to find out significance (Chi-square, χ^2) of pain occurrence in groups (adjuvant and neoadjuvant chemotherapy). Two-way repeated measure analysis of variance was used for tests of within-subject effect for numerical rating scale (NRS) pain versus groups (responders, partial responders, and NRs). Greenhouse-Geisser and Wilk's Lambda significance values ($P < 0.05$) were taken into consideration. The correlation coefficient (R2) was analyzed between pain experienced by the patients with their functional and physical wellbeing.

3. Results

The demographic profile of the patients shows a majority in the age range of 41-60 years (69.09%), school-educated (54%), Hindu (79.6%), married (76.1%), unemployed/housewives (73.5%) belonging to rural setup (64.6%) (Table 1) and their symptoms are represented in Figure 1. As per FIGO 2018 staging, most cases were of Stage III (82.02%), serious histology subtype (81%). After clinical evaluation 41, 44, and 25 patients were responders, partial responders, and NRs, respectively (Table 2). The NRS score of resting, movement, and sleep interference were non-significant within-subjects and in multivariate analysis (Wilk's Lambda $P > 0.05$). Movement-associated pain was significant ($P = 0.032$) in within-subject effect using Greenhouse-Geisser indicating a difference of pain in various chemotherapy response categories (Table 3). Frequencies of various types of neuropathic pain were recorded throughout 6 months of treatment for burning, pressure, cold sensation, pins and needles, and tingling (Table 4). There were no significant changes observed in the physical and functional well-being of the patients at the end of the study (Table 5). However, movement-associated pain had a strong positive correlation ($R^2 = 1$) with the physical and functional wellbeing of the patients (data not shown), indicating that higher pain scores diminish the normal physical activity and functionality. Anemia ($n = 65$), vomiting ($n = 26$), depression ($n =$

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Table 3. Mean NRS scores in responders, partial responders and non-responders at various time intervals

Groups	Baseline	2nd month	4th month	6th month	*P*-value[ł] (within groups)
Resting stage					
Rs (n=41)	3.00±3.30	3.07±3.27	3.34±3.36	3.15±3.36	0.385
PRs (n=44)	2.93±3.40	2.57±3.2	2.48±3.34	2.86±3.38	
NRs (n=25)	3.84±3.78	3.72±3.72	2.96±3.47	3.40±3.62	
P-value* (between groups)		0.343			
Movement stage					
Rs (n=41)	2.37±3.11	2.46±3.09	2.63±3.23	2.76±3.12	0.032
PRs (n=44)	2.16±3.02	2.30±3.08	2.36±3.12	3.09±3.48	
NRs (n=25)	3.92±4.06	3.88±4.10	2.96±3.55	3.36±3.70	
P-value* (between groups)		0.081			
Sleep interference					
Rs (n=41)	2.32±3.36	2.17±3.19	2.51±3.23	2.54±3.37	0.499
PRs (n=44)	2.63±3.61	2.84±3.72	2.37±3.55	2.35±3.30	
NRs (n=25)	3.20±4.03	3.08±4.03	2.80±3.73	2.72±3.69	
P-value* (between groups)		0.603			

Responders (Rs); Partial Responders (PRs); Non Responders (NRs). All values are expressed as mean±SD. The NRS scores of resting, and sleep were non-significant within-subject effect and multivariate analysis. Only the movement stage pain was significantly (P=0.032) associated with chemotherapy response.
* Multivariate analysis (Wilk's Lambda)
[ł] Greenhouse-Geisser
Mauchly's sphericity was significant (P<0.05).

Table 4. Patient distribution having different types of neuropathic pain at various time intervals

Neuropathic pain type	Baseline	2 months	4 months	6 months
Burning	18	18	15	20
Squeezing	21	16	17	14
Pressure	17	13	10	17
Electric Shock	16	11	10	15
Stabbing	13	11	10	15
Light touch	16	15	11	13
Pressing	18	14	14	20
Cold	18	14	12	24
Pins/Needles	14	19	19	23
Tingling	13	13	16	20

37), neuropathy (n = 43), weight loss (n = 30), constipation (n = 40), indigestion (n = 47), and renal toxicities (n = 25) were the most commonly noticed adverse effects in the patient population during the time of treatment (Table 6). 55 patients received adjuvant and 55 received neoadjuvant chemotherapy and significantly more occurrences of neuropathic pain were reported in the neoadjuvant chemotherapy arm (P = 0.001) (Table 7).

4. Discussion

Pain is one of the most distressing and constant symptoms reported in 60% of women with cancer and its treatment [24-27]. In this study, maximum patients (92.7%) reported abdominal pain similar to Ferrell *et al*. [27]. The persistent abdominal issues, including pain and distension, are one of the most prevalent and discomforting symptoms that lead patients to seek medical attention followed by the diagnosis with ovarian carcinoma. No Grade 4 abdominal pain was observed in our study similar to Coleman *et al*. [28] and Cristea *et al*. [29] but contradictorily, Dizon *et al*. [30] reported dose-limiting toxicity and Grade 4 abdominal pain with intraperitoneal cisplatin therapy.

The patients were assessed for different kinds of pain from baseline to 6 months of their treatment. 57 (51.8%), 49 (44.5%), and 44 (40%) of patients reported resting, movement, and sleep interference-associated pain, respectively, at the time of study recruitment. Upon follow-up, after 6 cycles of chemotherapy, the frequencies of resting and sleep interference-associated pain remained the same but movement-associated pain increased by 6.4%. Movement-associated pain includes physical activity related to household work, certain individualized exercises, and specific functional tasks that patients do during the day [31]. Similar results were observed in the study by Portenoy *et al*. that reported 60% of advanced-stage OC patients experiencing pain at disease onset [24].

There are strong reports of sleep disturbances linked to depression/anxiety in OC patients [32,33]. Pain, however, is also not an isolated symptom and is mostly linked with fatigue, worrying, and disturbed sleep. In the present study, we could not assess pain associated with depression/anxiety, but the population represented significant percentages of sleep interference-associated pain (40%) and depression/anxiety (33.6%) that needed interventions to manage.

The movement-associated pain was significantly different in the responders and NRs throughout the study duration with higher intensities in the NRs group. It was also found to be associated with their physical and functional well-being.

In the present study, the neuropathic pain was primarily felt in the extremities (fingers, toes, and legs) similar to Ezendam *et al*. [34]. Burning, pressing, cold sensation, pins/needles, and

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Table 5. Physical and functional QoL mean scores

Qol Domains	Groups	Baseline	2nd month	4th month	6th month	*P*-value[i]
Physical	Rs (n=41)	8.15±7.18	8.37±6.97	9.54±7.24	9.90±6.94	0.425
	PRs (n=44)	8.43±8.01	8.32±8.00	8.95±7.58	9.52±7.86	
	NRs (n=25)	8.32±8.76	7.52±8.75	7.8±8.06	6.72±7.03	
P-value*			0.347			
Functional	Rs (n=41)	7.07±6.59	7.24±6.71	8.22±6.72	8.73±6.67	0.846
	PRs (n=44)	7.55±7.17	7.64±6.93	7.68±6.63	7.70±6.63	
	NRs (n=25)	6.72±6.73	5.92±6.72	6.32±6.75	6.68±7.25	
P-value*			0.609			

All values are expressed as mean±SD. The physical and functional well-being were non-significant for within-subject effect test and multivariate analysis. *Multivariate analysis (Wilk's Lambda). [i]Greenhouse-Geisser. Rs: Responders; PRs: Partial responders; NRs: Non responders

Table 6. Grades of common side effects experienced by the patients (*n*=110)

Toxicities	Frequency (%) of grades 1-2	Frequency (%) of grades 3-4
Anemia (*n*=65)	56 (50.9)	9 (8.1)
Leukopenia (*n*=16)	15 (13.6)	1 (0.9)
Thrombocytopenia (*n*=6)	6 (5.45)	0 (0)
Granulocytopenia (*n*=3)	3 (2.7)	0 (0)
Nausea (*n*=18)	16 (14.5)	2 (1.8)
Vomiting (*n*=26)	22 (20)	4 (3.6)
Anxiety/depression (*n*=37)	31 (28.1)	6 (5.4)
Neuropathy (*n*=43)	40 (36.36)	3 (2.7)
Weight loss (*n*=30)	30 (27.2)	0 (0)
Diarrhoea (*n*=35)	29 (26.3)	6 (5.4)
Constipation (*n*=40)	31 (28.1)	9 (8.1)
Indigestion (*n*=47)	40 (36.36)	7 (6.3)
Abdominal pain/swelling (*n*=33)	31 (28.1)	2 (1.8)
Renal toxicity (*n*=25)	24 (21.8)	1 (0.9)

Table 7. Distribution of different grades (CTCAE) of neuropathy in adjuvant and neoadjuvant chemotherapy arms

Adverse effect grades	0	1	2	3	Total	Pearson Chi-square
Adjuvant chemotherapy	42	7	3	3	55	*P*=0.001
Neodjuvant chemotherapy	31	19	2	3	55	

tingling were the most reported chemo-induced peripheral neuropathy (CIPN) in the patient population. These symptoms were persistent throughout the study and did not significantly correlate with the treatment outcomes. The possible causes of persisting pain throughout the chemotherapy are tissue damage and nerve damage caused by carboplatin and paclitaxel [35,36] as common anti-neoplastic agents such as vinca alkaloids, platinum compound, and taxanes frequently induce a CIPN where both large and small primary afferent sensory neurons are injured.

Tumour-induced cancer pain tends to increase with advancing disease and can be driven by tumor-released products, acidosis, and direct injury to sensory nerve fibers present at the site of the primary tumor or the site of tumor metastasis. Our study reported 39.09% of patients with neuropathy. The observation was similar to the study by Nho *et al*. that reports about pain and CIPN symptom clusters to negatively impact general QoL, and Bonhof *et al*. reported worse global QoL and functioning in patients with high motor peripheral neuropathy [37,38]. Between-group differences were not observed in neuropathic pain. Quality-of-life improvement was not observed in our study previously published [39] similar to the outcome reported by Magnowska *et al*. [40]. Unlike our study, they also reported gabapentin to benefit CIPN.

The limitations of the present study include its non-randomized design and small sample size that may have a chance of bias. The study also failed to follow up the patients till the recurrence of the disease and conclude about the role of pain. However, these reports will encourage further studies on better pain management on a personalized setup to improve QoL. With the incorporation of cancer pain research into conventional oncology research, it is possible that analgesic and oncologic therapies can be parallelly evaluated with regard to the effects on survival, overall health, and QoL of both cancer patients and survivors.

5. Conclusion

The summary of results demonstrates no improvement of pain at diagnosis and after completion of six cycles of chemotherapy. There were significantly more occurrences of neuropathic pain in the neoadjuvant chemotherapy arm than adjuvant chemotherapy and did not improve with the treatment. Movement-associated pain was worse in chemotherapy NRs that debilitates the physical and functional well-being of patients.

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Conflict of Interests

The authors declare no conflict of interest.

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had undergone loop electrosurgical excision procedure (LEEP) as treatment for cervical dysplasia.

Methods: A retrospective analysis of 397 women who underwent LEEP at the University Hospital of Basel between 2015 and 2019 was conducted. Post-interventional pregnancies were analyzed regarding pregnancy outcome, time interval between conization and cone dimensions.

Results: Forty-nine women (12.3%) became pregnant within the observation period. The average interval between LEEP and pregnancy was 14 months (range 1-48 months; median 11 months). Thirty-two (97%) of all live births were at term (≥37 weeks); only one delivered preterm. Cervical shortening (≤20 mm) occurred in three pregnancies (9.1%), although all of these women carried to term. Early loss of pregnancy occurred in six patients (12.2%). There was no significant difference in the cone dimensions of women with term pregnancies compared to those with pregnancy loss (P=0.199 for length, P=0.205 for width and P=0.967 for depth).

Conclusions: While the number of preterm pregnancies following conization in this study was too low to make statistical conclusions, only one of the 33 women with live births delivered before term. The number of pregnancy losses did not differ significantly from the rate observed in the general population (P=0.163). Despite the low numbers, these results do not differ from larger studies on this subject and may still be useful for counseling young women planning future pregnancies regarding treatment of cervical dysplasia.

P0717 | ORAL APATINIB MAINTENANCE TREATMENT OF PERITONEAL PSEUDOMYXOMA: 1 CASE REPORT AND LITERATURE REVIEW

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/ SUB-THEME: AB 8.4 MEDICAL TREATMENT OF MALIGNANCIES

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Objectives: Pseudomyxoma of the Peritoneum (PMP) is a rare disease. Most PMP originates from the rupture of mucinous appendix tumors. Patients die of intestinal obstruction caused by pseudomyxoma of the peritoneum. Current treatments include complete cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC). There is still lack of effective treatment methods for patients whose lesions cannot be completely removed through above treatments. The anti-VEGF drug bevacizumab can prolong PFS and OS in all kinds of histologic types of appendix epithelial tumors, including peritoneal pseudomyxoma. As a VEGF-2 tyrosine kinase inhibitor, Apatinib can inhibit cellular proliferation and stimulate its apoptosis.

Methods: We report a patient oral apatinib treatment, who is still have residual lesions in the abdominal cavity after surgery and intraperitoneal hyperthermic perfusion treatment.

Results: Up to March 2021, the patient has been treated for 39 months. The disease has not progressed, and the patient's quality of life is satisfactory. As far as we know, this is the first successful application of apatinib alone in the treatment of pseudomyxoma of the peritoneum, and a large sample of cases needs to be further included in the study of the effectiveness and safety of apatinib in the treatment of pseudomyxoma of the peritoneum.

Conclusions: Apatinib can be considered as one of the maintenance treatments for peritoneal pseudomyxoma after cytoreductive surgery and intraperitoneal hyperthermic perfusion.

P0718 | MAD1L1 AND MAD2L2 POLYMORPHISMS IN ADVANCED OVARIAN CARCINOMA PATIENTS AND ITS CLINICAL IMPACT

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/ SUB-THEME: AB 8.4 MEDICAL TREATMENT OF MALIGNANCIES

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Objectives: The aim of this study was to investigate the associations between the MAD1L1 and MAD2L2 polymorphisms in advanced ovarian carcinoma (OC) patients and their clinical impact.

Methods: The study comprised of 45 patients with newly diagnosed advanced (Stage III and IV) OC who were undergoing the first-line treatment with surgery and chemotherapy (carboplatin and paclitaxel). DNA was isolated from whole blood to amplify genetic polymorphisms of Mad1L1 (rs1801368); Mad2L1 (rs1972014; rs1546120; rs3752830) using the PCR-restriction fragmentation length polymorphism (RFLP) method. Then the data were correlated with the clinical response of the patients which was evaluated by CA-125 blood biomarker and CT scan of abdomen and thorax. Patients were categorized as responders, partial responders and non-responders.

Results: In this study, there were 16, 19, and 10 responders, partial responders, and non-responders. The genotype distribution did not vary significantly among responders, partial responders, and non-responders. It was also not significant between adjuvant and neoadjuvant chemotherapy arms. The allele frequencies did not maintain Hardy-Weinberg equilibrium in the OC patients.

Conclusions: The MAD1L1 and MAD2L2 polymorphisms may not be a predictor for treatment outcomes of patients with advanced

ovarian cancer. However, further investigation is needed to confirm these findings in a larger sample size.

P0719 | DIFFERENT CHEMOTAXIS OF HUMAN MESENCHYMAL STEM CELLS TO CERVICAL CANCER CELLS

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/SUB-THEME: AB 8.5 NOVEL/ALTERNATIVE/HORMONAL TREATMENTS

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Objectives: Mesenchymal stem cells (MSCs) has been used as a drug-deliver for cancer therapy based on their unique tropism towards cancer cells. Despite similarities in morphology, immunophenotype, and differential potent in vitro, MSCs sourced from different tissues do not necessarily have equivalent biological behaviors. It is of great significance to screen the most chemotactic MSCs to cancer cells.

Methods: Different MSCs were isolated from various human tissues including adipose, umbilical cord, amniotic membrane, and chorion. Trilineage differentiation, flow cytometric and western blot analysis were performed to identify all isolated cells. Cell viability was detected by CCK-8 assay. Transwell assay was conducted to investigate the tropism of MSCs to cervical cancer cells. ELISA and western blot analysis were performed to detect the expression of CXCL12 from cervical cancer cells and CXCR4 from MSCs, respectively.

Results: MSCs derived from distinct sources can be differently recruited to cervical cancer cells, among which chorion-derived MSC (CD-MSC) possessed the strongest tropic capacity. Furthermore, CXCL12 was found to be highly secreted by cervical cancer cells, in parallel with the expression of CXCR4 in all MSCs. Consistently, CD-MSC displayed the highest level of CXCR4. These results indicated that CXCL12/CXCR4 pathway contributed to the different chemotaxis to cervical cancer cells of each MSCs.

Conclusions: CD-MSC with the highest CXCR4 expression is the best therapeutic vehicles for targeted therapy in cervical cancer.

P0720 | KRUKENBERG'S TUMOR DURING PREGNANCY: A CASE REPORT

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/ SUB-THEME: AB 8.3 SURGICAL TREATMENT OF MALIGNANCIES

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Objectives: Responsible for 1-2% of ovarian cancers, Krukenberg's tumor during pregnancy is extremely rare and its management is usually a dilemma. Our objective is to increase the knowledge about this rare presentation, by reporting this case.

Methods: The patient was a 41-year-old woman, admitted to a reference service for high-risk maternal care at the gestational age of 27 weeks and 4 days, presenting a pelvic mass yet to be diagnosed. Imaging exams showed left ureterohydronephrosis and expansive formations of probable ovarian origin measuring 19.4 x 20.4 x 21.3 cm and 15.8 x 11.0 x 14.4 cm at the left and right sides, respectively. A cesarean section was performed with bilateral anexectomy at a gestational age of 30 weeks and 3 days. Surgical inspection was suggestive of a bilateral Krukenberg's tumor implanted in the stomach and peritoneum.

Results: The anatomopathological analysis of the lesions showed a stage IV gastric adenocarcinoma with signet ring cells. The patient received palliative chemotherapy and died of acute obstructive abdomen.

Conclusions: The distinction between primary and metastatic malignant ovarian disease, although often difficult, is important due to management and prognostic implications. The management of the condition should take into account maternal and fetal aspects and the staging of the disease. Although the management of such cases is a challenge, the delay in starting treatment is linked to poor maternal outcomes.

P0721 | GRANULOSA CELL TUMOR: A CASE REPORT

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/ SUB-THEME: AB 8.3 SURGICAL TREATMENT OF MALIGNANCIES

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Objectives: Report a case of granulosa cell tumor (GCT), a rare type of cancer, corresponding to 2-5% of malignant ovarian neoplasms.
Methods: Descriptive observational study analyzing medical records.
Results: Female, 59y, with severe abdominal pain, nausea and vomiting for 1 year. Globose abdomen, umbilical hernia, left pelvic mass palpable, firm, painless and lobulated. Abdominal USG (02/2020): heterogeneous expansive solid lesion in the abdominopelvic cavity, on the right rejecting neighbouring structures. Multiple nodules in abdominal and retroperitoneal fat. Solid hepatic nodule suggesting secondary lesion. Abdominal tomography (02/2020): Nodules suggesting neoplasia and peritoneal carcinomatosis; umbilical hernia containing fat, ascites and peritoneal nodule. Performed tumor resection, mesocolon and mesorectal topography, and omentectomy. Anatomopathological: GCT, adult type. Immuno-histochemical: GCT infiltration, adult type. Abdominal pelvic tomography after surgery: moderate ascites, peritoneal nodules, suggesting carcinomatosis. Performed a left colostomy with nodulations in the subcutaneous fat due infiltrating disease into the abdominal wall. Mild lymph node enlargement in retroperitoneum. Discharge on the 10th postoperative day.
Conclusions: The GCT adult type is the most common in women aged 50-54 years, corresponding to 95% of these neoplasms. The prognosis depends on the stage and residual presence after surgery, given the metastatic potential and late recurrence. There is no standardized treatment, and the surgery is among the best options for initial management, due to its curative nature. Therefore, screening for ovarian neoplasms in patients with abdominal symptoms is very important, in addition to early intervention for better prognosis.

P0722 | MANAGEMENT OF A RARE RECURRENT VULVAL MYXOID SARCOMA

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/ SUB-THEME: AB 8.3 SURGICAL TREATMENT OF MALIGNANCIES

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Objectives: Malignant tumors of the female reproductive system are serious health and social problem. Vulvar tumors represent only 4% of all gynecological cancers. Vulval Sarcomas are rare tumors and comprise approximately 1-3% of all vulvar cancers. Leiomyosarcomas, epithelioid sarcomas, and rhabdomyosarcomas are among the most common variants. Only a few cases have been reported. In this case report, we describe a rare case of recurrent vulval sarcoma.
Methods: A 42-year-old multiparous postmenopausal woman presented with swelling of 2*2 cm in the left labia majora 5 years back for which she was operated with local wide excision and diagnosed with vulval sarcoma on histopathology. One year after surgery patient presented with recurrent swelling of 6*6 cm with an ulcerative surface in the same area.
Results: The patient was further managed and diagnosed with vulval myxoid epithelioid sarcoma by wide local excision followed by adjuvant therapy.
Conclusions: Only a few cases of vulval sarcoma have been reported with the presence of focal myxoid changes. Early diagnosis is difficult and optimal treatment is not well established due to its rarity. These are very aggressive tumors. We hereby highlight the key points of management in a recurrent case of myxoid sarcoma.

P0723 | OUTCOME OF THE VISUAL INSPECTION WITH ACETIC ACID OF THE CERVIX OF HIV POSITIVE WOMEN IN ANYIGBA, NORTH CENTRAL NIGERIA

THEME: AB 8 GYNAECOLOGICAL ONCOLOGY/SUB-THEME: AB 8.2 DIAGNOSIS AND SCREENING OF MALIGNANCIES

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Objectives: To determine the prevalence of premalignant lesions of the cervix among HIV positive women in Anyigba, North central Nigeria.
Methods: This prospective study was done between December 2020 and February 2021. 125 counseled women participated. HIV positive women that attend clinics at the Kogi State University Teaching Hospital and the Holley Memorial Hospital had a Visual

184MO Efficacy and safety of sintilimab plus paclitaxel and cisplatin as neoadjuvant therapy for locally advanced cervical cancer: A phase II trial

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Background: The KEYNOTE 826 study has demonstrated significant survival benefits with first-line pembrolizumab (anti PD-1) plus chemotherapy for recurrent/metastatic cervical cancer (CC). This phase II study aims to evaluate the efficacy and safety of Sintilimab (anti PD-1) plus paclitaxel and cisplatin as neoadjuvant therapy for locally advanced CC.

Methods: This is a single arm, phase II study (NCT04799639). Pts with newly confirmed locally advanced CC (stage IB3 or IIA2) received neoadjuvant chemotherapy with paclitaxel (150mg/m^2, iv) and cisplatin (70mg/m^2, iv) plus Sintilimab (200mg, iv) Q3W for 3 cycles, followed by radical surgery. For Pts evaluated PD without distant metastases after 2 cycles of neoadjuvant therapy underwent surgery immediately. The primary endpoint was pathological complete response (pCR) rate. Key secondary endpoints included ORR, PFS, 2y PFS rate and adverse events (CTCAE 5.0) and biomarkers.

Results: As of data cutoff on Jun 16, 2022, 21 eligible pts were enrolled, all pts received 3 cycles of neoadjuvant therapy. 20 pts underwent radical surgery and were evaluable. The primary endpoint of pCR rate was 35% (7/20). Except for one patient with SD, 19 pts had objective responses (95%), including 4 CR and 8 PR. At the date cutoff, the median follow-up time was 5 months (range 1-11), no patient recurred. In terms of hematological toxicity, 4 patients presented with grade 3-4 neutropenia and no other grade 3-4 adverse events. Possible immune-related side effects included skin rash (8/20, G1-2), alanine aminotransferase increased (7/20, G1), creatinine increased (3/20, G1), hyperlipidemia (1/20, G1), and hypothyroidism (3/20, G1-2). No treatment-related death were observed.

Conclusions: Sintilimab plus paclitaxel and cisplatin as neoadjuvant therapy had encouraging antitumor activity with 35% pCR rate and manageable toxicities in patients with locally advanced CC. The study is ongoing and more data will be reported in the future.

Clinical trial identification: NCT04799639.

Legal entity responsible for the study: The authors.

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185MO Preliminary results of niraparib combined with brivanib or toripalimab dual therapy evaluation in recurrent, metastatic and persistent cervical cancer (CQGOG0101): An open-label, two cohorts, phase II clinical trial

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Background: The aim of this study (CQGOG 0101, NCT04395612) is to evaluate the safety and activity of Niraparib (an oral PARP1/2 inhibitor) combined with brivanib (an anti-angiogenesis inhibitor) or Toripalimab (a PD-1 inhibitor) in patients with recurrent, metastatic, or persistent cervical cancer.

Methods: 30 patients were planned to be enrolled.Eligible patients were aged 18—70 years with measurable lesions and had an ECOG performance status of 0-2. Two cohorts were included in the study: Cohort 1 : Subjects received oral Niraparib 200 mg and Brivanib 400 mg once daily. Cohort 2 : Subjects received oral Niraparib 200 mg once daily and Intravenous Toripalimab 240 mg every 21 days. Treatment until until disease progression, intolerable toxicity, or withdrawal of consent. Primary endpoint was the objective response rate (ORR) assessed by RECIST version 1.1. Secondary endpoints included disease control rate (DCR), duration of response (DOR) and safety.

Results: Between May 8th, 2020 and June 22nd, 2022, 23 patients (median age, 50 years old [28-73]) were enrolled. Patients had received a median of two (1-3) previous lines of platinum-based therapy. All of 23 patients had distant metastatic lesions. In Cohort 1, 9 patients had underwent at least one post baseline tumor assessment (To deadline for submission), including 1 confirmed partial response, 4 with stable disease, 4 with progressive disease, the ORR is 11%.No drug-related grade 3 or worse treatment-emergent adverse events were detected. In Cohort 2,13 patients had underwent at least one post baseline tumor assessment (To deadline for submission),including 8 confirmed stable disease, 4 with progressive disease, 1 withdrawal of consent. The median duration of treatment has not met yet. Treatment of Cohort 2 is still ongoing (Median follow-up is 2 months). Grade 3 or worse TEAEs were detected in 3 subjects.

Conclusions: The Cohort 1 (Niraparib combined with Brivanib) seems to show a similar efficacy compared to other recurrent cervical cancer late-line therapies. The treatment of the Cohort 2 (Niraparib combined with Toripalimab) is still ongoing and final data will be reported later.

Clinical trial identification: NCT04395612.

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186P Degradation of BRCA2 expression by hyperthermia sensitizes HRD-negative (BRCA2 wild-type) ovarian cancer cells to niraparib

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Background: Poly (ADP-ribose) polymerase (PARP) inhibitors have significantly improved survival for advanced ovarian cancer patients as maintenance therapy and have become an important component of standard treatment for ovarian cancer. Ovarian cancer cells with BRCA mutations are particularly sensitive to PARP inhibitors, but how to improve the sensitivity of BRCA wild-type ovarian cancer to PARP inhibitors has become a focus of research. We identified if down-regulation of BRCA2 expression by hyperthermia (HT) sensitizes HRD-negative ovarian cancer to Niraparib.

Methods: BRCA2 mutations of human ovarian cancer OVCAR3, A2780 and mouse ID8 cells were identified by whole genome sequencing, and the expressions of BRCA2, PARP1, and γ-H2AX were detected by qPCR and Western Blot assays after HT treatment. The formation of RAD51 foci was observed by immunofluorescence microscopy and the apoptosis was measured with FCM, and colony formation was detected by crystal violet assay after combination treatment of HT and Niraparib. In vivo, female mice were implanted with BRCA2^{wt} and BRCA2^{kd} ID8 cells in the right hind leg; The mice bearing tumor received local HT treatment (for 1.5 h at 42°C) twice a week, with an interval of 48 h for 3 weeks and Niraparib p.o. at the dosage of 50 mg/kg 2 h prior to HT treatment. The tumor growth and survival were observed.

Results: Genome sequencing showed that A2780 and ID8 cells were BRCA2^{wt} and OVCAR3 cells were BRCA2^{mt}; Hyperthermia induced degradation of BRCA2 of A2780 and ID8 cells, leading to HRD status; RAD51 expression has no significant change but PARP1 expression was elevated. Hyperthermia inhibited the formation of RAD51 foci in these two cells, Niraparib, a PARP inhibitor, combined with HT treatment synergistically increased DSBs of DNA and the rate of apoptosis and inhibited the growth of A2780 and ID8 cells. The in vivo experiment showed that the treatment of Niraparib combined with HT slowed down the tumor growth rate and prolonged the survival of the mice bearing tumors.

Conclusions: Hyperthermia could enhance the sensitivity of BRCA2^{wt} ovarian cancer to PARP inhibitors.

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187P BUB1 (2530C>T) polymorphism and expression affects chemotherapy response and predicts poor prognosis in advanced epithelial ovarian cancer

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Background: Ovarian cancer (OC) is a frequent gynecological cancer with a complex pathophysiology and high mortality. This study determines roles of 2530C>T SNP in BUB1, a key component of spindle assembly checkpoint (SAC), and its expression in advanced OC pathology & treatment outcome.

Methods: 77 advanced epithelial OC patients were recruited, who received post-operative adjuvant [I.V. doses of 175mg/m2 paclitaxel+ carboplatin AUC 5-6mg.min/mL] or neoadjuvant chemotherapy with interval debulking surgery. Their demographics, clinical parameters, toxicity and survival were recorded. BUB1(2530C>T)

SNP was detected by the PCR-RFLP followed by sequencing. BUB1 expression, BUB1mRNA and miRNA-495 were assessed using IHC & qRT-PCR.

Results: Stages III (82·02%) and IV (17·98%) OC patients were mostly 41-60 (60%) years old. 37%, 40%, 23%% were categorized as responders (Rs), partial responders (PRs) and non-responders (NRs) with significantly different ($p<0.05$) survival outcomes. The polymorphic homozygous genotypes (CC) and (TT) of 2530C>T were most prevalent in NRs & PRs respectively showing significant association in chemotherapy response ($p=0.021$). The allele frequencies were found to be C= 0.2215, and T= 0.7784. The standard regimen was well tolerated but no significant relationship was observed between SNP and toxicities. The survival outcome was nearly significant ($p=0.06$) showing association of CC genotype with higher risk (HR=9.938, 95%CI= 1.19-82.9) when compared to CT (HR=0.885, 95%CI= 0.109-7.19) and TT (HR=0.409, 95% CI= 0.049-2.209). 97.6% of biopsy samples showed low to moderate BUB1 IHC expression and did not have significant association with clinical response ($p=0.32$). BUB1 mRNA and miR-495 analysis revealed a Ct mean of 37.42 and 27.614 respectively. There was a negative correlation between BUB1mRNA and miR-495 levels ($r=-0.551$, $p=0.16$).

Conclusions: The homozygous polymorphic phenotypes of 2530C>T are significantly associated with chemotherapy response and poor survival outcomes but not related to chemo-induced toxicity. The very low expression of BUB1 may be attributed to regulation by miR-495. The negative expression also suggests a deficient SAC response in the advanced tumour.

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188P Spatial transcriptomic analysis of tumor tissue in ovarian cancer patients treated with neoadjuvant chemotherapy

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Background: Ovarian cancer (OC) is one of the most common gynecological cancers, with the worst prognosis and the highest mortality rate out of gynecological diseases. Despite a good response to the first line of standard neoadjuvant chemotherapy (NAC), the relapses in patients with sporadic ovarian cancer are detected within a short period of time in 70% of cases. The most relevant task is to identify the key features of tumor tissue of non-responders and to find out how these features can affect the development and outcome of OC.

Methods: We applied 10x Visium technology for spatial transcriptomic analysis of FFPE samples with ovarian cancer tissue. Eight patients, treated with neoadjuvant chemotherapy were included: 5 had unfavorable outcomes (local or distant recurrence or metastasis) and 3 did not experienced progression during 2 years after the treatment. All patients had initial partial response after the completion of NAC course assessed by the CRS score system. Sequencing of 10x Visium libraries was performed using NextSeq2000 platform (Illumina). The Spaceranger pipeline was used for the raw sequencing data processing, namely, the Fastq files generation, the quality control of the raw reads, mapping of the reads and counting of the reads mapped to the individual genes. The filtered expression matrixes were analyzed via the Seurat package in R. Filtered data was normalized via SCTransform, merged and additionally re-normalized with SCTransform. The Harmony R package was used for batch effect reduction. The batch-corrected data were used for non-linear dimension reduction and the SNN clustering.

Results: Bioinformatics analysis allowed us to reveal 16 clusters in combined 8 samples. Patients who experienced unfavorable outcomes had clusters with significantly more pronounced expression of genes, belonging to processes of angiogenesis, extracellular matrix remodeling, invasion and immune activation. They include collagens, matrix metalloproteases and other matrix proteins as well as immunoglobulins.

Conclusions: For the first time we performed spatial transcriptomic analysis of NAC-treated patients with two distinct outcomes, favorable and unfavorable.

Legal entity responsible for the study: Larionova Irina.

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189P LncRNA NKILA as a negative regulator of NFkB in ascites in ovarian cancer

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Background: NF-κB-interacting LncRNA (NKILA) plays an inhibitory role in the NF-κB pathway, which is a key regulator of inflammatory cytokines. These mediators, freely floating in ascitic fluid (AF), reflecting the molecular genetic characteristics of tumor cells in ovarian cancer (OC), and associated with epigenetic switches of key pathways of carcinogenesis remain poorly understood. The aim of the work was to study the expression of LncRNA NKILA and marker cytokines of the NF-kB pathway in ascites of patients with OC.

Methods: The study included 22 patients with ascitic OC at stage III-IV according to FIGO. Isolation of circulating nucleic acid was performed from 2 ml of the cell-free fraction of ascites before treatment using SileksMagNaDirect magnetic particles (Sileks, Russia). lncRNA NKILA expression in AF was determined by qPCR (CFX-96, BioRad). Determination of cytokines VEGFA, IL-6, MCP-1 in AF was performed by ELISA. According to the effect of platinum-containing chemotherapy (CT), the patients were divided into groups: 1 - progression against the background of CT and early relapse; 2 - nonrelapsive. Statistical data processing was carried out using Statistica 13.0.

Results: The study included 22 patients with ascitic OC at stage III-IV according to FIGO. Isolation of circulating nucleic acid was performed from 2 ml of the cell-free fraction of ascites before treatment using SileksMagNaDirect magnetic particles (Sileks, Russia). lncRNA NKILA expression in AF was determined by qPCR (CFX-96, BioRad). Determination of cytokines VEGFA, IL-6, MCP-1 in AF was performed by ELISA. According to the effect of platinum-containing chemotherapy (CT), the patients were divided into groups: 1 - progression against the background of CT and early relapse; 2 — non-relapsive. Statistical data processing was carried out using Statistica 13.0.

Conclusions: Thus, the study of the lncRNA NKILA profile in tumor ascites in relation to inflammatory cytokines of the NF-kB signaling pathway requires further study in the context of understanding the signatures in the formation of chemoresistance in advanced OC.

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190P Real-world applications of poly (ADP-ribose) polymerase inhibitors for ovarian cancer: A single-center study in China

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Background: Olaparib and niraparib have been approved for first/second-line maintenance treatment of ovarian cancer patients in China for more than 3 years. In this study, we analyzed the clinical application characteristics of PARPi in the maintenance therapy of ovarian cancer in real world to promote their rational application.

Methods: Retrospective chart review identified patients prescribed Ola, Nira for maintenance therapy of newly diagnosed or recurrent ovarian cancer from Sichuan cancer hospital in China between 1 July 2018 and 30 November 2021. Their medical records including pathologic, treatment and genetic information were reviewed.

Results: 131 patients were finally enrolled (67 Ola 51%; 64 Nira, 49%), data collection time was up to 7 May, 2022. 63% (42/67) of patients were detected to have BRCA mutations in the Ola group especially higher in the 1st line maintenance setting (92% 32/35). More than 90%(58/64) of patients were BRCA wild type or unknow in the Nira group. The median follow-up time was 16.9 months in the Ola group, and 16.3 months in Nira group. The median duration of treatment (DOT) of was 14.3 months in the Ola group, and 13.5 months in the Nira group. At the time of data censoring, 87 (66.4%) patients were still on treatment. The PFS rate at 24-month (PFS 24) was 56.2%(95CI:0.40-0.78) in the Ola group, and 58.8%(95CI:0.47-0.74) in the Nira group. The PFS 24 of 1 Lm was 60.4%(95CI:0.37-0.88) in the Ola group, and 66.3%(95CI:0.54-0.82) in the Nira group. PFS rate at 12-month (PFS 12) of recurrence patients was 80% (95%CI:0.68-0.95) in the Ola group, and 50% (95%CI:0.30-0.82) in the Nira group. No new safety signal was observed. Dose discontinuations were observed in 1 patient with Nira due to ALL,2 patients with Ola due to thrombocytopenia and AML. We also observed that patients with skin pigmentation had a reduced probability of AEs.

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Abstract	Purpose: This study aims to determine the roles of SAC signaling components in advanced ovarian cancer pathology and patients' treatment outcome. Experimental Design: Advanced epithelial OC patients (FIGO Stages III & IV) were recruited, who received postoperative adjuvant chemotherapy [I.V. doses of 175mg/m2 paclitaxel+ carboplatin AUC 5-6mg.min/mL] or neoadjuvant chemotherapy with interval debulking surgery. BUB1 & MAD2 expression was observed by immunohistochemistry and their respective mRNA with miRNA-495 expressions were assessed using qRT-PCR. BUB1B and MAD2 SNPs were detected using PCR-RFLP followed by sequencing. All the data was correlated with the clinical response, toxicity and survival of the patients. In silico approaches were utilised for molecular docking analysis. Results: 80 enrolled patients among whom 29, 32 and 19 were evaluated as responders, partial and non-responders respectively with significant survival outcomes (p=0.019). BUB1 was downregulated with miR-495 involvement while MAD2 was upregulated in majority of advanced tumours with significant survival impact (p=0.001). The novel identification of BUB1B and MAD2L1 polymorphism were significantly associated with chemotherapy induced anemia, anxiety, diarrhoea, constipation, and renal toxicity (p<0.05). *In silico* approaches revealed mutant BUB1B to be highly deleterious and resulting protein to have high binding energy and stable interaction with paclitaxel than its wild type. MAD2L1 has much higher binding energy and stability with carboplatin than paclitaxel. Conclusions: Our investigation suggests that dysregulation of SAC in cancer pathophysiology may be responsible for treatment and survival outcomes. **Word Count: 232**
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