PROGNOSTIC SIGNIFICANCE OF GLUTATHIONE LEVELS IN CERVICAL CANCER PATIENTS UNDERGOING RADIOTHERAPY

By

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MBBS

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Dr. ARPITHA SHRUTHI A

LIST OF ABBREVIATIONS USED

HPV : Human Papillomavirus Infection
ROS : Reactive Oxygen Species
SOD : Superoxide Dismutase
GPX : Glutathione Peroxidase
GSH : Glutathione
SCJ : Squamocolumnar Junction
IUD : Intrauterine Device
WHO : World Health Organization
HLA : Human leukocyte Antigen
SCC : Squamous cell carcinoma
LSIL : Low grade squamous intraepithelial lesion
HSIL : High grade squamous intraepithelial lesion
VIA :Visual Inspection with Acetic Acid
VILI : Visual inspection with lugol's iodine
IVP : Intravenous Pyelogram
EBRT : External Beam Radiotherapy
LVSI : Lymphovascular Space Invasion
GTV : Gross Tumour volume
CTV : Clinical target volume
LDR : Low dose radiotherapy
HDR : High dose radiotherapy
GFR : Glomerular filtration rate
WBC : White Blood Cells
RBC : Red Blood Cells
RT : Radiotherapy
CT : Chemotherapy
DNA :Deoxyribonucleic Acid
SCGE :Single Cell Gel Electrophoresis
GSSG :Glutathione disulfide
RNA : Ribonucleic acid
PMN :Polymorphonuclear Neutrophilis
APC :Antigen Presenting Cells
GST :Glutathione S – Transferases
MDA : 3,4- Methylenedioxyamphetamine
SOD : Superoxide Dismutase
NR :Non Responders
CR :Complete Responders
PR : Partial Responders
HIV :Human Immunodeficiency Virus
SPSS : Statistical Package for Social Sciences
TNB :5 Thio 2 Nitrobenzoic Acid
FIGO :The international federation of gynecology and obstetrics

ABSTRACT

INTRODUCTION:

Cervical cancer is the most prevalent gynaecological cancer in the world , including India. It is attributed as a multifactorial disease process.

Cervical carcinoma does not arise de novo , but is preceded by a spectrum of various abnormal epithelial changes which is known as dysplasia an important precancerous lesion. Evidence has indicated that the reactive oxygen species (ROS) are necessary for both the initiation and progression of carcinogenesis. The most commonly used therapeutic modality for treatment of cervical cancer is radiotherapy, effect of radiation therapy is mediated by free radicals production. Intracellular GSH counteracts the radiation induced free radical–mediated biomolecular damage . Hence response to radiation is dependent on GSH redox status of the system. SO we can postulate that tumour radio response can be predicted using the variations in GSH values before and immediately after few fractions of radiotherapy so that it may be useful in the future for treatment planning on individual basis.

OBJECTIVES:

1. To estimate serum glutathione levels between Pre Radiotherapy and day 3 of treatment i.e. after two fractions of radiotherapy and one cycle of chemotherapy in cervical cancer patients being treated with Radical Radiotherapy and Chemotherapy.

2. To correlate glutathione levels with clinical response at 3 months after completion of treatment.

MATERIALS AND METHOD:

It is a prospective hospital based study conducted at R.L Jalappa hospital , Kolar.40 Patients in the age group of 30 yrs – 70 yrs visiting the department of OBG and clinically diagnosed with cervical cancer were enrolled in the study group.

METHOD:

Patients underwent routine investigations and were staged as per FIGO guidelines. The patients were administered with Inj. Cisplatin 40 mg/m2 BSA once per week as IV infusion for 5 weeks with an initial dose on first day of the week and concurrently subjected to external beam radiotherapy of 50Gy in 25 fractions, 5 days per week for 5 weeks delivered using Tele-cobalt. A standard four-field box technique involving anterior and posterior and two lateral fields was employed. After 2 weeks of rest, two applications of remote after loaded HDR intracavitary brachytherapy of 6.5 Gy x 2 fractions to point A was delivered once per week. Response to radiotherapy was assessed after 3months of completion of treatment as WHO guidelines.

Two blood samples were collected from patients first sample before treatment and second after 2 fractions of RT and one cycle of chemotherapy. Blood sample was centrifuged and stored at -20 degree Celsius. Serum glutathione was estimated using Cayman's Assay kit and post treatment Fall in glutathione levels were calculated .

Various outcome results were recorded and tabulated. The results were statistically analysed using parameters like mean, standard deviation and chi square test.

RESULTS:

After completion of treatment, Complete response was seen in 60% of patients, Partial response in 12 % and 4 % of patients were non responders.Complete response was in higher in age groups 41-50yrs and 51-60 yrs. There was also significant fall in GSH noted in the above age groups .($P<0.0001$ and $P<0.0001$).The response rates were similar in premenopausal and post-menopausal patients, but the fall in GSH was significant in postmenopausal women ($p<0.0001$).

Response rates were comparable in underweight, normal weight and obese patients. GSH value showed significant fall in BMI <18.5 kg/m2 and in BMI 18.5- 24.9 kg/m2 with no difference between the two groups (P<0.0004 and P<0.0001).

Complete response was significantly higher in patients with squamous cell carcinoma as compared to patients with adenocarcinoma (P=0.032) and also significant fall in GSH value was seen only in squamous cell carcinoma (P<0.0001), whereas patients reported with adenocarcinoma did not show any significant fall (P=0.06).Statistical difference was seen in fall in GSH between complete, partial and Non-responders. In complete responders, there was significant fall in GSH values after initiation of radiotherapy (P<0.0001).In partial responders too there was significant fall in GSH (P<0.0001) but in non responder, though there was small fall in GSH values, it was not statistically significant (P=0.393).

CONCLUSION:

This study concludes that serum glutathione concentration in carcinoma cervix patients before treatment and fall after two fractions of radiotherapy and one cycle of chemotherapy significantly helps to predict response to treatment. Better response to radiotherapy was also observed in patients of age group 40-60yrs and BMI <18.5kg/m^2. However , further studies with larger sample size and longer follow up will be required to assess long term response and sub group analysis.

KEYWORDS:

Glutathione; cancer of cervix; chemo-radiotherapy; predictive factors; tumor response; free radicals; radio-sensitivity; cisplatin

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Introduction

INTRODUCTION

Cervical cancer is the most prevalent gynaecological cancer in the world, including India. It is attributed as a multifactorial disease process and several risk factors include multiparty, poor genital hygiene, early age at first intercourse, multiple sex partners ,low socioeconomic status and Human papillomavirus (HPV) infection. Chronic inflammation and infection over a prolonged period of time have been recognized as major risk factor for disease initiation[1].

Cervical carcinoma does not arise de novo, but is preceded by a spectrum of various abnormal epithelial changes which is known as dysplasia an important precancerous lesion. Several studies have also suggested the role of antioxidant elements in diet for prevention of cervical cancer. Diet deficient in anti-oxidants (vitamin A, vitamin C and folic acid) appears to have a role in the aetiology of carcinoma. Lipid peroxidation is responsible for damage to tissues in vivo and has been implicated as one of the probable causes of cancer [2] .Evidence has indicated that the reactive oxygen species (ROS) are necessary for both the initiation and progression of carcinogenesis. This might be due to the damage caused by immunological defence mechanisms in the body. Superoxide, hydroxyl radicals are the oxygen-free radicals, responsible in producing oxidative stress. This oxidative stress can be associated with various other factors which might lead to neoplastic transformations [1].

To control and reduce the lipid peroxidation, nature invokes the use of antioxidants such as enzyme anti-oxidants-Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and non enzyme antioxidants - reduced glutathione or Gamma Glutamyl cysteinyl glycine (GSH). Glutathione (GSH) functions as an important cellular

antioxidant in the destruction of hydrogen peroxide and lipid peroxides by providing substrate for the glutathione peroxidases (GPX)[1]. Hence the concentration of glutathione is distributed in humans in the ratio of 90:10 reduced/oxidized forms.

The most commonly used therapeutic modality for treatment of cervical cancer is radiotherapy, effect of radiation therapy is mediated by free radicals production. Since, the most important antioxidant is glutathione, a consumer of free radicals, it can be postulated that those cell systems containing a high ratio of glutathione as reflected by the plasma and erythrocyte glutathione levels, might be found to be radio-resistant. Studies on plasma/serum GSH levels in various malignancies have reported to have an altered GSH redox system [2].

Intracellular GSH counteracts the radiation induced free radical–mediated biomolecular damage [2]. Hence response to radiation is dependent on GSH redox status of the system. SO we can postulate that tumour radio response can be predicted using the variations in GSH values before and immediately after few fractions of radiotherapy so that it may be useful in the future for treatment planning on individual basis.

Objectives

OBJECTIVES

1. To estimate serum glutathione levels between Pre Radiotherapy and day 3 of treatment i.e. after two fractions of radiotherapy and one cycle of chemotherapy in cervical cancer patients being treated with Radical Radiotherapy and Chemotherapy.

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Review of Literature

REVIEW OF LITERATURE

HISTORY :

The origin of word cancer is credited to Hippocrates (460-370BC) who used the terms carcinos and carcinoma to describe non –ulcer forming and ulcer forming tumours.

In 1886, John Williams first described the lesion which was eventually known as carcinoma in situ [3].

Between 1908 and 1912, Schauenstein, Pronai, Rubin, Schottlander and Kermauner conceptualised that cervical cancer was preceded by an intraepithelial growth phase [4].

Later Walter Schiller first proposed the term pre invasive carcinoma. He defined pre-invasive carcinoma as cytological atypia without invasion of basement membrane [5].

Until 1940's microscopic examination of tissue biopsies was used to diagnose cervical carcinoma and premalignant lesions were rarely diagnosed. In 1940, cervical smear was developed which diagnosed premalignant lesions of cervix and concluded that preinvasive abnormalities precede invasive carcinoma by several years [6].

In 1941, Papanicoloau and Traut reported that cervical cancer in asymptomatic women and indemonstrable by tissue biopsy could be detected by use of vaginal smear [7].

Until the beginning of 19th century surgery was termed as the only cure for cervical carcinoma.

In 1898, following Marie and Pierre Curie's discovery of the advantages of radium, the scenario changed.

American surgeon Robert Abbe in 1910 was the first to use radium for the treatment of cervical carcinoma.

In 1912, Gosta Forsell from Stockholm used radium in patients with inoperable cases of cervical carcinoma and found good response in several patients. Later together with James Heyman they developed the Stockholm method of treatment.

In 1927, James Heyman advocated that all patients with cervical carcinoma in Sweden should be treated with primary radiation irrespective of the age of patient or operability.

In 1993, Meigs advocated that the key for cure of cervical cancer is early detection.surgery and radiation were termed as the second key for cure.

CERVICAL CANCER

The leading cause of adult deaths worldwide is cancer. Every year about 14 million newly diagnosed cancer cases are detected and around 8 million people die of cancer [8]. However, cervical cancer is certainly a public health problem in developing countries in contrast to the developed countries where the incidence is very low.

India alone accounts for one-quarter of the worldwide burden of cervical cancer [9].It is one of the leading cause of cancer mortality, accounting for 17% of all cancer deaths among women aged between 30 and 69 years. Cervical cancer is estimated to occur in approximately 1 in 53 Indian women during their lifetime compared with 1 in 100 women in developed regions of the world [9].

According to a review article on the magnitude of cancer cervix in India published by Nandakumar et. al,age reported incidence rates per 100000 women is 22.8% in 2004-05. 90708 new cases are identified on an average every year. In the hospital based registers cancer cervix is the leading cause of death in the urban and rural areas [10].

ANATOMY OF UTERINE CERVIX:

Cervix is located in the lower portion of uterus. It is cylindrical in shape and the length is 2.5 to 3cm and diameter is 2.5 to 3 cm .

It consists of two portions:

1. Portio vaginalis

2. Portio supravaginalis which is divided according to the vaginal reflection.

Portio vaginalis outer portion is covered by ectocervix which is lined by stratified squamous epithelium and the endocervical canal is lined by mucin producing columnar epithelium. It has two openings - the external and internal os.

Squamocolumnar junction is the point at which the ectocervical squamous epithelium and endocervical columnar epithelium join. Transformation zone is the zone between the SCJ at puberty and SCJ after squamous metaplasia that occurs as the age advances.

Epithelium of the ectocervix:

The lining epithelium is non keratinising stratified squamous epithelium. It consists of superficial, intermediate, para basal and basal layers. The cells of the basal layer has a vertically oriented oval nuclei with a dense chromatin and scanty cytoplasm. The cells of this layer are inactive mitotically. Above this layer is the para basal layer .They are larger with increased amount of cytoplasm. Mitosis is seen in this layer and they express Ki-67.

The next layer is the intermediate cell layer.

These cells have vesicular nuclei and these have abundant and clear cytoplasm due to glycogen accumulation. The superficial cells have round and small nuclei with a clear cytoplasm. There is abundant glycogen in these cells. During menstrual cycle, there are changes in the epithelium due to the influence of hormones. Superficial cells predominate when there is estrogen in the preovulation stage. In the post ovulatory phase, progesterone increases and hence there is predominance of intermediate cells.

Endocervical epithelium and endocervical glands:

It is covered by a layer of columnar cells (mucin producing) .The nucleus is basally situated oval small nuclei with dense chromatin.

Epithelium of the transformation zone:

Squamous epithelialisation and metaplasia are responsible for the endocervical epithelium transformation to squamous epithelium. In epithelialisation, mature squamous cells move below the endocervical epithelium and push the endocervical cells off the basement membrane and there is extension of these process to the clefts. Squamous metaplasia is the proliferation and differentiation of the endocervical reserve cells to the squamous cells. The reserve cells initially look like parabasal and basal cells. When these cells acquire cytoplasm, they are called as immature squamous metaplasia. When these cells acquire glycogen, they are called as mature squamous metaplasia. The nuclei of these cells are uniform, smooth contours and nuclear abnormalities are minimal.

Cervical stroma:

It consists of a denser fibrous stroma admixed with 10 to 15% of the elastin and smooth muscle fibres. Many blood vessels and lymphocytes are seen.

Changes associated with pregnancy:

Immature squamous metaplasia and decidual reaction can be seen in pregnancy.

Etiology of cervical cancer:

HPV continues to be the most common etiology for cervical cancers. HPV types most common are types 16,18,45,31,33,52,58 and 3510. High risk types are 16,18,31,33,35,39,45,51,52,56,58,59,68,73 and 82, low risk types are 6,11,40,42,43,44,54,61,70,72,81 and CP6108. HPV 16 and 18 are strongly associated with CIN III and invasive cervical cancer [11].

According to sherman et al, 93% of tumours expressed HPV DNA. HPV 16 was found in 50%,HPV 14 in 14%,HPV 45 in 8%,HPV 31 in 5% of specimens. HPV 16

predominated in squamous cell carcinomas(51% of such specimens),HPV 18 predominated in adenocarcinomas(56% of such specimens) and adenosquamous tumours (39% of such specimens) [12] .

Multiparty and younger age of having first child is associated with cervical cancer. According to a study conducted by Louise et al, risk increased to 5.1 (95% confidence interval 2.7–9.7) for those with 4 or more pregnancies [13].

Oral contraceptive usage had more incidence of invasive cancer than the the Intrauterine devices [14].

Cervical cancer is also associated with long duration of smoking [15].

According to latest WHO report, 70% of cases are in the developing countries. This difference is due to lack of access to effective screening and lack of facilities for early detection and treatment.

Genetic susceptibility for cervical cancer is related to HLA class II, HLA B7 and DQB1 16.

Pathogenesis:

Transformation zone recedes into the distal endocervical canal as the age advances [16].

SCC and adenocarcinoma of the cervix accounts for most common malignancies normally encountered in the cervix. In this, more than 70% of the cervical cancers are due to SCC which develops from the transformation zone [17].

The second most common histology in cervical cancer is adenocarcinoma which arises from the endocervical cells .The other types of cancers arising in cervix are adenosquamous and lymphoma [18].

Precancerous lesion of cervix is known as CIN which is of 3 types.

CIN 1(mild dysplasia), CIN 2(moderate dysplasia), CIN3 (severe dysplasia), CIS and invasive carcinomas [19]. According to Bethesda system, CIN 1 are LSIL and both CIN 2 and CIN 3 are together called as HSIL [20].

The natural history of cervical cancer is that the precancerous lesions of cervix do not progress to invasive cervical cancers suddenly.

According to Holowaty et al, risk of severe dysplasia developing from mild dysplasia was only 1% per year but moderate dysplasia progresses to severe dysplasia or worse was 16% within 2 years and 25% within 5 years. The risk of progression was more within the first 2 years after a dysplastic smear [21].

In another study conducted by Arends et al, approximate likelihood of regression of CIN1 is 60%, persistence is 30%, progression to CIN3 is 10% and progression to invasive cancer is 1%. Similarly, corresponding approximations for CIN 2 are 40%, 40%, 15% and 5 %. Likelihood of CIN3 going for regression is 33% and progression to invasive cancer is 12%[22].

DEGREE OF DYSPLASIA	REGRESSION %	PERSISTENCE %	PROGRESSION TO CIN 3 (%)	PROGRESSION TO INVASIVE CANCER (%)
CIN I	60	30	10	1
CIN II	40	40	15	5
CIN III	33	55	NOT APPLICABLE	12

So, if the cervical cancer is identified at an early micro invasive cervical cancer stage and confirmed by biopsy, it can be treated early and it does not present with metastatic disease. Whereas if the patients come with later stages treatment is difficult and it has a poor outcome[23].

Figure 1 : The timeline and natural history of cervical pre-cancer and cancer development

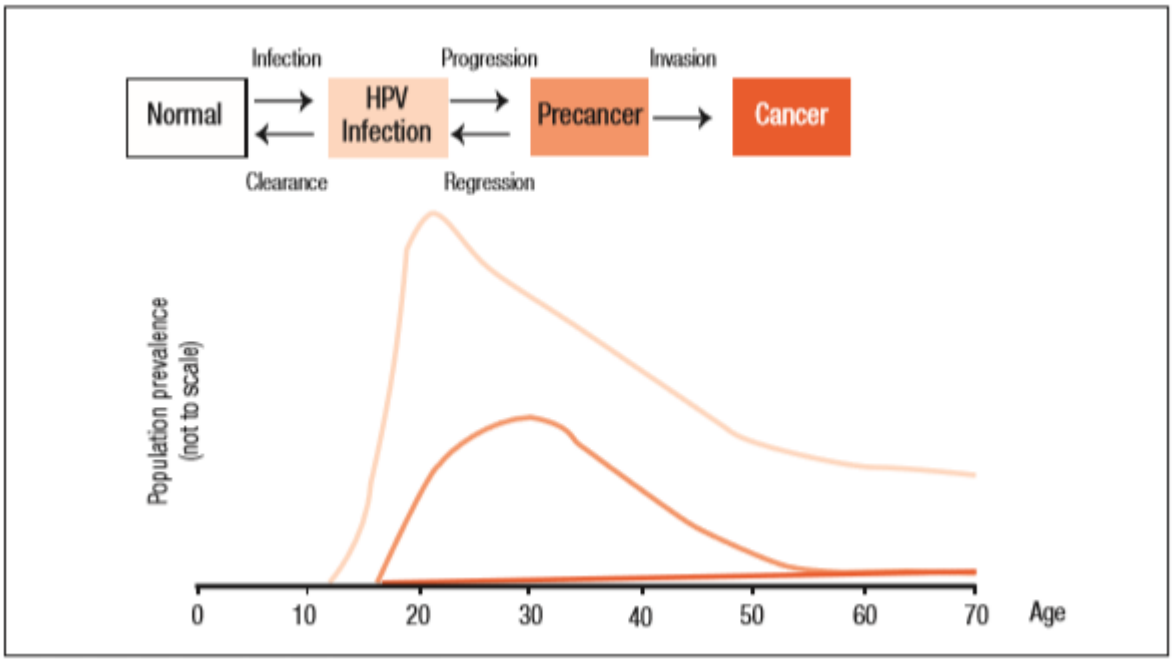


Figure 1 - depicts the timeline of the progression from a normal (uninfected) cervix to HPV-infected cervix to pre-cancer and invasive cancer. Note that changes occur in both directions because a large proportion of HPV-infected cells return to a normal state and a large proportion of cervical pre-cancers do not become cancer.

Figure 2: Progress from normal epithelium to invasive cancer

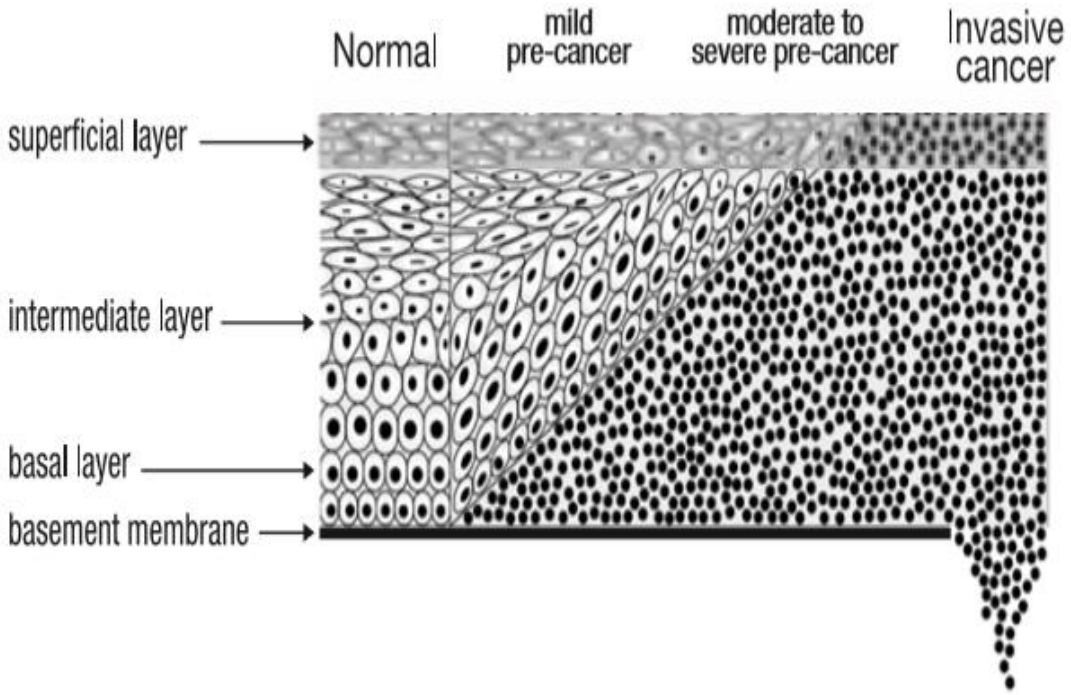


Figure 2: Illustrates normal cervical squamous epithelium on the left and progressively thicker layers of new abnormal small cells involving the epithelium in the large intermediate section. As this section in the middle involves more and more of the thickness of the normal epithelium, the epithelium is considered to have mild, then moderate, and finally severe pre-cancer. This sequence leads to invasive cancer if the abnormal cells invade the bottom layer of the epithelium (basement membrane), as shown on the right of the figure.

Routes taken by invasive cancer through the body as it progresses:

There are four, usually sequential, routes through which invasive cancer progresses.

i. Within the cervix: Spread occurs from a tiny focus of micro invasive cancer until it involves the entire cervix, which can enlarge to 8 cm or more in diameter. The cancer can be ulcerating, exophytic (growing outwards) or infiltrating (invading inwards).

ii. To adjacent structures: Direct spread in all directions is possible – downwards to the vagina, upwards into the uterus, sideways into the tissues supporting the uterus in the pelvis and the ureters, backwards to the rectum, and forwards to the bladder.

iii. Lymphatic: Spread to pelvic lymph nodes occur in 15% of cases when the cancer is still confined to the cervix, and increases as the cancer spreads. Lymph-node metastases are at first confined to the pelvis and are later found in the chain of nodes along the aorta, eventually reaching the space above the collarbone (supraclavicular fossa). The lymph nodes, once invaded with cancer, are enlarged and, if close to the skin, can be palpated. For example, if the cancer has advanced into the lower third of the vagina, the groin nodes may become involved and will be palpably enlarged, and the supracervical nodes will also feel noticeably enlarged.

iv. Distant metastases through the bloodstream and lymph channels. Cervical cancer cells may spread through the blood stream.

Prevention and control of cervical cancer:

Owing to the huge burden of cervical cancer, prevention and control of cervical cancer becomes important. For this, different methods have been developed for the early diagnosis and treatment of the cervical precancerous lesions which has led to drastic reduction of the disease burden.

The screening can be done by several methods including cervical cytology

(Pap smear and newly developed Liquid based cytology) and VIA or VILI and HPV DNA detection. So, awareness should be brought for reducing the incidence of cervical cancer worldwide.

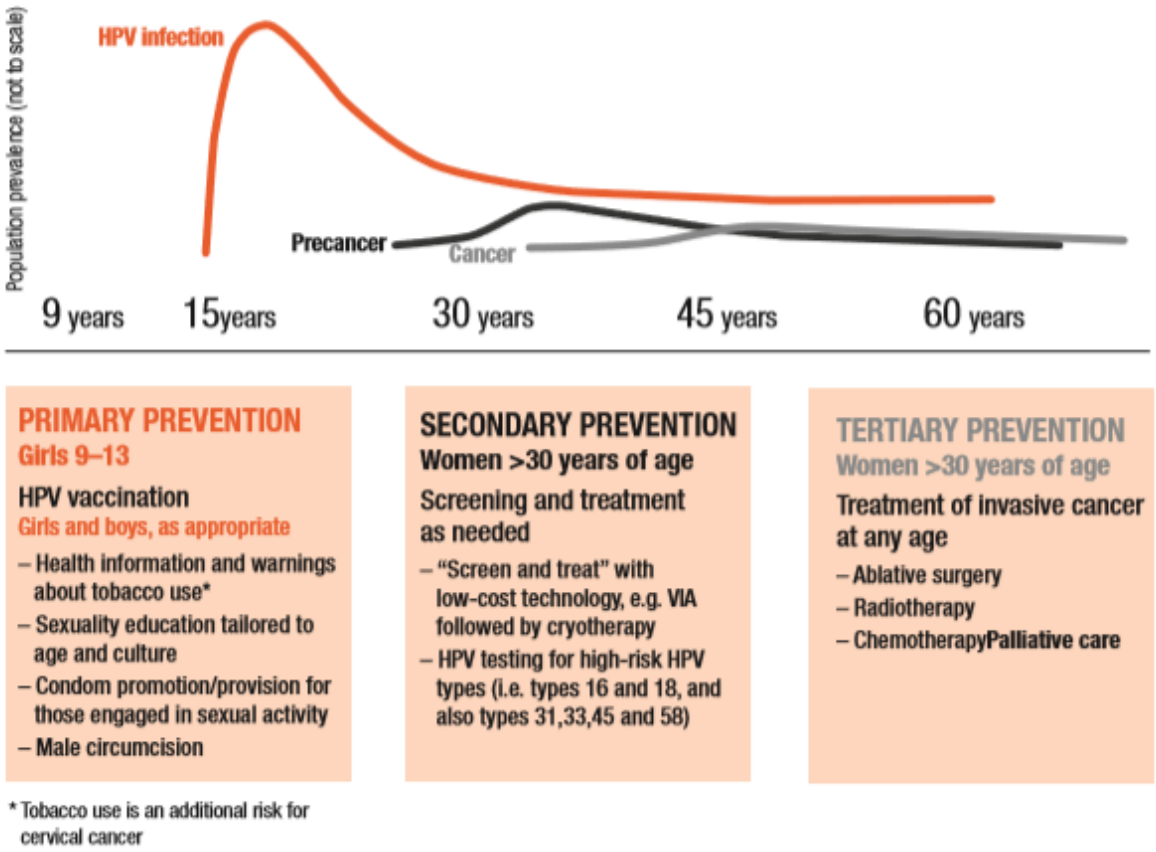


Figure 3: The WHO comprehensive approach to cervical cancer prevention and control: Overview of programmatic interventions over the life course to prevent HPV infection and cervical cancer.

Symptoms of invasive cervical cancer:

Early Symptoms:

- Vaginal discharge, sometimes foul-smelling
- Irregular bleeding (of any pattern) in women of reproductive age
- Postcoital spotting or bleeding in women of any age, even young women
- Postmenopausal spotting or bleeding
- In the case of abnormal perimenopausal bleeding, cervical cancer should always be considered, particularly if the bleeding fails to respond to appropriate treatment

Advanced Symptoms:

- Urinary frequency and urgency
- Backache
- Lower abdominal pain
- Severe back pain
- Weight loss
- Decreased urine output (from obstruction of the ureters, or renal failure)
- Leakage of urine or faeces through the vagina (due to fistulae)
- Swelling of the lower limbs
- Breathlessness (due to anaemia or, rarely, lung metastases or effusion).

Investigations for staging and treatment for cervical cancer

Mandatory for staging:

- Speculum, vaginal and rectal examination
- Intravenous pyelogram (IVP) or abdominal ultrasound

Supplementary for staging:

- Cystoscopy
- Proctoscopy
- Cone biopsy
- Endocervical curettage or smear
- Chest X-ray
- Skeletal X-ray or bone scan (if bone pain)

CERVICAL CANCER - FIGO STAGING

STAGE	
STAGE 0	Carcinoma in situ
STAGE I	Invasive carcinoma confined to the cervix
STAGE IA	Dignosed only by microscopy
STAGE IA1	Microinvasive carcinoma with stromal invasion <3mm in depth and <7mm in wide
STAGE IA2	Microinvasive carcinoma not exceeding 5mm in depth / 7mm in width
STAGE IB	Clinically visible or microscopic lesion >IA2
STAGE IB1	Clinical lesion not exceeding 4cm in diameter
STAGE IB2	Clinical lesion more than 4cm in diameter
STAGE II	Extension beyond the cervix but not to the pelvic wall
STAGE IIA	Involvement of vagina but not the lower third
STAGE IIA1	Clinically visible lesion not more than 4cm
STAGE IIA2	Clinically visible lesion more than 4cm
STAGE IIB	Parametrial Involvement not reaching the pelvic side wall
STAGE III	Carcinoma that has extended into the pelvic sidewall
STAGE IIIA	No extension into the pelvic sidewall but involvement of the lower third of the vagina
STAGE IIIB	Extension into the pelvic sidewall or hydronephrosis or non functioning kidney.
STAGE IV	Carcinoma that has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum
STAGE IVA	Spread of the tumour into adjacent pelvic organs
STAGE IVB	Spread to distant organs

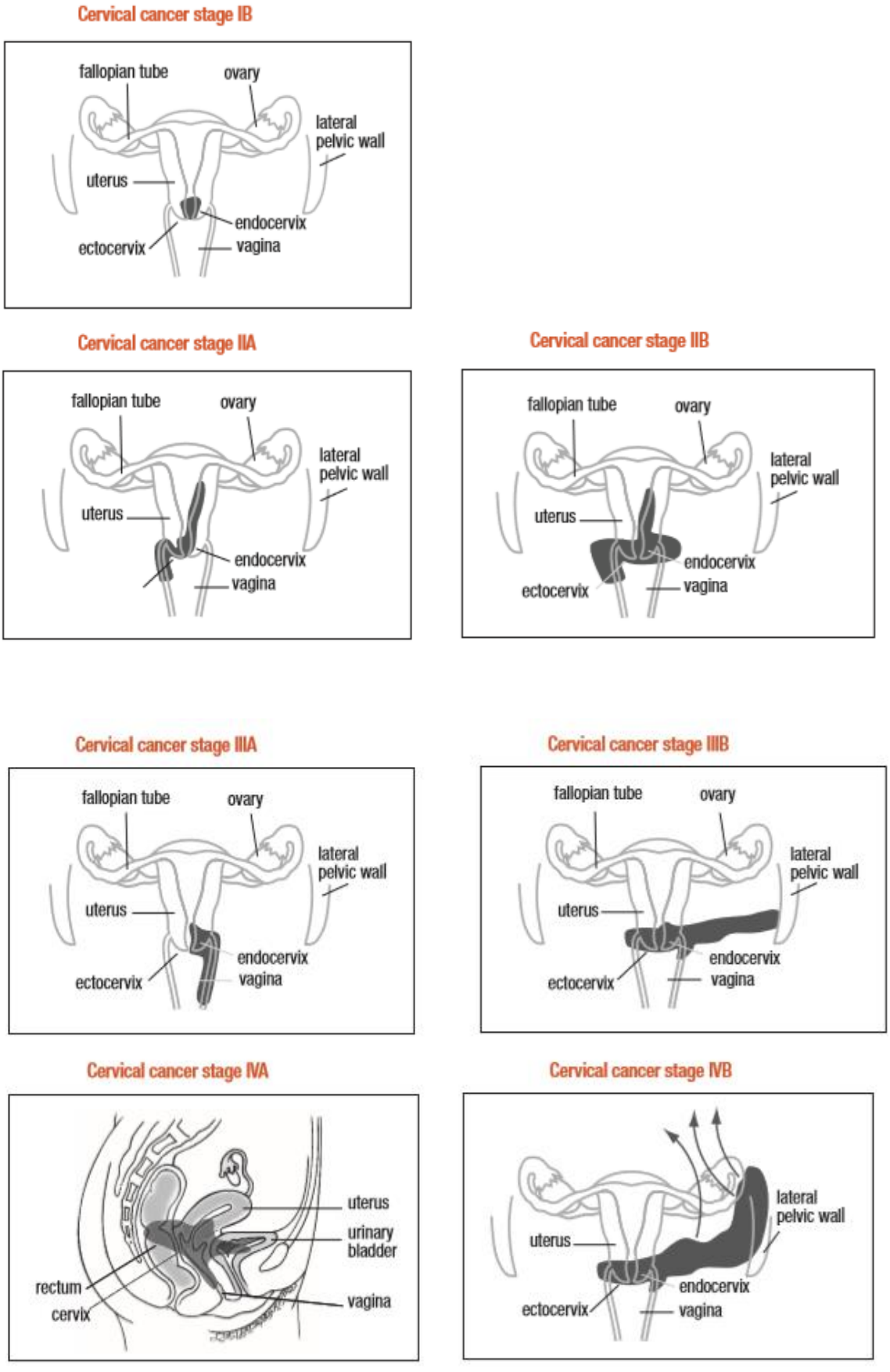


Figure 4 : Cervical Cancer – FIGO staging.

TREATMENT:

Early stage disease

Stage Ia2 (3–5mm deep stromal invasion and no greater than 7mm diameter) and Ib1 (4cm or less in size) are treated with radical hysterectomy and bilateral pelvic lymphadenectomy. In selected patients with tumours less than 2cm who are keen to preserve fertility, radical vaginal trachelectomy (removal of cervix, the upper part of the vagina, and parametrial tissue) and laparoscopic pelvic lymphadenectomy may be carried out [24].

Stage Ib1 and IIa (tumour 4cm or less) are treated equally effectively with radical surgery or combined radical EBRT and brachytherapy with both treatments giving 80–90 per cent 5year survival rates.

Stage Ib2 or IIa tumours (>4cm tumour size) have deep stromal invasion and an increased risk of parametrial and lymph node involvement. Surgery is performed in selected patients with large tumours, lymphovascular space invasion (LVSI) and adenosquamous or high grade histology, followed by postoperative radiotherapy. Ovarian transposition may minimise the chances of radiation-induced menopause.

Primary radical radiotherapy is preferable for other stage Ib2 and IIa patients. This avoids the increased morbidity seen when surgery is followed by postoperative radiotherapy. In patients who have residual macroscopic disease, positive surgical margins, parametrial involvement and positive lymph nodes, concomitant chemotherapy with radiotherapy improves local control and survival rates compared with postoperative radiotherapy alone, but late morbidity is also increased.

Locally advanced disease:

Primary radiotherapy is the treatment of choice for loco regionally advanced disease with a careful balance of EBRT and brachytherapy to maximise dose to tumour and avoid normal tissues [25].

Neoadjuvant chemotherapy shows no benefit when given before radiotherapy. Data from randomised trials show that cisplatin-based chemotherapy improves survival, especially in stage II, III, IVa disease. It is commonly delivered as a single agent, weekly during EBRT.

Where a vesico- or recto-vaginal fistula is present, a urinary diversion procedure or diversion colostomy should be performed prior to radiotherapy.

Stage IVb metastatic disease:

Short course pelvic radiotherapy is successful in relieving bleeding and pelvic pain in patients with metastatic disease. Palliative radiotherapy is used to relieve pain from bone secondaries and symptoms from brain and nodal metastases.

Palliative chemotherapy is beneficial for cervical cancer patients with distant metastasis.

Primary radiotherapy:

Primary radiotherapy is delivered to the primary cervical tumour (GTV with a margin for local microscopic spread [CTV-T]) as well as to potential sites of pelvic lymph node involvement (CTV-N), with or without concurrent chemotherapy.

Brachytherapy:

Brachytherapy allows delivery of a very high dose to the central tumour volume to obtain maximal local control without exceeding the tolerance of surrounding normal tissues. It is feasible because the normal uterus and upper vagina are relatively radio-resistant and there is rapid fall-off of dose at a distance from the cervix, protecting the adjacent rectum, bladder and small bowel.

Primary radiotherapy

Stage IB2 and IIA

EBRT 45Gy in 25 daily fractions of 1.8Gy given in 5 weeks followed by intracavitary brachytherapy.

Intracavitary brachytherapy

LDR

27Gy to point A single insertion.

Or

HDR

14Gy in 2 fractions given in 5–8 days to point A.

Stage IIB or above

EBRT

50.4Gy in 28 daily fractions of 1.8Gy given in 5 1⁄2 weeks followed by intracavitary brachytherapy.

Intracavitary brachytherapy

LDR

22.5–25Gy to point A single insertion.

HDR

21Gy in 3 fractions over 5–8 days to point A.

Concurrent chemotherapy (weekly Inj. cisplatin 40mg/m2) is given for both high risk early stage disease and locally advanced tumours unless patients are medically unfit for chemotherapy or have a Glomerular filtration rate of 50mL/min. Overall treatment time should not exceed 56 days including brachytherapy and should ideally be 49 days or less.

The major side effect associated with cisplatin chemo is nausea and vomiting. Two forms of vomiting are observed: acute (within the first 24 hours) and delayed (>24 hours).

Early form begins within 1 hour of starting cisplatin therapy and may last for 8–12 hours. The delayed form can last for 3–5 days. Nephrotoxicity is the dose-limiting toxicity, seen in up to 35%–40% of patients. Myelosuppression is another major problem, occurs in 25%–30% of patients, with WBCs, platelets, and RBCs equally affected. Ototoxicity with high-frequency hearing loss and tinnitus has also been observed with cisplatin use [26].

Cisplatin is believed to augment the effects of radiation by inhibiting the repair of radiation-induced sublethal damage and by sensitizing hypoxic cells to radiation. Because of its cytotoxic effect, the drug reduces the bulk of tumours, which leads to

reoxygenation of the tumour and entry of the cells into a radiation-sensitive phase of the cell cycle[27].

According to Elena Pereira et al, all patients with tumours < 4 cm experienced complete clinical response to RT+CT, compared to 72 (68.6%) in 142 patients with tumour size> 4 cm. Histologic subtype did not appear to be a prognostic indicator. Overall 76/135 patients with SCC demonstrated 56.3% complete clinical response rate, which was comparable to the complete clinical response of 18/30 (60%) among adenocarcinoma cases [28].

The radio-response of tumours varies across a wide spectrum, and this phenomenon has a significant effect on the treatment outcome. The difference in radio-response of tumours is attributed to intrinsic factors of tumour such as DNA aneuploidy, S phase fraction, proliferation kinetics, tumour hypoxia & intracellular thiols. Several studies have reported about the role of anti-oxidant status, GSH content, survival fraction, potential doubling time and micronuclei for predicting tumour radio-response. Among these, GSH content estimation and assessment of DNA damage by Single cell gel electrophoresis (SCGE) have shown promising results [29].

GLUTATHIONE

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is a tripeptide present in virtually all animal cells. It is usually the most abundant intracellular small molecular weight thiol, present in the millimolar range in mammalian cells. The peptide γ-linkage between glutamic acid and cysteine is thought to protect the tripeptide from degradation by aminopeptidases.

Glutathione is also less prone to oxidation than cysteine, making it an ideal compound for maintaining intracellular redox potential. Glutathione exists either in reduced (thiol, GSH) or oxidized (disulphide, GSSG) form. GSH is the predominant form, and GSSG content is usually less than 1% of GSH. In the cell, almost 90% of glutathione is in the cytosol, 10% in the mitochondria and a small percentage in the endoplasmic reticulum and in the nucleus.

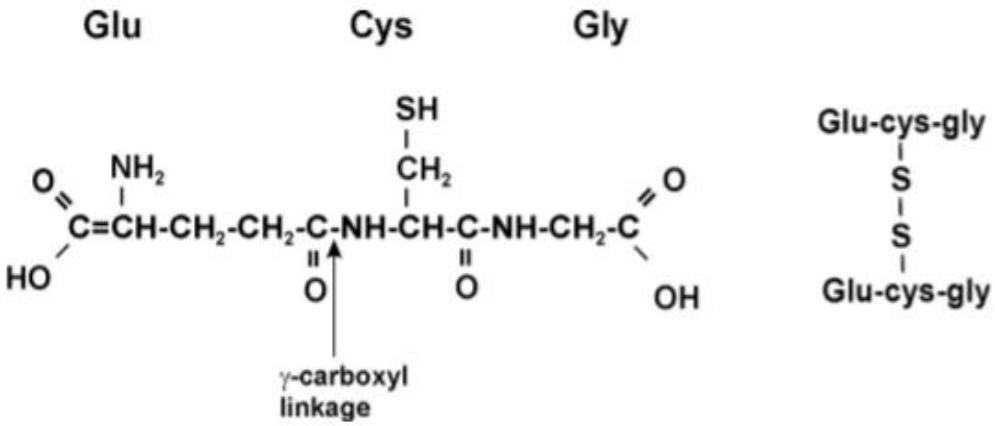


FIGURE 5: GLUTATHIONE AND GLUTATHIONE DISULPHIDE STRUCTURE

Glutathione is made up of 3 amino acids namely L-glutamic acid, L-cysteine and glycine. This tri-peptide has gamma peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) & the carboxyl group of the glutamate side-chain[30].

Molecular formula: C10H17N3O6S

Molar mass: 307.32 g/mol

Glutathione is commonly abbreviated GSH (because of the sulfhydryl group of its cysteine, which is the active functional group of the molecule). A number of

potentially toxic electrophilic xenobiotics (such as certain carcinogens) are conjugated to the nucleophilic GSH in reactions that can be represented as follows: 2GSH +R = G-S-S-G+RH

Where R = an electrophilic xenobiotic. The enzymes catalysing these reactions are called glutathione S transferases and are present in high amounts in liver cytosol and in lower amounts in other tissues. A variety of glutathione S-transferases are present in human tissue. They exhibit different substrate specificities and can be separated by electrophoretic and other techniques. If the potentially toxic xenobiotics were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage. GSH is therefore an important defence mechanism against certain toxic compounds, such as some drugs and carcinogens[31].

The glutamyl and glycinyl groups belonging to glutathione are removed by specific enzymes, and an acetyl group (donated by acetyl-coA) is added to the amino group of the remaining cysteinyl moiety. The resulting compound is a mercapturic acid, a conjugate of L-acetylcysteine, which is then excreted in the urine [62].

Glutathione is synthesized in the cytoplasm. Since mitochondria do not contain the enzymes required for glutathione synthesis, about 10-20% of total cellular glutathione is transported into mitochondria from the cytoplasm by at least two transport systems. Activity of γ-glutamylcysteine synthetase and availability of cysteine are the rate limiting factors for glutathione synthesis. Glutamate and glycine are readily synthesized via several metabolic pathways, and are not believed to limit the rate of glutathione synthesis. The most important source of cysteine is dietary, but it can also be supplied by cleavage of cysteine or by trans-sulphuration of methionine via the

cystathionine pathway in the liver. Although glutathione is exported from many cells under normal conditions, and exported glutathione enters plasma, the liver is the main source of plasma glutathione. Sources of glutathione in the intestine include the diet, hepatic glutathione exported into bile, desquamated epithelial cells, and export from epithelial cells of the stomach and intestine. Generally, glutathione does not freely enter cells, but uptake depends on γ-glutamyl trans peptidase, an enzyme present on the external surface of the cell membrane in many tissues [32].

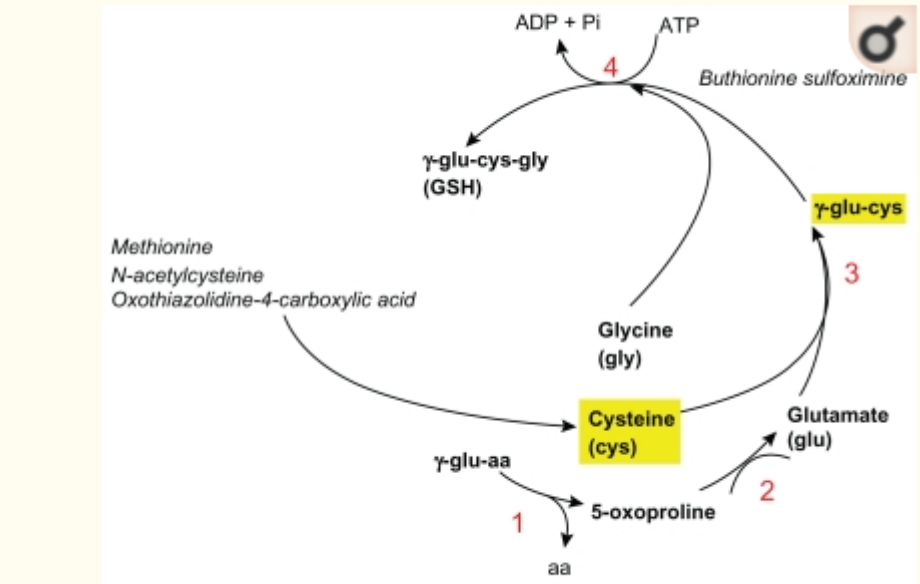


FIGURE 6: Glutathione (GSH) biosynthesis . 1) gamma-glutamyl cyclotransferase; 2) 5-oxoprolinase; 3) gamma-glutamylcysteine synthetase; 4) Glutathione synthetase. Outlined in yellow are the limiting factors in GSH biosynthesis.

FUNCTIONS:

1. It participates in the decomposition of potentially toxic hydrogen peroxide in the reaction catalysed by glutathione peroxidase.

2. It is an important intracellular reductant, helping to maintain essential SH groups of enzymes in their reduced state.
3. A metabolic cycle involving GSH as a carrier has been implicated in the transport of certain amino acids across membranes in the kidney. The first reaction of the cycle is shown below.
4. Amino acid + GSH ⟹ γ- Glutamyl amino acid+ Cysteinylglycine.

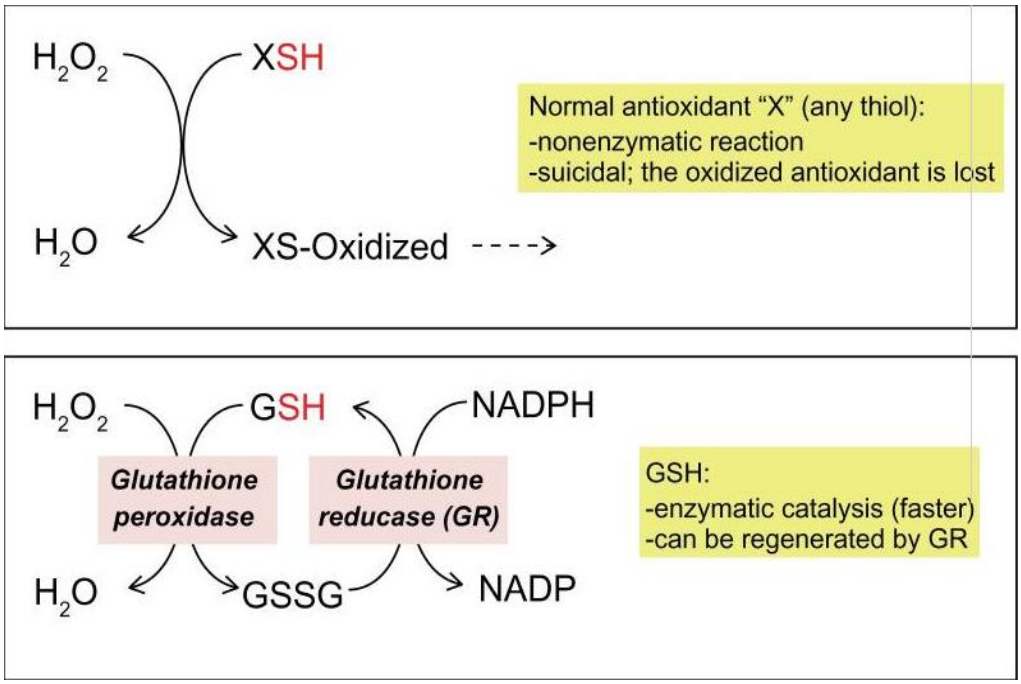


Figure 6: METABOLIC CYCLE OF GLUTATHIONE (Glutathione: a very efficient antioxidant).

GSH is essential for some functions of the immune system, both innate and adaptive, including T-lymphocyte proliferation, phagocytic activity of polymorphonuclear neutrophils (PMN) and dendritic cell functions and is also important for the first step of adaptive immunity, consisting of the antigen presentation by antigen-presenting cells (APC). Indeed, cell-mediated immunity requires that protein antigens be first degraded in the endocytic vesicles of APCs (e.g., macrophages, dendritic cells), so

that the smaller peptides can be presented on the cell surface by the major histocompatibility complex to activate proliferation of antigen-specific T cells. One of the first steps in antigen degradation and processing is the reduction of disulfide bonds, which requires GSH[33].

ROLE OF GLUTATHIONE IN CANCER:

1. Glutathione by its free radical scavenging action reduces the level of reactive oxygen species in non-cancerous cells, which reduces the risk of cancer development.

2. Glutathione is required for DNA repair.

3. Glutathione is a natural detoxifying agent. It gets conjugated with carcinogenic and toxic agents that enter into the body and neutralize them hence preventing cancer.

4. Glutathione is an integral component of immune system. It is involved in many steps of immune reactions and facilitates its functions. Intact immune system reduces the risk of development of cancer. Immunocompromised patients have an increased risk of development of cancer.

FUNCTION OF GLUTATHIONE IN CANCER CELLS:

1. Tumours possess a higher glutathione content than normal tissues which help them survive in adverse conditions.

2. Tumours with higher glutathione content are more aggressive and possess higher tendency to metastasise.

3. Metastatic deposits were found to have higher glutathione content than the primary tumour. This means that tumour clonogens which were capable of producing more glutathione were more successful in reaching distant sites and growing there. Higher glutathione content helps them in surviving the high oxygen concentration during transit through blood and lymphatics.

4. Glutathione (GSH) plays a critical role in cellular mechanisms that result in cell death. The studies have found that cancer cells resistant to apoptosis had higher intracellular GSH levels. Depletion of glutathione in these tumour cells makes them more vulnerable to the effects of anticancer drugs and radiotherapy.

The reduction-oxidation (redox) balance in tissue is controlled in part by the relative concentrations of reduced glutathione and its oxidized disulfide counterpart that can influence gene expression, cellular differentiation, proliferation and apoptosis. Many of the chemotherapeutic agents and radiation treatments used today induce some form of oxidative stress in the cancer cell and alter this redox balance. Therefore,

antioxidants play a key role in determining cellular response to wide range of therapies[34].

Induction of GSH deficiency in tumours was shown to be potentially useful in cancer therapy[34]. Elevation of intracellular GSH levels is associated with mitogenic stimulation and regulation of DNA synthesis[35].We found that GSH controls the onset of tumour-cell proliferation by regulating protein kinase C activity and intracellular pH [36], and that a direct increase of intracellular GSH levels promotes survival and metastatic growth of B16M cells in the liver [37, 38].

The intracellular GSH content of melanoma cells can be modulated by the tumour GGT-dependent cleavage of extracellular GSH. This is important because multidrug and/or radiation resistance, which are characteristic features of malignant tumours, frequently associate with high GSH content in the cancer cells[39]. Indeed, exogenous GSH may protect cells from oxidative damage[40].GGT activity generates cysteine, the rate-limiting amino acid for GSH synthesis, promoting an increase in tumour GSH and thereby, facilitating metastatic growth.

GLUTATHIONE: TO EVALUATE TUMOUR RESPONSE TO TREATMENT

Glutathione prevents free radical damage arising from radiation and chemotherapy administration and has been shown to directly bind and inactivate electrophilic intermediates [41]. Depletion of cellular glutathione leads to sensitization of tumours to chemotherapy and to radiation [42], and increase of cellular glutathione often augments resistance to these modalities. In addition to preventing free radical damage, glutathione provides cells with reducing power utilized for maintenance of thiol groups of intracellular proteins and other molecules[44].

Analysis of tumours for glutathione content may predict chemo sensitivity, as the cellular level of glutathione determines the degree of drug resistance of the tumour.

When the glutathione levels are depressed, there is markedly increased sensitivity to radiation [46].

Platinum compounds can be inactivated by the process of glutathione conjugation. The "glutathione pathway" is highly genetically polymorphic which includes enzymes responsible for glutathione synthesis and redox status, as well as glutathione S-transferases (GSTs) and transporters that remove glutathione conjugates from cells[47]. This pathway is responsible for cellular resistance to cancer chemotherapy by glutathione conjugation of active metabolites which is followed by the excretion of glutathione conjugates [45].

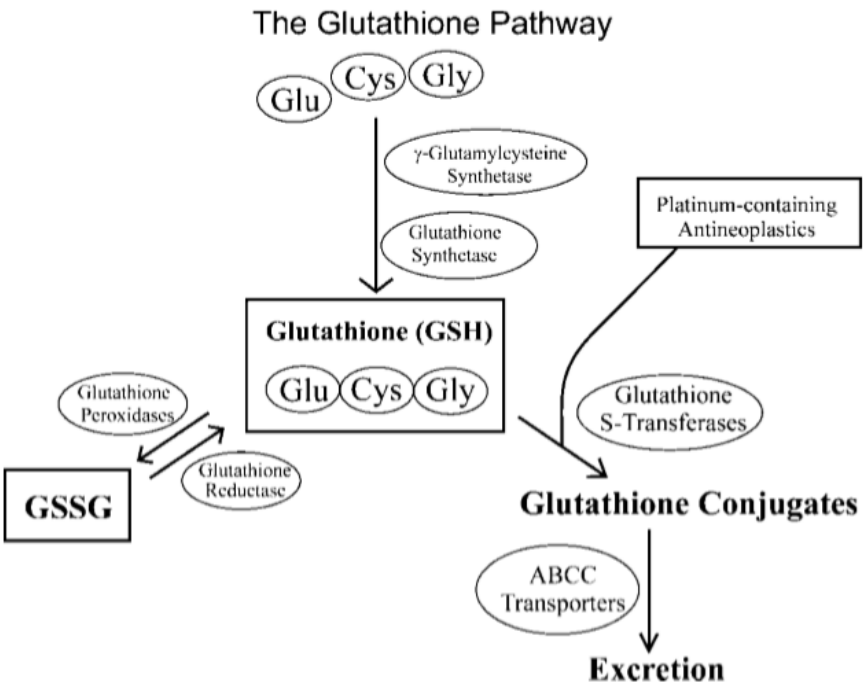


FIGURE 7: Schematic representation of glutathione pathway. Glutathione is synthesized by glycine, cysteine and glutamate. Glutathione red-ox state is regulated

by glutathione peroxidases. Glutathione is conjugated to substrates and excreted by cell membranes through ABCC transporters.

Studies have shown that it is possible to achieve very high levels of cisplatin resistance (>1000-fold) in cultured human tumour cells. Increase in cellular glutathione is accompanied by Cisplatin resistance which is essentially linear with resistance. The increase in glutathione may initially be accomplished by increases in γ-glutamyl trans peptidase steady-state mRNA, but as resistance and glutathione levels increase, the expression of γ -glutamylcysteine synthetase is elevated [43] These changes occur in the absence of gene amplification. Thus, it was established that there is a direct causal relationship between glutathione and resistance produced by classical alkylating agents, platinum drugs and irradiation[48] .

ROLE OF GLUTATHIONE IN CERVICAL CANCER :

Experimental evidence shows the important contribution of free radicals to carcinogenesis that is caused by damaging DNA synthesis and repair mechanisms. The oxidant-antioxidant system balance and the levels of antioxidant system enzymes and molecules have been intensively investigated in head and neck, breast, and bladder cancers. Along with other malignancies, the antioxidant levels in patients with cervical cancer have been investigated.

A prospective study conducted in 1993 at Taiwan to investigate the intratumour GSH distribution and variation, as well as the difference between tumour and its normal counterpart showed the GSH content was two fold higher in squamous cell carcinoma of the cervix than its normal counterpart, but there was no obvious difference in a case

with adenoid cystic carcinoma. The tumour margin adjacent to normal tissue had a higher GSH content than the tumour center in a large tumour [49].

A study conducted 1994 by Subramaniyan et al on status of antioxidant levels in carcinoma cervix, showed a remarkable reduction in glutathione content and in the activities of catalase and superoxide dismutase in neoplastic tissue in stages II, III and IV ($P < 0.001$) whereas the activities of glutathione peroxidase and glutathione reductase were significantly lower in stage III and IV patients than that of normal controls. The tissue level of lipid peroxides and glutathione-S-transferase were found to be significantly higher than that of normal from stage II onwards. These suggested the impaired antioxidant status in cervical cancer patients [50].

A study was conducted in 1997 to determine Glutathione S-transferase activity as an early marker for malignant tumours of corpus uteri. Glutathione (GSH) concentrations and glutathione S-transferase (GST) activities were determined in 30 paired malignant human corpus uteri tumour samples and in samples of adjacent normal tissues. For GSH concentrations no difference was found between normal (126.0 +/- 52.4 nmol/mg protein) and tumour tissues (110.1 +/- 46.4 nmol/min/mg protein; $p = 0.219$). The GST activities were significantly higher in tumour tissues (322.4 +/- 135.54 nmol/min/mg protein) than in corresponding normal tissues (224.6 +/- 95.64 nmol/min/mg protein; $p = 0.005$). This activities were independent on the pathohistological and clinical factors, except for positive lymphovascular invasion and myometrial invasion (over 50%), where significantly lower GST activities were found. For normal tissues the positive correlation between GSH concentrations and GST activities was found (correlation coefficient = 0.50, $p = 0.005$), but not for tumour tissue (correlation coefficient = 0.20, $p = 0.281$). The prognosis of patients

(according to the well-established prognostic factors, such as tumour type, myometrial invasion and grades) who had lower GSH concentration and GST activity in normal tissue was similar to those with higher GSH concentration and GST activity [51].

In 1998 , a case control study was conducted to determine the predictive role of GSH for determining response to RT . Blood and tissue samples were collected from 45 cancer patients before and after the first fraction of RT. They concluded that GSH levels in blood and tissue were significantly decreased after the first fraction, and they found a correlation between the tumour response and the rate of GSH depletion [53].

A study conducted in 1999 to estimate Lipid peroxides, glutathione content and the activities of antioxidant enzymes, with vitamin C and E content, in patients who had carcinoma of the cervix, and these values were compared with those of normal women. The results showed a significant reduction in the content of glutathione, vitamin E and C. Amount of glutathione peroxidase and superoxide dismutase were also reduced in cervical cancer compared to normal controls , this reduction was more marked in late stages (III, IV) than in early stages (I, II). Glutathione levels were significantly reduced in poorly differentiated tumours (grade III) compared to moderate and well differentiated tumours (grade I and II) [52].

A prospective study conducted in 1999 by Mukundan et al. studied GSH and GPX levels in 30 patients with cervical cancer pre- and post-RT. They showed that the GSH and GPX levels of patients with cervical cancer were significantly lower than those of the control group [54].

In 2005, Hedley et al. Investigated the relation between nuclear non-protein sulfydryls (NPSH) glutathione and cysteine and the clinical outcome of radical radiotherapy

(RT). Results showed Nuclear and tissue NPSH values correlated closely in all cases. No statistically significant associations were noted between NPSH levels and tumour size, stage . Tissue and nuclear NPSH are not predictive of the outcome of patients with locally advanced cervical carcinomas treated with RT [55].

A study was conducted in 2007 to investigate the blood lipid peroxide, GSH, GPx, glutathione-s -transferase (GST) and catalase levels in 60 patients with cervical cancer before neoadjuvant chemotherapy, 2 weeks after the second cycle of chemotherapy and 2 weeks after the completion of RT. They reported that lipid peroxide levels were higher in patients with cancer, and before treatment, the GSH, GPx, GST and catalase levels were lower. They also reported that the elevated lipid peroxide levels before chemotherapy decreased after treatment, and the GSH, GPx, GST and catalase levels returned to normal after the completion of Radiotherapy [56].

In 2007, Wozniak et al. investigated the catalase and GPx levels in patients with cervical cancer. Individuals were classified into 3 groups based on treatment options: group 1, patients who had brachytherapy before surgery; group 2, patients who had brachytherapy after RCT, and group 3, patients who had external treatment after brachytherapy. Catalase activity was higher and GPx activity lower before and after treatment in all groups compared to the control group. In cancer patients, enzyme levels had returned to normal values six months after treatment. The authors stated that the normalization of catalase and GPx activities after treatment shows the efficacy of treatment. Moreover, several other studies have also shown that GSH and GPx activities are decreased and MDA levels elevated in patients with cervical cancer compared to a control group [57].

A decreased GSH level and GPx activity in cancer patients may lead to the accumulation of free radicals and induction of tumorigenesis in patients. However, another possible mechanism is that carcinogenesis causes decreased GSH synthesis and disruption of defence mechanism. Decreased SOD activities in various cancer patients have been shown by researchers. Investigators from India evaluated the status of SOD in cervical cancer patients treated with RT and chemotherapy and they found that the SOD activity of the cancer patients were lower than those of the control subjects [58].

In 2008, Kasapovic et al. evaluated antioxidant status and lipid peroxidation in breast cancer patients and demonstrated that the SOD activities of breast cancer patients were decreased compared to controls [60].

In 2009, Kacakci et al. evaluated SOD activity in laryngeal cancer patients and compared it with that of healthy controls; they found that the SOD activity was lower in the cancer patients [59].

In 2010, Vidyasagar *et al.*[61]demonstrated that serum GSH depletion may be a predictor of treatment response for cervical cancer patients treated with RCT. Study was conducted to assess the predictive significance of serum glutathione (GSH) and tumour tissue DNA damage in the treatment of cervical cancer patients undergoing chemo radiotherapy. Cervical cancer patients who underwent chemo radiotherapy, blood samples were collected after 2 fractions and after 5 fractions of radiotherapy (RT) both blood and tissue samples were collected.

The results showed Serum GSH content significantly depleted after a total dose of 4Gy and 10Gy of radiotherapy with a single dose of cisplatin, which was significantly lesser in non responders (NR) than of complete responders (CR) patients. Similarly,

Olive Tail Moment, the index of DNA damage, indicated higher values in the fifth fraction of radiotherapy (5-RT) than in pre-treatment. The DNA damage after 5-RT in the NR subgroup was significantly lower than that of CR[61].

Table 1 : Comparison of pre and post 2 RT serum glutathione levels by vidyasagar et al.

	Serum Glutathione level (mcg/ml)		
Tumour response	Pre-treatment levels (mcg/ml)	Post-treatment levels (mcg/ml)	P value
Complete responders	148.63 +/- 15.64	110.67 +/- 6.1	<0.001
Partial responders	167.79 +/- 2.81	144.56 +/- 15.45	<0.01
Non responders	172.69 +/- 7.61	151.93 +/- 1.01	NS

Table 2 : Comparison of pre and post 5 RT serum glutathione levels by vidyasagar et al.			
	Serum Glutathione level (mcg/ml)		
Tumour response	Pre-treatment levels (mcg/ml)	Post-treatment levels (mcg/ml)	P value
Complete responders	149.12 +/- 7.2	99.52 +/- 11.76	<0.001
Partial responders	159.94 +/- 6.24	138.04 +/- 13.8	<0.01
Non responders	167.66 +/- 3.45	158.15 +/- 2.3	NS

In a case control study conducted in 2011 showed that in the patient group, pre-radiotherapy glutathione and glutathione peroxidase levels were significantly lower (P <0.01 and P <0.0001, respectively) than the control group. Pre-radiotherapy levels of superoxide dismutase were significantly higher in cancer patients (P <0.01). In general, no difference was observed between pre- and post-radiotherapy antioxidant levels in cancer patients. However, when post-radiotherapy glutathione levels were analysed, patients who did not respond to treatment had significantly higher levels than those who did respond (P <0.01) [63].

Table 3: comparison of Pre and Post RT serum glutathione by Demecri et al.		
Tumour response	Pre-treatment levels (mcg/ml)	Post-treatment levels (mcg/ml)
Complete responders	434.07 +/- 154.63	614.64 +/- 193.13
Partial responders	542.21 +/- 108.06	352.46 +/- 133.13
Non responders	442.23 +/- 114.50	465.90 +/- 148.61
P value	0.08	< 0.01

A study was conducted in 2016 in Karnataka to investigate whether the fall in serum glutathione predict the long-term outcome to concurrent chemo radiation for cervical cancer patients. Thirty eight patients with cervical cancer were treated with concurrent chemo radiation followed by brachytherapy. Serum GSH was measured before and after two fractions of radiation and first chemotherapy. Patients were followed for a median follow up of four years. Fall in GSH was correlated with response at six weeks and disease status at four years. Results showed that Median fall in serum GSH was 171.16 µg per ml. fall in GSH was 170.42, 103.54 and 37.25 µg per ml (*P* value of <0.0001, 0.05 and 0.18) in patients showing complete response, partial response and no response respectively. Among 26 patients who had no disease at six weeks, 22 women remained disease free at four years ($P < 0.0001$), two recurred ($P < 0.05$) and two died of other causes respectively. Non bulky tumours and patients more than 50 years of age showed a fall of 190.69, 265.17 µg per ml respectively. The study

concluded that Greater fall in serum GSH predicts better early response as well as long term disease control [64].

ROLE OF GLUTATHIONE IN OTHER CARCINOMAS:

Glutathione levels in brain cancers have found to be lower compared to normal brain tissue. An exception for this is seen in meningomas, where higher levels of glutathione was found in tumour tissue compared to normal brain tissue. A study conducted by kudo et al in 1990 concluded that there are low levels of glutathione in tumours and these low levels make the tumours more sensitive to treatment [65].

Glutathione levels was found to be higher in the breast tumour tissue compared to the adjacent normal tissue. Buser et al conducted a study in 1997 and concluded that there were no differences in disease free or overall survival rate in patients with high levels glutathione and various drug resistance parameters. In fact good prognosis was associated with higher levels of glutathione [66]. In another study by Woolston et al, there was no co-relation between glutathione levels and overall recurrence or survival rates [67].

Skrzydlewska et al. conducted a study on 2003 and the results showed a increase in glutathione levels in oesophageal cancer compared to normal tissue [68]. Contradicting these above results, Sivho et al and Levy et al, reported that there were significantly lower levels of glutathione in oesophageal tumours compared to healthy tissue [69,70].

To evaluate tumour therapy response in relation to glutathione levels, Barranco et al concluded that patients with a high level of glutathione the survival level at 24 months was 54% compared with 89% for the low level group. There was a significant association between colorectal tumour levels found at the time of diagnosis and patient survival [71].

Glutathione levels have also been studied in ovarian carcinomas and their relation to therapy response. Cheng et al monitored glutathione levels in ovarian carcinoma patients before and after chemotherapy and concluded that there was no difference in the two groups , concluding that glutathione does not play a role in therapy response [72].

Materials and methods

MATERIALS AND METHODS

Study Design: It is a prospective hospital based study.

Source of Data: Study conducted in department of OBG, in association with department of radiotherapy and department of biochemistry, R.L Jalappa hospital , Kolar. Patients in the age group of 30 yrs – 70 yrs visiting the department of OBG and clinically diagnosed with cervical cancer were enrolled in the study group after obtaining written consent form from the patients.

The duration of study period was from January 2017 to June 2018.

Sample size:

Was estimated based on the outcome of Serum glutathione levels (110.67 ± 6.1) in the cervical cancer (Type Ib to type IIIb) subjects at Post radiotherapy period from the study by Mamidipudi et al. Considering SD of 6. 1, at 5% alpha error and at 95% Confidence level and 2% precision, sample size of 36 was obtained and will be included in the study.

$$\text{SAMPLE SIZE} = Z1\text{-}\alpha/22SD2 / d2$$

Z = = Is standard normal variate as mentioned in previous section= at 5% alpha error =1.96

SD = 6.1

d= Absolute error or precision as mentioned in previous section=2% error

N = 36

Considering 10% non-response a sample size of 36 + 3.6 ≈ 40 cases will be included in the study.

Inclusion criteria:

- Patients with biopsy positive for cervical cancer.
- Age between 40-70yrs.
- Cervical cancer patients with FIGO staging IB- IIIB

Exclusion criteria:

- Cancer cervix patients with HIV , active tuberculosis.
- Cancer cervix patients previously treated .
- Patients operated for cervical cancer.
- Recurrent cases of cancer cervix.

METHOD:

The study sample consisted of 40 patients who underwent routine investigations such as haemoglobin, white blood cell and platelet count, renal functional tests, ultrasound of abdomen and pelvis, cervical biopsy, chest X-ray and staged as per recommendations of International Federation of Gynaecology and Obstetrics classification. The patients were administered Inj. Cisplatin 40 mg/m2 BSA once per week as IV infusion for 5 weeks with an initial dose on first day of the week and concurrently subjected to external beam radiotherapy of 50Gy in 25 fractions, 5 days per week for 5 weeks delivered using Tele-cobalt. A standard four-field box technique involving anterior and posterior and two lateral fields was employed. After 2 weeks of rest, two applications of remote after loaded HDR intracavitary brachytherapy of 6.5 Gy x 2 fractions to point A was delivered once per week. Response to radiotherapy was assessed after 3months of completion of treatment. The

clinical response assessment was done as per World Health Organization guidelines. Degree of tumour volume shrinkage was considered as an index of radio responsiveness. Patients with 100% regression of tumour at the primary site are considered as complete responders (CR), whereas partial responders (PR) have more than 50% regression and non responders (NR) have a lower than 50% regression .

Statistical Analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were compared using Independent t test (as the data sets were normally distributed) between the two groups and ANOVA test between more than two groups. Paired t test was used for comparison between pre RT GSH and post RT GSH.

2. Qualitative variables were correlated using Chi-Square test/Fisher's exact test.

A p value of < 0.05 was considered statistically significant.

The data was entered in MS EXCEL spreadsheet and analysis was done using

Statistical Package for Social Sciences (SPSS) version 21.0.

Sample collection and processing:

Two millilitre of blood was collected from the patients from ante-cubital vein under aseptic conditions , first sample was pre-treatment i.e. . on Day 1 and second sample on Day 3 after two fractions of radiotherapy and one cycle of chemotherapy. Blood was allowed to clot soon after collection in room temperature for 30minutes. Then the samples were centrifuged at 3000rpm for 20 minutes to obtain clear serum that was stored at -20 degree Celsius until analysis.

GLUTATHIONE ESTIMATION BY OPTIMISED ENZYMATIC RECYCLING METHOD:

Glutathione present in the serum sample was estimated as per the procedure supplied by Cayman's chemicals USA.Cayman's GSH assay utilizes a carefully optimised enzymatic recycling method, using glutathione reductase , for the quantification of GSH. The sulfhydryl group of GSH reacts with DTNB and produces a yellow coloured 5-thio-2-nitrobenzoic acid(TNB). The mixed disulphide, GSTNB (between GSH and TNB) that is concomitantly produced , is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 405nm provided an accurate estimation of GSH in the sample.

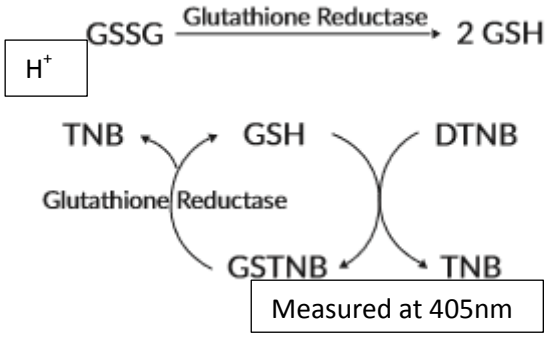


Figure 9: Shows glutathione recycling

REAGENTS USED:

1. GSH MES Buffer – the buffer consists of 0.4 M 2-(N-morpholino) ethanesulphonic acid , 0.1 M phosphate and 2nM EDTA, pH6.0.
2. GSSG Standard – consists of 2ml of 25µM GSSG in MES buffer.
3. GSH Co-factor Mixture – consists a lyophilized powder of NADP+ and glucose-6-phosphate. This is reconstituted with 0.5ml of water and used.
4. GSH Enzyme Mixture – consists of glutathione reductase and glucose-6-phosphate dehydrogenase in 0.2ml buffer.
5. GSH DTNB – consists a lyophilised powder of DTNB. The contents are reconstituted with 0.5ml of water and used.

SAMPLE PREPARATION :

The serum sample which was stored at -20 degree Celsius and was kept at 4 degree Celsius for 30minutes before starting the procedure.

DEPROTEINATION OF SAMPLES:

All biological samples used for GSH measurement contain large amounts of proteins. Hence, it is necessary to remove as much protein possible from the sample to avoid interferences due to particulates and sulfhydryl groups of proteins.

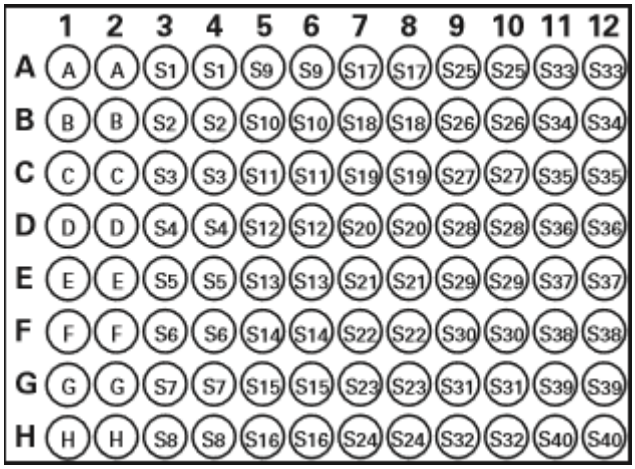
Reagents required were MPA (Metaphosphoric acid) reagent, 5g of MPA dissolved in 5oml of water and 4M TEAM reagent (prepared by mixing 531 µl of triethanolamine with 469 µl of water).

Equal volume of MPA (800 µl) and sample (800 µl) was added and vortexed for mixing , vortexed sample was kept at room temperature for 5 minute, later centrifuged at 2000rpm for 2-3 minutes and supernant was collected.

To the above 1ml of supernant, 50 µl of 4M TEAM reagent was added and vortexed immediately. Addition of TEAM reagent increases the pH of the supernant .thus the supernant obtained was ready for assay of total Glutathione (both reduced and oxidized).

ASSAY PROTOCOL:

Plate set up:



A-H = Standards

S1-S40 = Samples

STANDARD PREPARATION:

Eight clean test tubes are taken and marked from A-H. GSSG standard and MES buffer is added to each tube as described.

Tube	GSSG Standard (µl)	MES Buffer (µl)	Final Concentration (µM GSSG)	Equivalent Total GSH (µM)*
A	0	500	0	0
B	5	495	0.25	0.5
C	10	490	0.5	1.0
D	20	480	1.0	2.0
E	40	460	2.0	4.0
F	80	420	4.0	8.0
G	120	380	6.0	12.0
H	160	340	8.0	16.0

PERFORMING THE ASSAY:

1. 50µl of standard (tubes A-H) was added to wells as designated on the plate.

2. 50µl of sample was added to each of the sample wells.

3. Plate was covered for 5 minutes using plate cover.

4. Assay cocktail was prepared by mixing the following reagents in a 20ml vial: MES buffer (11.25ml) , reconstituted cofactor mixture (0.45ml) , reconstituted enzyme mixture (2.1ml) , water (2.3ml) and reconstituted DTNB (0.45ml).

5. The cover of the plate was removed and 150 µl of freshly prepared assay cocktail was added to each of the wells containing standards and samples. The cover was replaced and plate incubated in the dark for 25minutes.

6. GSH concentration of the sample was measured by end point method at 405nm where the average absorbance from the 25minutes measurement for each sample and standard was calculated.

7. Absorbance value of standard A was subtracted from itself and from all other values. This value was the corrected absorbance.

8. The corrected absorbance values were plotted and GSH values were calculated from the standard curve.

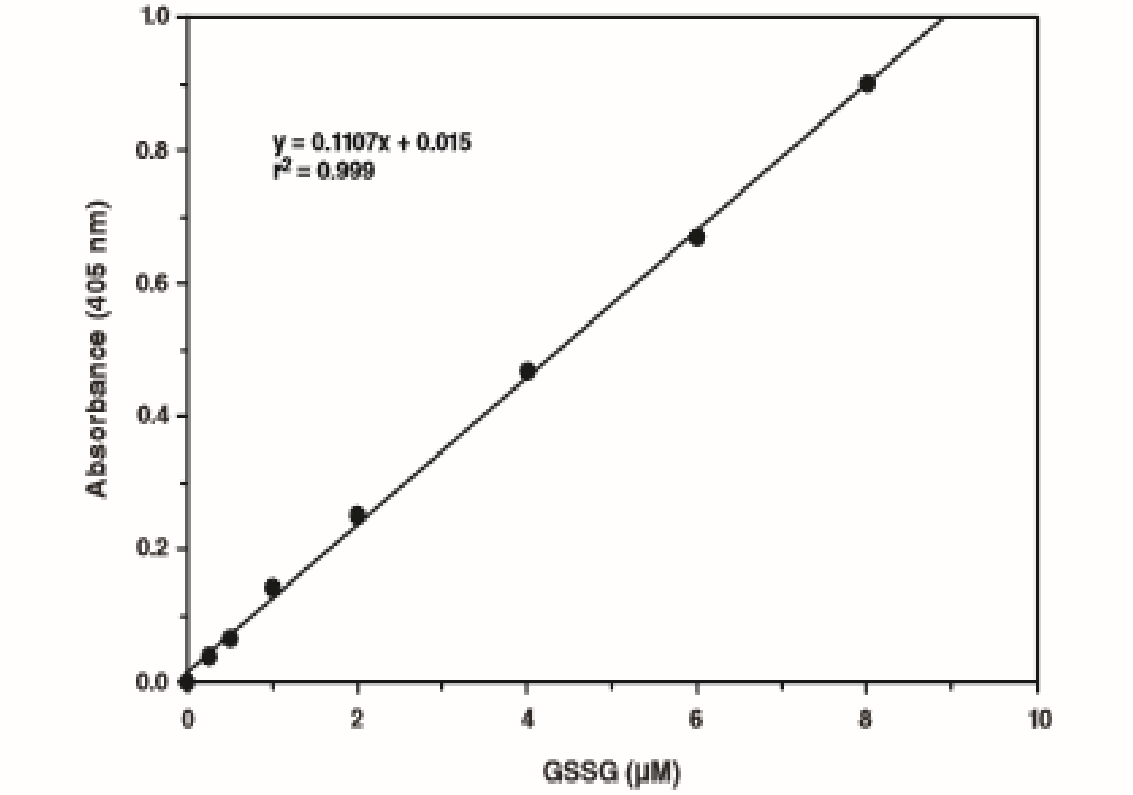


Figure 10: plot of corrected absorbance at 405nm versus GSSG concentration (µM)

Total GSH = (absorbance at 405nm)- (y-intercept) x 2 x sample dilution

slope

Report =____µM of GSH in the given sample.

Figure 11 : Plate placed in the Elisa machine for estimation of serum glutathione.

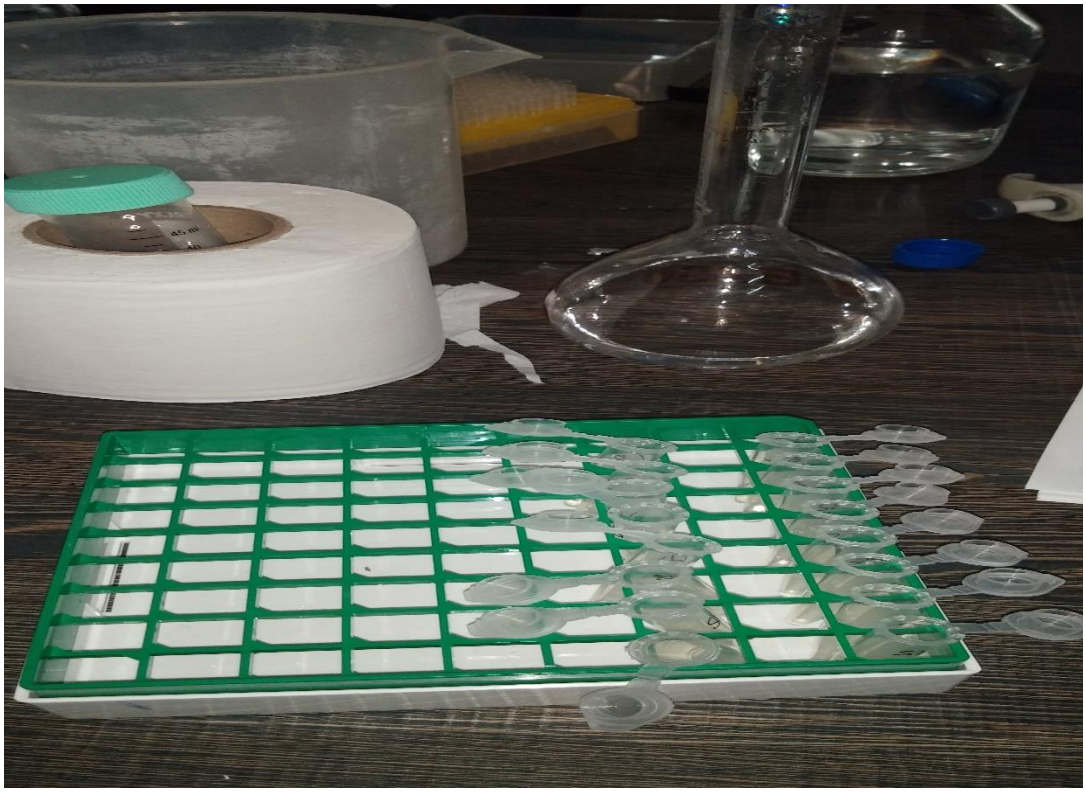


FIGURE 12: Standard tubes from A- H.

Figure 13: Vortex with centrifuge machine.

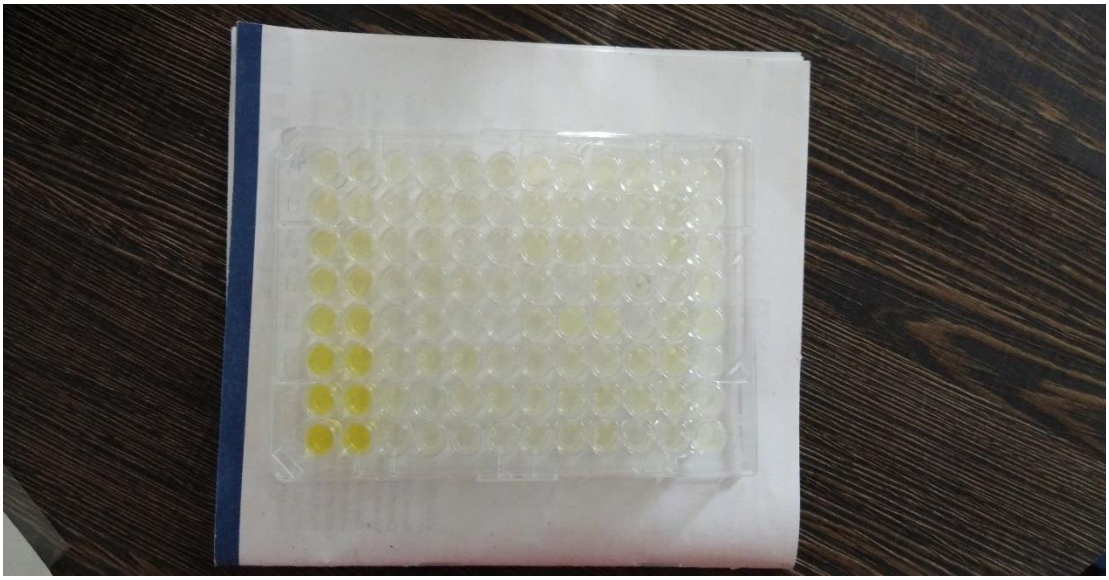


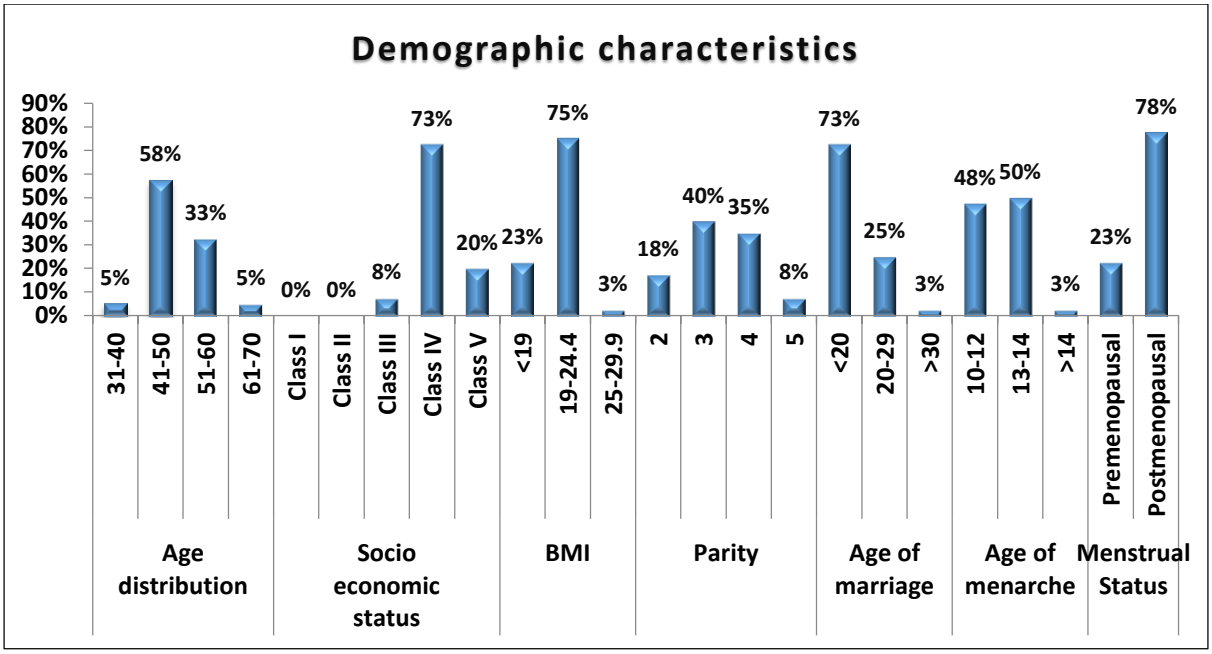
Figure 14 : standard and samples showing colour change

Results

RESULTS AND OBSERVATIONS

Table 4:- Demographic characteristics

Demographic characteristics	Frequency	Percentage
Age distribution in years		
31-40	2	5.00%
41-50	23	57.50%
51-60	13	32.50%
61-70	2	5.00%
Socio economic status		
Class I	0	0.00%
Class II	0	0.00%
Class III	3	7.50%
Class IV	29	72.50%
Class V	8	20.00%
BMI(kg/m2)		
<18.5	9	22.50%
18.5-24.9	30	75.00%
25-29.9	1	2.50%
Parity		
2	7	17.50%
3	16	40.00%
4	14	35.00%
5	3	7.50%
Age of Marriage in years		
<20	29	72.50%
20-29	10	25.00%
>30	1	2.50%
Age of menarche in years		
10-12	19	47.50%
13-14	20	50.00%
>14	1	2.50%
Menstrual Status		
Premenopausal	9	22.50%
Postmenopausal	31	77.50%



Graph 1:- Demographic characteristics

In the present study, majority of the patients enrolled were in the age group 41-50 years, 32.50% of patients were in the age group 51-60 years and only 2 patients (5%)were in the age group 31-40 years and 61-70 years each.

Socio economic status of 72.50% of patients were Class IV and very few patients belonged to Class III . None of the patient belonged to Class I and Class II.

Around 75% of patients had BMI between 19-24.4 kg/m^2, and only one had BMI more than 25 kg/m^2.

Majority of patients had parity 3 followed by parity 4 and very few patients had 2 and 5 parity.

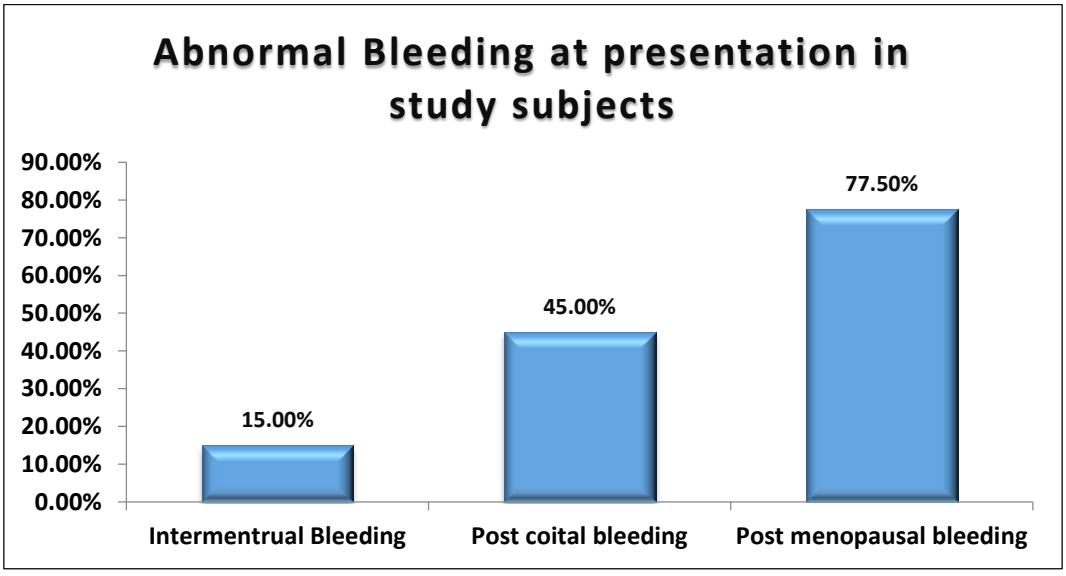
Age of marriage of around 72.50% of patients were below 20 years, 25% of patients got married in the age of 20-29 years and only one patient was married at the age of more than 30 years.

Almost half of the patient's age of menarche was between 13-14 years and 47.50% of patient's age of menarche was between 10-12 years.

Menstrual status of majority of patients was postmenopausal compared to only 22.50% of patients were premenopausal.

Table 5:- Abnormal bleeding at presentation in study subjects

Abnormal bleeding	Frequency	Percentage %
Intermenstrual Bleeding	6	15.00
Post coital bleeding	18	45.00
Post-menopausal bleeding	31	77.50

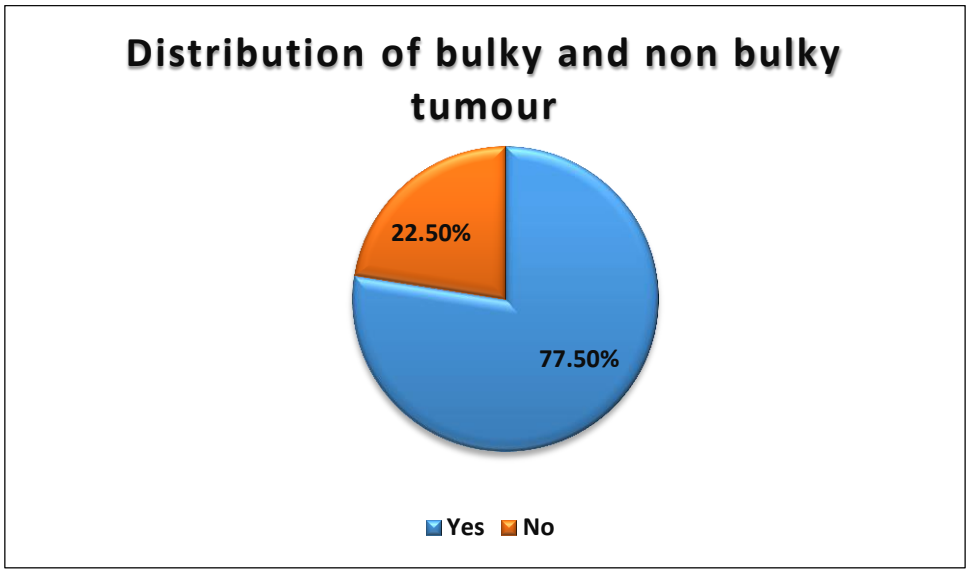


Graph 2:- Distribution of abnormal bleeding at presentation in study subjects

In this study, 77.50% of patients had postmenopausal bleeding, 45% of patients experienced post coital bleeding and only 15% of patients experienced intermenstrual bleeding as shown in table 5 and Graph 2.

Table 6:- Distribution of Size of tumour in study subjects

Bulky Tumour	Frequency	Percentage
Yes	31	77.50%
No	9	22.50%
Total	40	100.00%

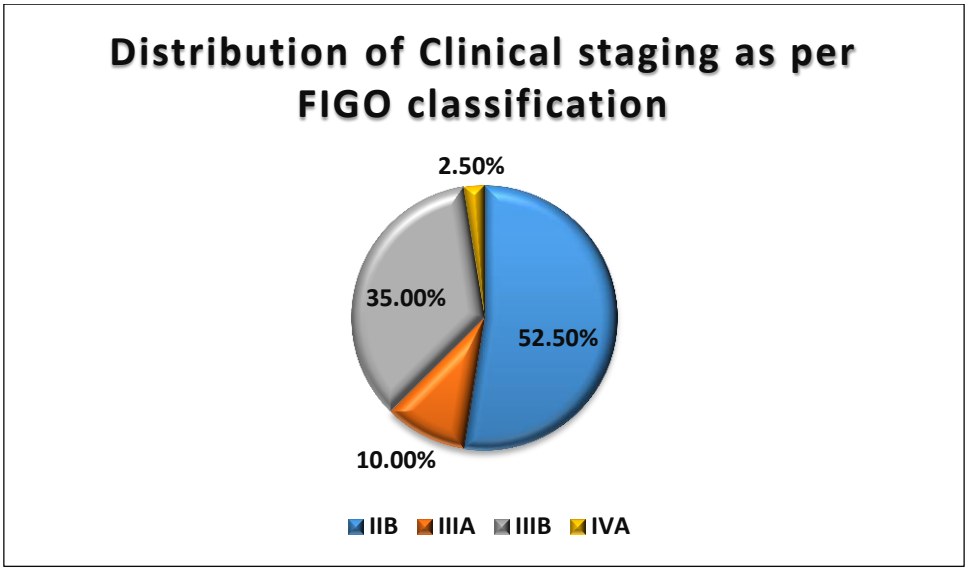


Graph 3:- Distribution of bulky and non bulky tumour

In this study, 77.50% of the patients had bulky tumour and rest had non bulky tumour as shown in table 6 and Graph 3.

Table 7:- Distribution of Cervical Cancer according to FIGO staging in study subjects

Clinical staging FIGO	Frequency	Percentage
IB1	**0**	**-**
IB2	**0**	**-**
IIA	**0**	**-**
IIB	21	52.50%
IIIA	4	10.00%
IIIB	14	35.00%
IVA	1	2.50%
Total	40	100.00%

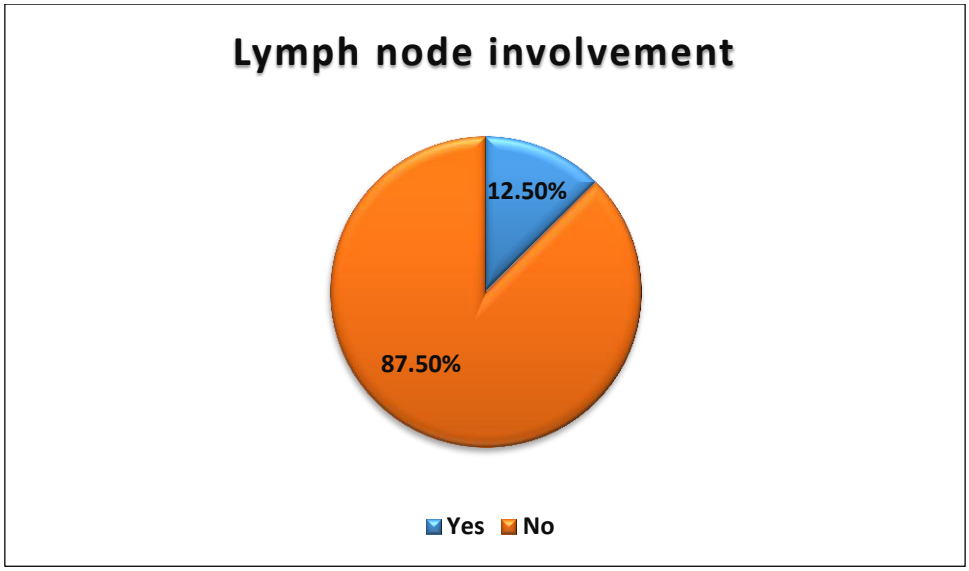


Graph 4:- Distribution of Clinical staging as per FIGO classification

52.50% of the patients in the study had clinical staging IIB and 35% had clinical staging IIIB. Very few patients had clinical staging IIIA and IVA as shown in table 7 and Graph 4.

Table 8:- Distribution of lymph node involvement in study subjects

Lymph node involvement	Frequency	Percentage%
Yes	5	12.50
No	35	87.50
Total	40	100.00

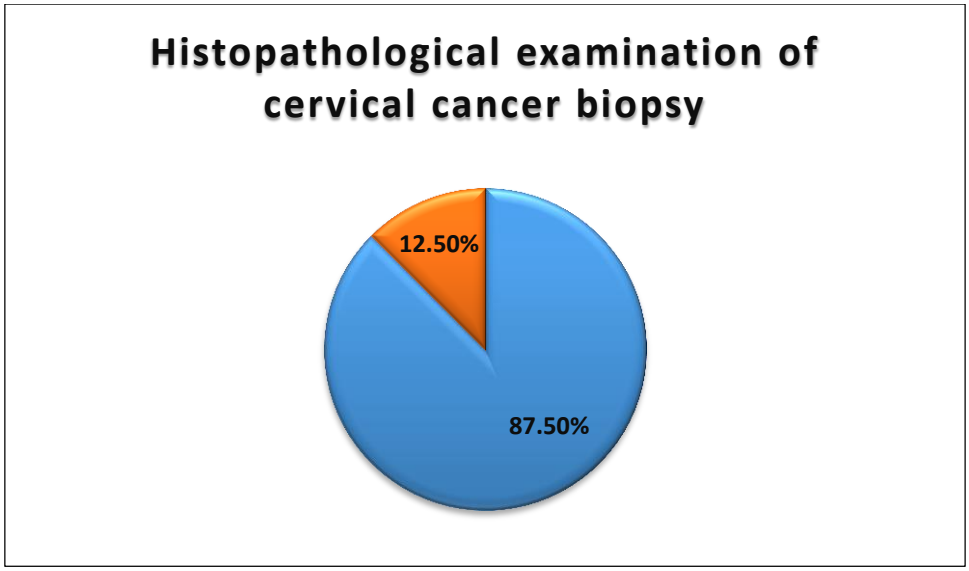


Graph 5:- Lymph node involvement in study subjects

Lymph node involvement was not present in 35 (87.50%) of the patients and was present in 5 (12.50%) of patients as shown in table 8, Graph 5.

Table 9:- Distribution of study subjects as per Histopathological Examination

HPE	Frequency	Percentage %
Squamous	35	87.50
Adenocarcinoma	5	12.50
Total	40	100.00

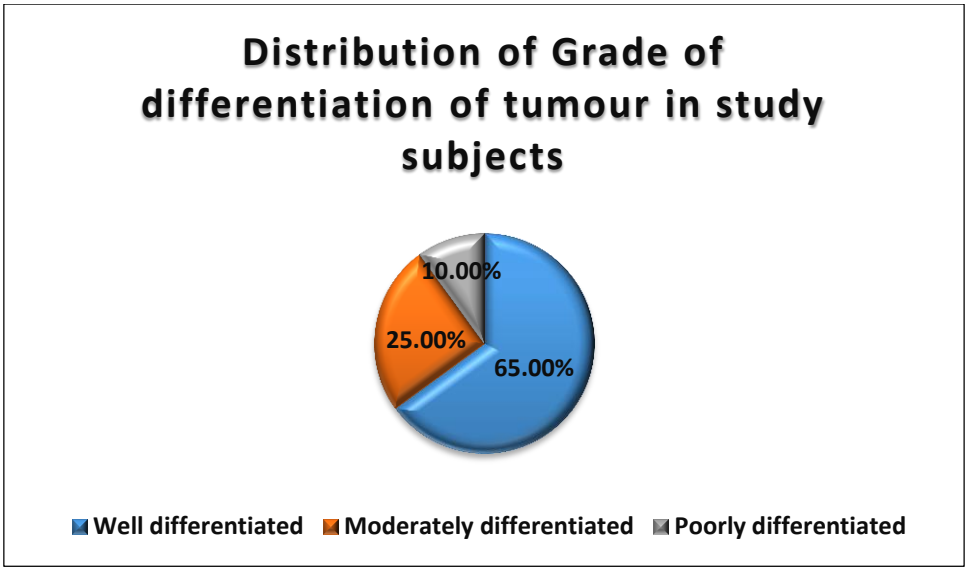


Graph 6:- Distribution of Histopathological examination in study subjects

In Histopathological examination report, squamous was reported in 87.50% and adenocarcinoma was reported in 12.50% of patients as shown in table 9 and Graph 6.

Table 10:- Distribution of Grade of differentiation of tumour in study subjects

Grade	Frequency	Percentage%
Well differentiated	26	65.00
Moderately differentiated	10	25.00
Poorly differentiated	4	10.00
Total	40	100.00

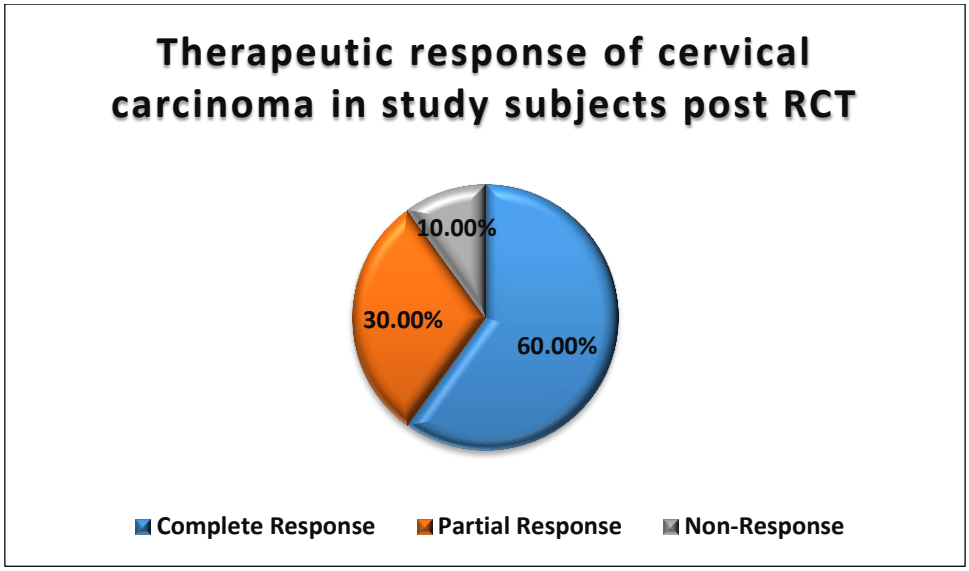


Graph 7:- Distribution of grade of differentiation of tumour in study subjects

Majority (65%) of patients had well differentiated tumour, 25% of patients had moderately differentiated tumour and very few fall in poorly differentiated tumour group as shown in table 10 and Graph 7.

Table 11:- Distribution of response in study subjects

Response	Frequency	Percentage%
Complete Response	24	60.00
Partial Response	12	30.00
Non-Response	4	10.00
Total	40	100.00



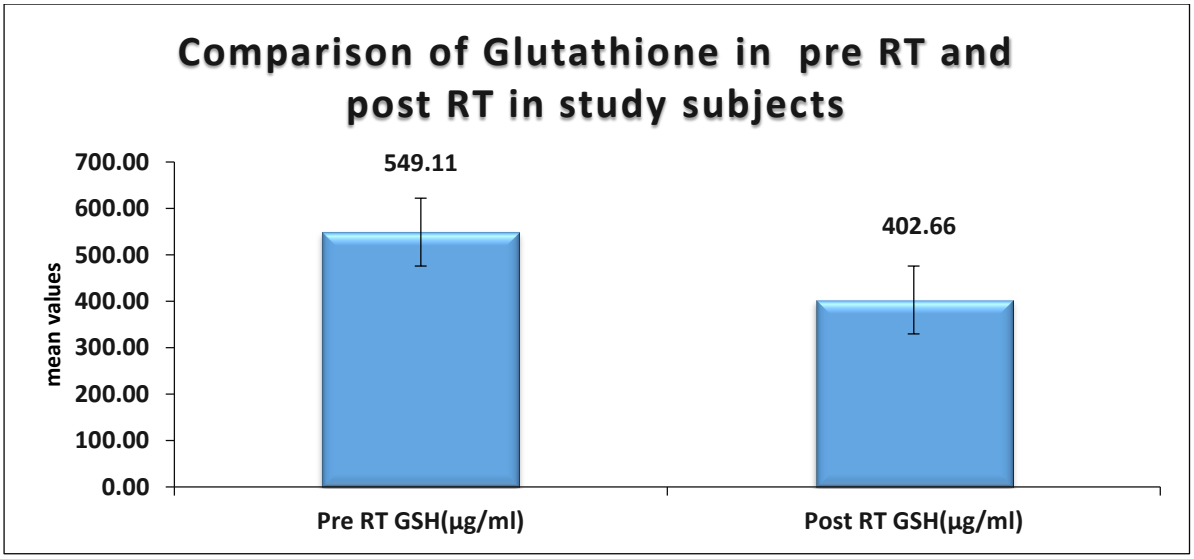
Graph 8:- Distribution of therapeutic response in study subjects.

In this study, complete response was seen in 60% of patients, 12 patients were partial responder and 4 patients were categorised as non-responders as shown in table 11 and Graph 8.

Table 12:- Comparison of pre RT and post RT Glutathione in study subjects

GSH	Mean ± Std	Median(IQR)	P value
Pre RT GSH(µg/ml)	549.11 ± 84.03	562.47(473.958 - 624.744)	<.0001
Post RT GSH(µg/ml)	402.66 ± 101.8	398.25(340.142 - 462.169)	

Note : level of significance <0.05



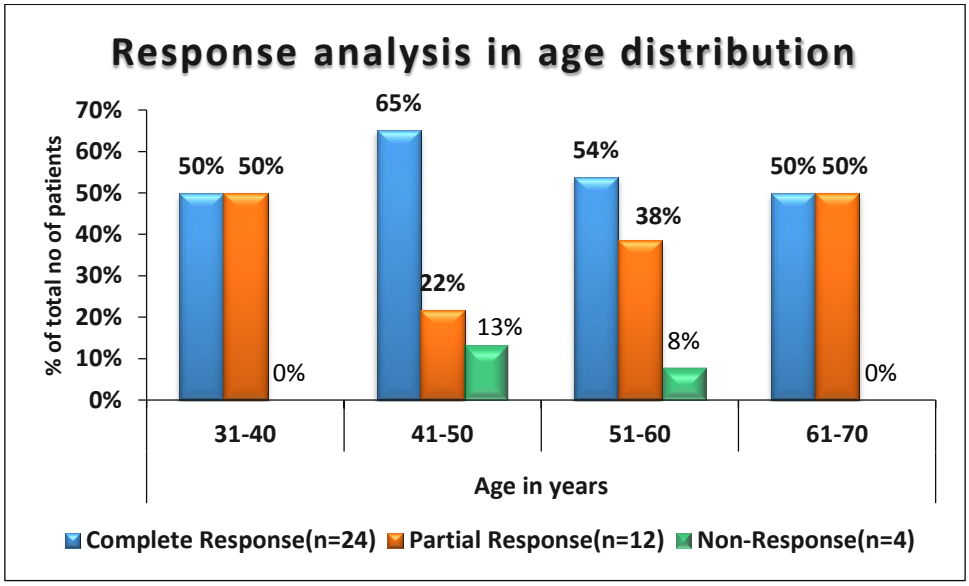
Graph 9:- Comparison of pre RT and post RT GSH in study subjects.

In this study mean pre and post RT GSH values in the study group was 549.11 ± 84.03 µg/ml and was 402.66 ± 101.8 µg/ml respectively. Hence the mean fall in GSH was 146.43 ± 85.6 µg/ml .The fall in GSH was statistically significant with p value <.0001 as shown in table 12 and Graph 9.

Table 13 :- Response analysis in age distribution

Age in years	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
31-40	1 (50.00%)	1 (50.00%)	0 (0.00%)	2	0.890
41-50	15 (65.22%)	5 (21.74%)	3 (13.04%)	23	
51-60	7 (53.85%)	5 (38.46%)	1 (7.69%)	13	
61-70	1 (50.00%)	1 (50.00%)	0 (0.00%)	2	

Note : P value <0.05 is considered significant

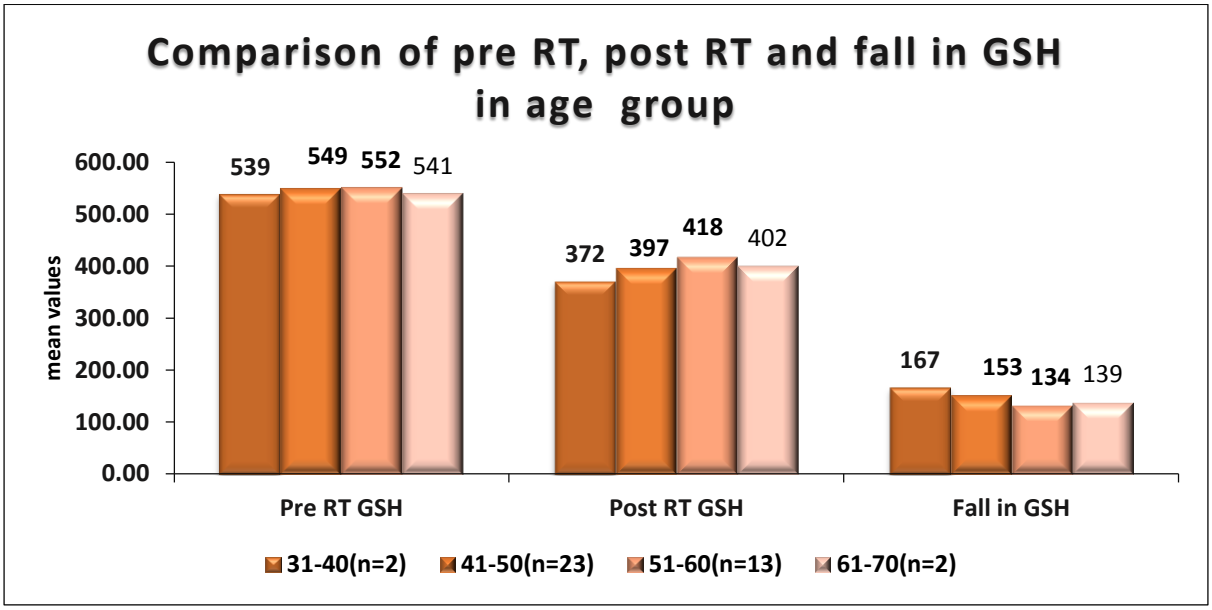


Graph 10:- Response analysis in age distribution

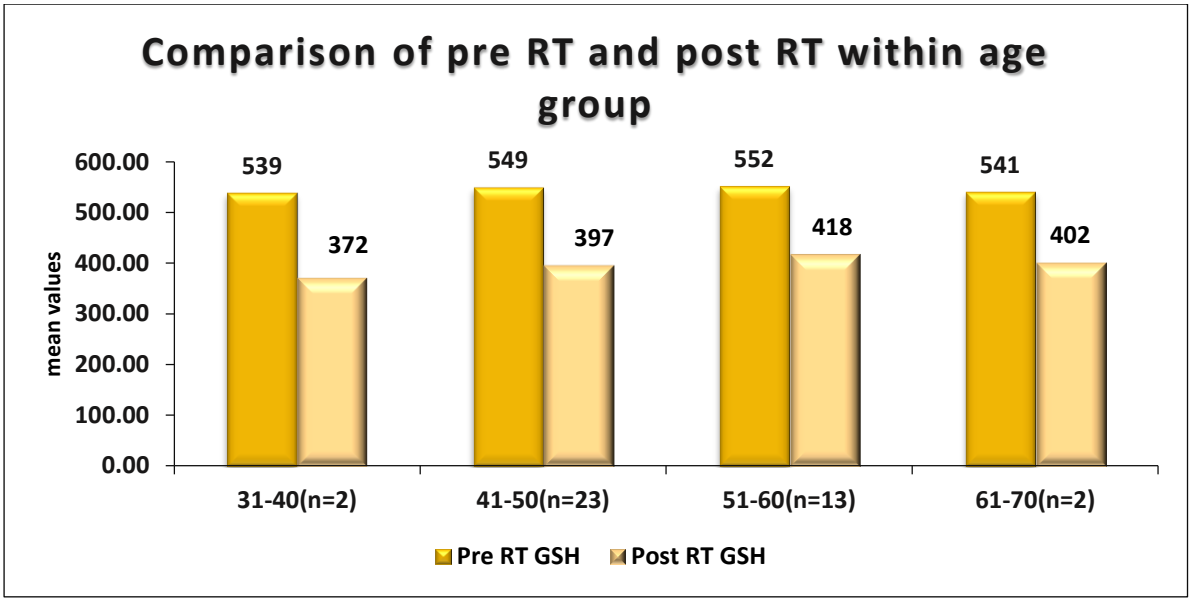
The percentage of patients achieving complete response was greater in age group 41-50 years as compared to other age groups but the difference did not reach statistical significance. (P=0.89) as shown in table 13 and Graph 10.

Table 14:- Comparison of pre RT, post RT and mean fall in GSH in age groups

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Std	Median(IQR)	Mean ± Std	Median(IQR)	Mean ± Std	Median(IQR)	
31-40(n=2)	538.99 ± 122.1	538.99(452.65 - 625.32)	371.60 ± 12.01	371.60(363.09 - 380.07)	167.41 ± 110.09	167.41(89.56 - 245.25)	0.277
41-50(n=23)	549.30 ± 74.4	562.50(488.23 - 581.48)	396.71 ± 103.53	401.62(339.79 - 444.77)	152.53 ± 105.39	125.63(86.85 - 209.07)	<.0001
51-60(n=13)	551.70 ± 101.64	524.40(479.83 - 633.78)	418.12 ± 107.18	395.30(341.20 - 518.36)	133.60 ± 44.65	129.1(101.78 - 167.24)	<.0001
61-70(n=2)	540.80 ± 119.5	540.80(456.25 - 625.25)	401.70 ± 156.52	401.70(291.02 - 512.36)	139.06 ± 37.01	139.06(112.89 - 165.23)	0.118
P value	0.996		0.912		0.916		



Graph 11.1:- Comparison of pre RT, post RT and fall in GSH in age



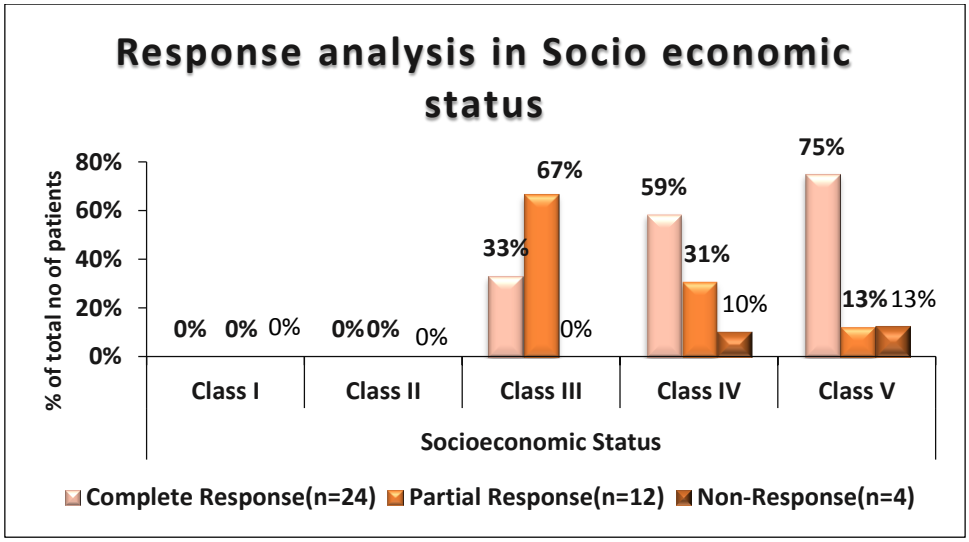
Graph 11.2:- Comparison of pre RT and post RT within age group

In this study there was no significant difference seen in the values of pre RT GSH, post RT GSH and fall in GSH between different age groups as shown in table 14 and Graph 11.1.

There was significant fall in value of GSH in age group 41-50 years and 51-60 years with p value <.0001 whereas in age group 31-40 years and 61-70 years the value of GSH was comparable between pre RT and post RT with no significant fall as shown in table 14 and Graph 11.2

Table 15 :- Response analysis in Socio economic status

Socioeconomic Status	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Class I	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0.527
Class II	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	
Class III	1 (33.33%)	2 (66.67%)	0 (0.00%)	3 (100.00%)	
Class IV	17 (58.62%)	9 (31.03%)	3 (10.34%)	29 (100.00%)	
Class V	6 (75.00%)	1 (12.50%)	1 (12.50%)	8 (100.00%)	



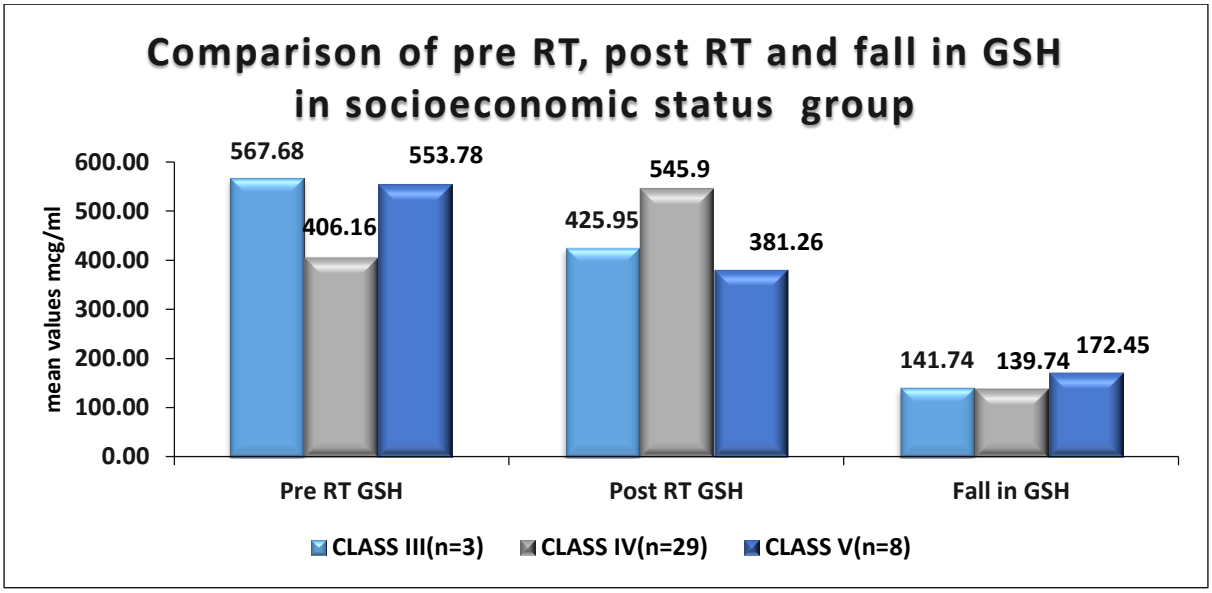
Graph 12:- Response analysis in Socio economic status

The percentage of patients achieving complete response was greater in Class V as compared to other classes of socio economic status but the difference did not reach statistical significance. (P=0.527) as shown in table 15 , Graph 12.

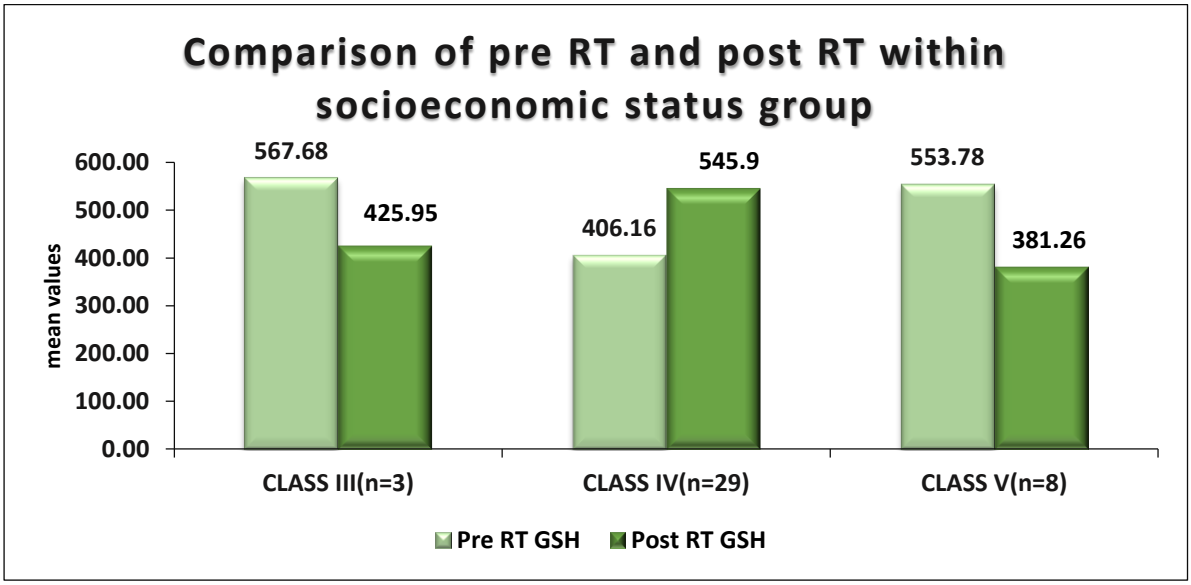
Hence the socio economic status did not influence the response rate.

Table 16:- Comparison of pre RT, post RT and fall in GSH in socioeconomic status group

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median (IQR)	Mean ± Stdev	Median(IQR)	
CLASS III(n=3)	567.68 ± 100.08	625.36(495.435 - 625.513)	425.95 ± 54.4	442.26(384.506 - 463.311)	141.74 ± 49.52	155.24(103.962 - 176.135)	0.038
CLASS IV(n=29)	406.16 ± 105.14	399.52(341.198 - 461.075)	545.9 ± 84.51	562.48(479.760 - 624.490)	139.74 ± 79.59	125.25(93.364 - 185.785)	<.0001
CLASS V(n=8)	553.78 ± 87.72	543.36(490.130 - 591.400)	381.26 ± 108.94	378.89(294.901 - 436.312)	172.45 ± 117.56	147.17(105.548 - 206.646)	0.004
P value	0.903		0.771		0.641		



Graph 13.1:- Comparison of pre RT, post RT and fall in GSH in socioeconomic status group.



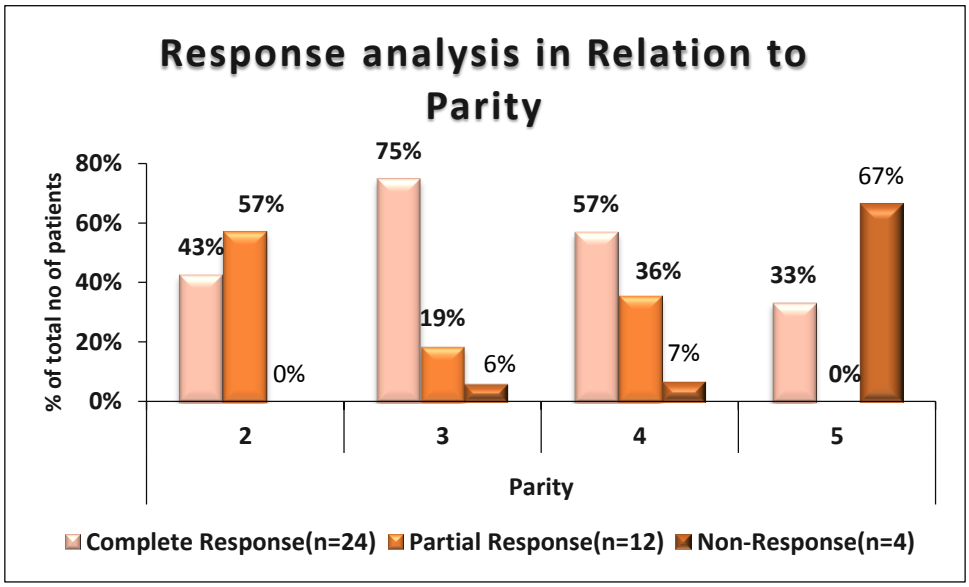
Graph 13.2:- Comparison of pre RT and post RT within socioeconomic status group

In this study there was no significant difference seen in the values of pre RT GSH, post RT GSH and fall in GSH between different socio economic status as shown in table 16 , and Graph 13.1.

Table 16 shows the comparison of pre and post RT GSH levels. Paired t-test was used for the analysis since data was normally distributed. In all the classes of socio economic status there was a statistically significant fall in value of GSH after RT compared to baseline GSH levels(P<.05) as shown in table 16 and Graph 13.2

Table 17 :- Response analysis in relation to parity

Parity	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
2	3 (42.86%)	4 (57.14%)	0 (0.00%)	7 (100.00%)	0.017
3	12 (75.00%)	3 (18.75%)	1 (6.25%)	16 (100.00%)	
4	8 (57.14%)	5 (35.71%)	1 (7.14%)	14 (100.00%)	
5	1 (33.33%)	0 (0.00%)	2 (66.67%)	3 (100.00%)	

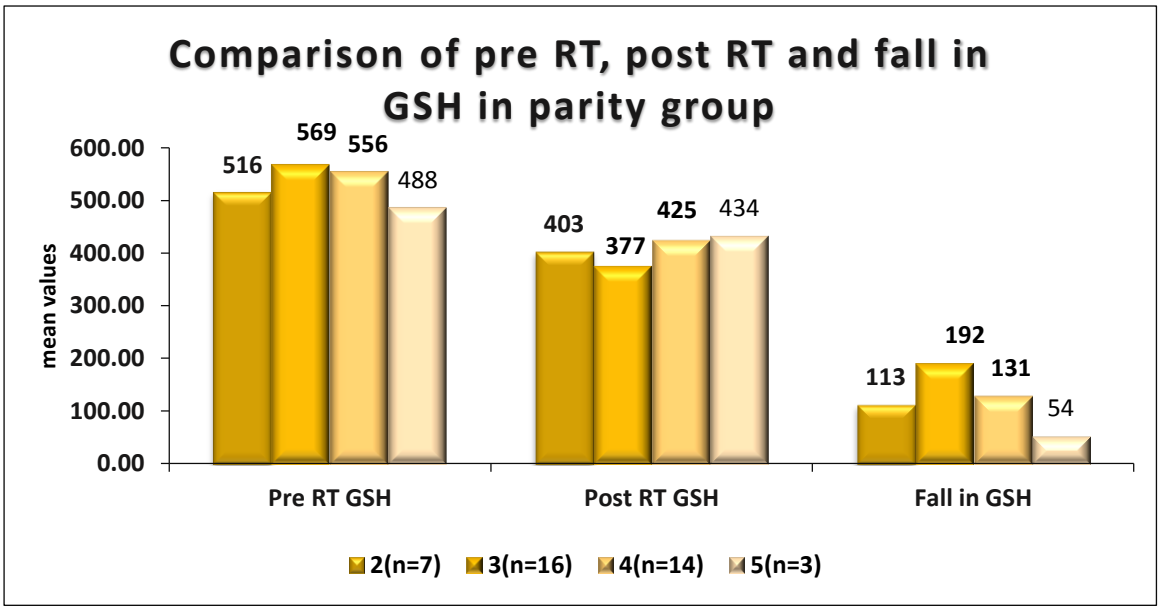


Graph 14:- Response analysis in relation to parity

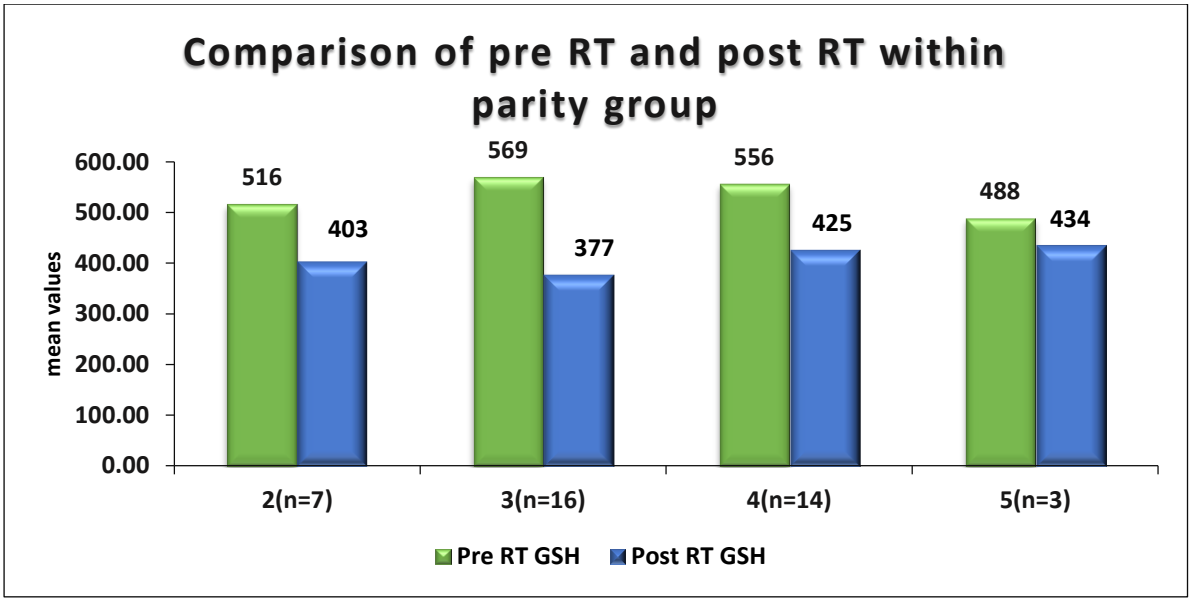
Patients with parity 3 had significantly higher complete response as compared to other parity groups. 66.67% of patients with parity 5 were non responder as compared to 0.00% in parity 2, 6.25% in parity 3 and 7.14% in parity 4.It is evident that response rate was significantly different in parity groups(P=0.017) as shown in table 17, Graph 14.

Table 18:- Comparison of Pre RT, post RT and mean fall in GSH in parity

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
2(n=7)	516.19 ± 65.72	521.36(454.213 - 557.256)	402.96 ± 97.1	397.11(348.004 - 473.014)	113.23 ± 48.88	110.4(83.945 - 122.499)	0.0009
3(n=16)	568.92 ± 83.69	564.42(491.914 - 625.345)	376.71 ± 112.32	371.3(299.058 - 442.798)	192.21 ± 99.23	174.18(114.211 - 255.253)	<.0001
4(n=14)	556.02 ± 91.1	563.36(496.254 - 625.562)	425.42 ± 84.33	433.27(379.730 - 470.327)	130.56 ± 50.22	126.05(94.631 - 173.197)	<.0001
5(n=3)	488.06 ± 73.43	485.65(433.309 - 543.404)	434.21 ± 149.76	370.86(337.622 - 546.639)	53.84 ± 101.13	45(-20.683 - 130.582)	0.454
P value	0.311		0.583		0.016		



Graph **15.1:- Comparison of Pre RT, post RT and mean fall in GSH within parity group.**



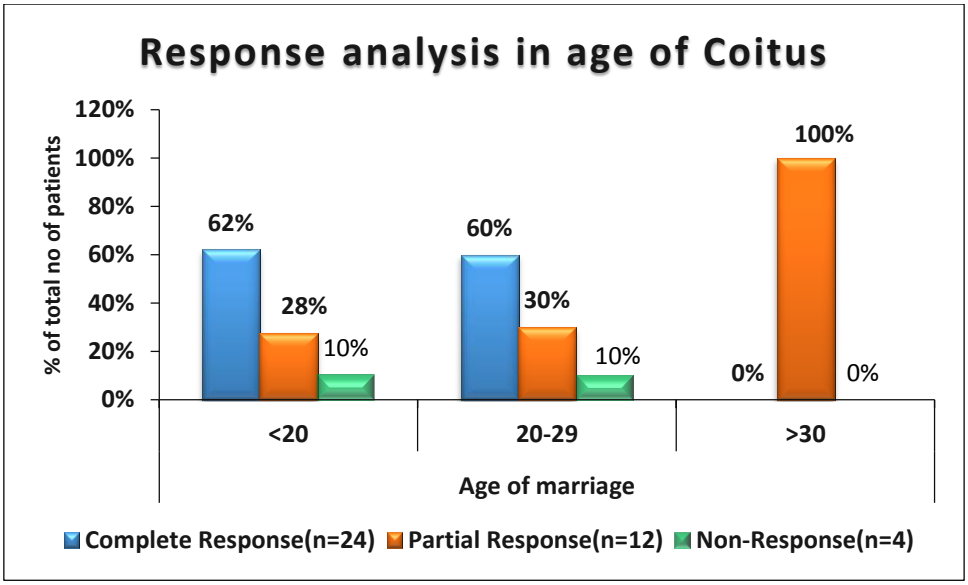
Graph 15.2:- Comparison of pre RT and post RT within parity group

In this study there was no significant difference seen in the values of pre RT GSH and post RT GSH between parity groups. However fall in GSH was significantly higher in patients with parity 3 and was significantly lower in patients with parity 5(P=0.016) as shown in table 18, and Graph 15.1.

There was significant fall in value of GSH in patients with parity 2,3 and 4 with P value <0.05 whereas in patients with parity 5, value of GSH was comparable between pre RT and post RT with no significant fall as shown in table 18 and Graph 15.2

Table 19 :- Response analysis in age of Coitus

Age of marriage	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
<20	18 (62.07%)	8 (27.59%)	3 (10.34%)	29 (100.00%)	0.660
20-29	6 (60.00%)	3 (30.00%)	1 (10.00%)	10 (100.00%)	
>30	0 (0.00%)	1 (100.00%)	0 (0.00%)	1 (100.00%)	

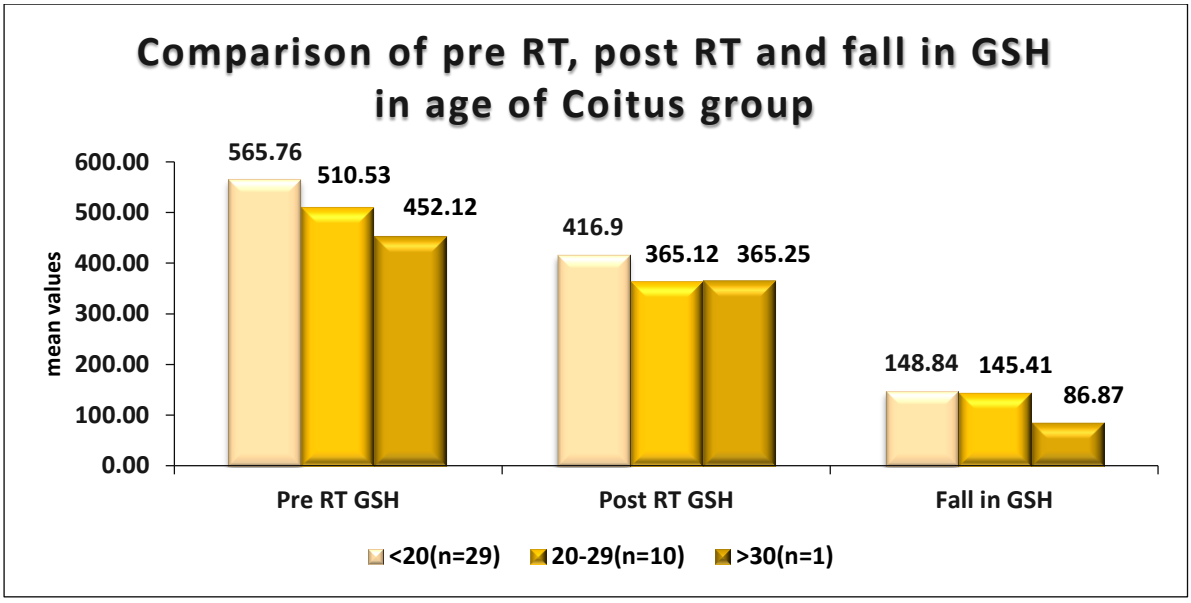


Graph 16:- Response analysis in relation to age of Coitus in study subjects

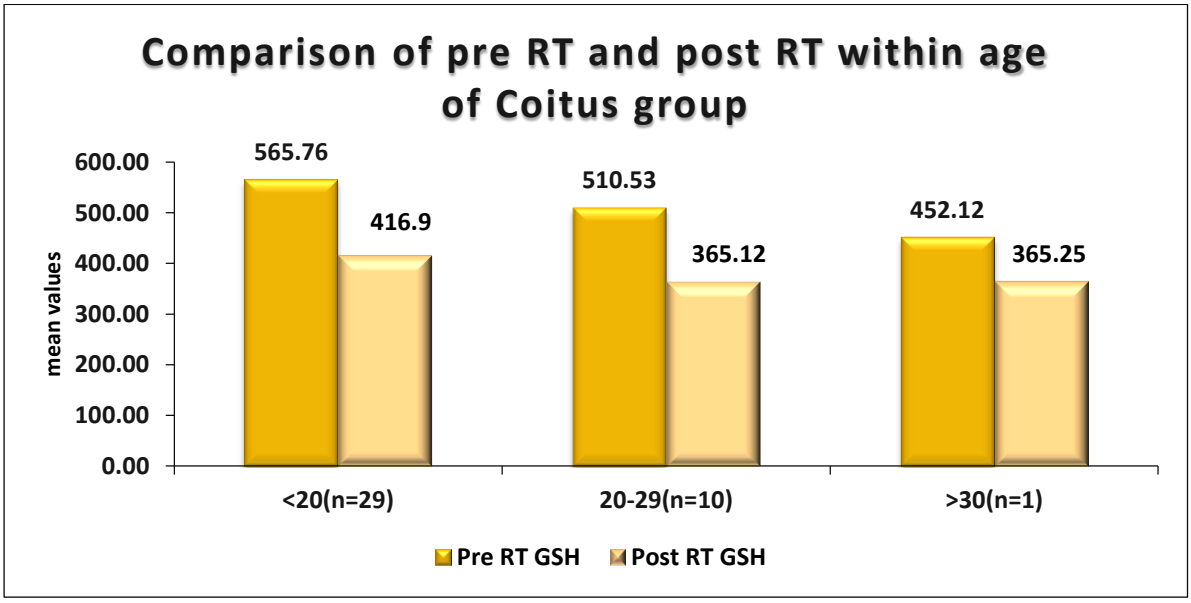
The percentage of patients achieving complete response was comparable in all the age groups of Coitus with P value 0.660 as shown in table 19 and Graph 16.

Table 20:- Comparison of pre RT, post RT and mean fall in GSH in age of Coitus groups

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
<20(n=29)	565.76 ± 87.2	564.25(493.581 - 625.414)	416.9 ± 111.08	435.6(341.198 - 489.792)	148.84 ± 86.7	129.1(108.389 - 189.619)	<.0001
20-29(n=10)	510.53 ± 57.84	509.82(456.254 - 562.652)	365.12 ± 64.35	375.3(326.542 - 399.387)	145.41 ± 89.2	111.5(86.842 - 186.265)	0.0006
>30(n=1)	452.12 ± 0	452.12(452.125 - 452.125)	365.25 ± 0	365.25(365.254 - 365.254)	86.87 ± 0	86.87(86.871 - 86.871)	-
P value	0.099		0.366		0.784		



Graph 17.1:-Comparison of pre RT, post RT and mean fall in GSH in age of Coitus group.



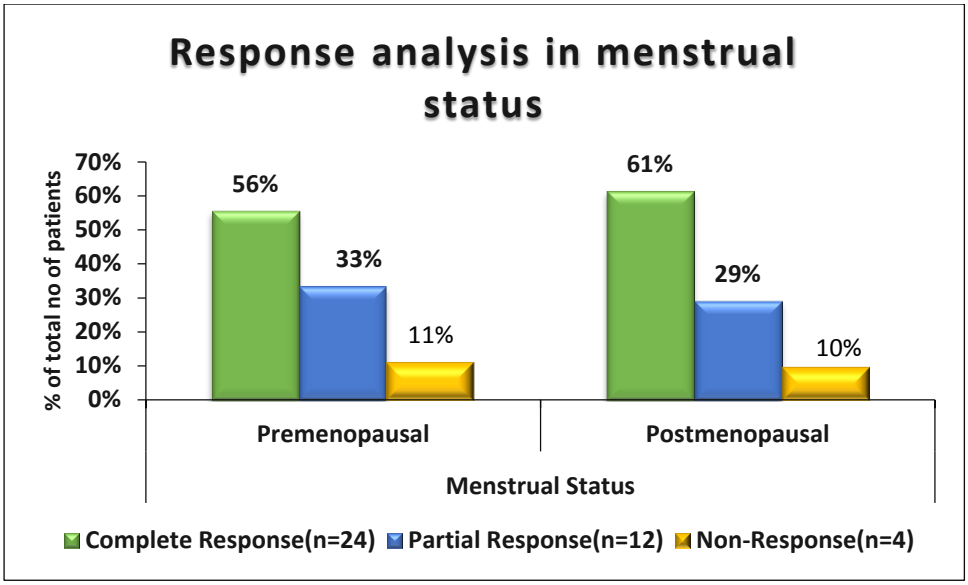
Graph 17.2:- Comparison of pre RT and post RT within age of Coitus group

In this study no significant difference was seen in the values of pre RT GSH, post RT GSH and fall in GSH between different age group of coitus as shown in table 20 and Graph 17.1.

There was a statistically significant fall in GSH values both in less than 20 years and 20-29 years group of age of Coitus. Since there was only one patient who had coitus after 30 years so analysis could not be performed for that group (table 20 and Graph 17.2)

Table 21:- Response analysis based on menstrual status.

Menstrual Status	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Premenopausal	5 (55.56%)	3 (33.33%)	1 (11.11%)	9 (100.00%)	0.953
Postmenopausal	19 (61.29%)	9 (29.03%)	3 (9.68%)	31 (100.00%)	

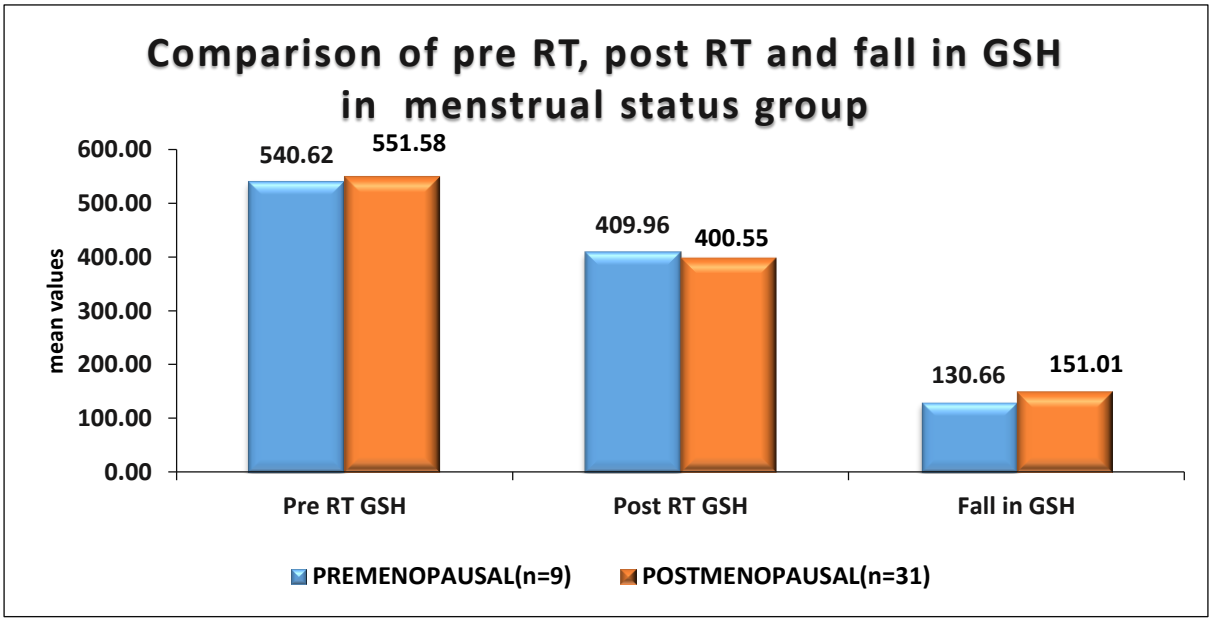


Graph 18:- Response analysis based on menstrual status in study subjects

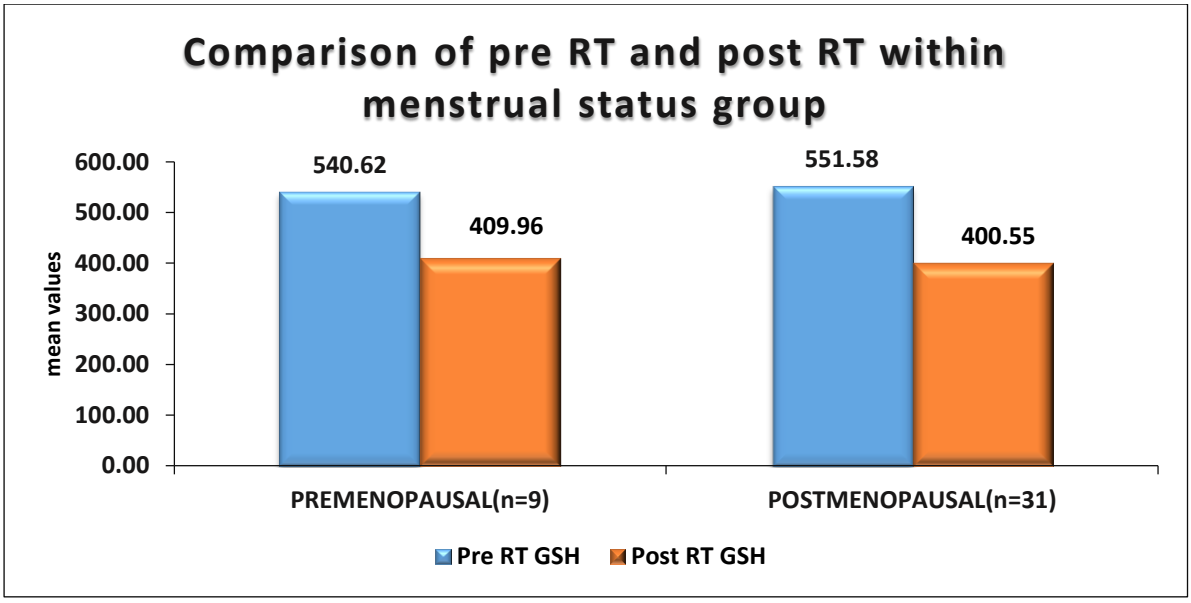
The difference in response rates between premenopausal and postmenopausal were not statistically significant as shown in table 21 and Graph 18.

Table 22:- Comparison of Pre RT, post RT and mean fall in GSH in menstrual status

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
Premenopausal (n=9)	540.62 ± 74.55	562.48(485.354 - 579.781)	409.96 ± 56.93	430.93(368.919 - 447.442)	130.66 ± 73.69	110.25(81.995 - 190.235)	0.0007
Postmenopausal(n=31)	551.58 ± 87.57	562.45(468.156 - 624.999)	400.55 ± 112.19	395.26(332.980 - 474.216)	151.01 ± 89.34	129.1(99.754 - 186.105)	<.0001
P value	0.735		0.811		0.537		



Graph 19.1:- Comparison of Pre RT, post RT and mean fall in GSH in menstrual status



Graph 19.2:- Comparison of pre RT and post RT within menstrual status group

GSH values showed significant fall in both group with no difference between premenopausal and postmenopausal group as shown in table 22 and Graphs 19.1,19.2.

Table 23 :- Response analysis in BMI distribution

BMI	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
<19	3 (33.33%)	5 (55.56%)	1 (11.11%)	9 (100.00%)	0.358
19-24.4	20 (66.67%)	7 (23.33%)	3 (10.00%)	30 (100.00%)	
25-29.9	1 (100.00%)	0 (0.00%)	0 (0.00%)	1 (100.00%)	

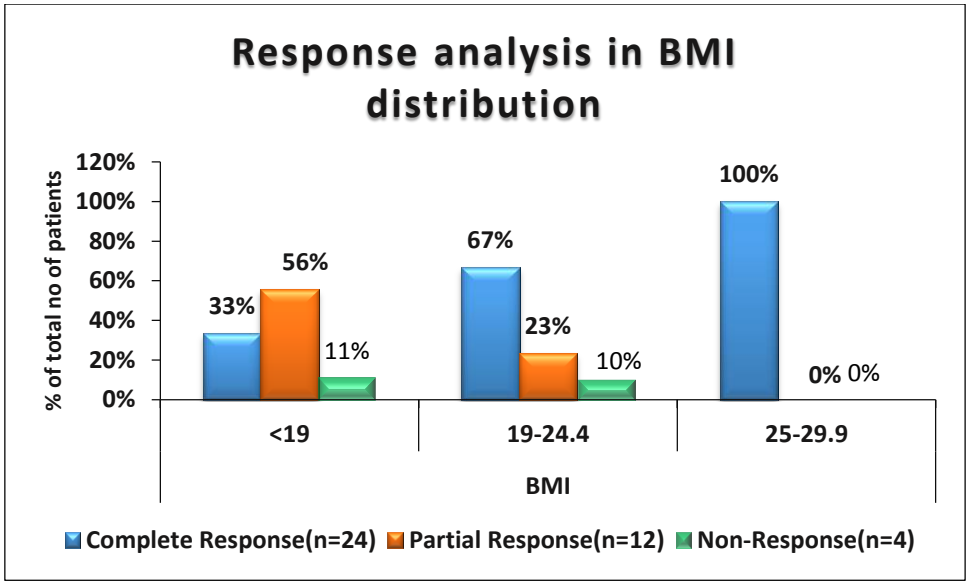


Figure 20:- Response analysis in BMI distribution

Response rate was comparable between all the three groups of BMI as shown in table 23 and figure 20.

Table 24 :- Comparison of Pre RT, post RT and mean fall in GSH in BMI

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
<19(n=9)	549.22 ± 98.43	523.65(455.802 - 633.778)	407.36 ± 134.1	363.09(341.198 - 512.365)	141.86 ± 73.54	112.89(99.484 - 176.304)	0.0004
19-24.4(n=30)	548.58 ± 82.55	562.47(485.652 - 618.546)	399.54 ± 94.16	398.25(331.297 - 443.334)	149.01 ± 91.13	127.98(86.871 - 201.600)	<.0001
25-29.9(n=1)	564.26 ± 0	564.26(564.265 - 564.265)	454.01 ± 0	454.01(454.011 - 454.011)	110.25 ± 0	110.25(110.254 - 110.254)	-
P value	0.984		0.866		0.896		

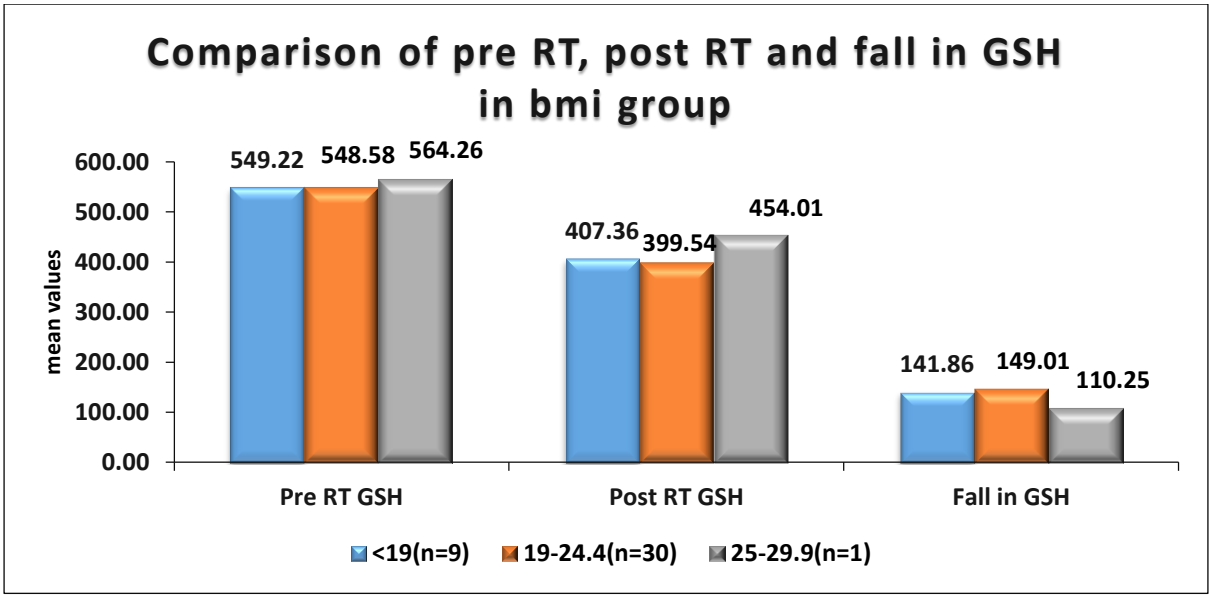


Figure 21.1:- Comparison of Pre RT, post RT and mean fall in GSH in BMI

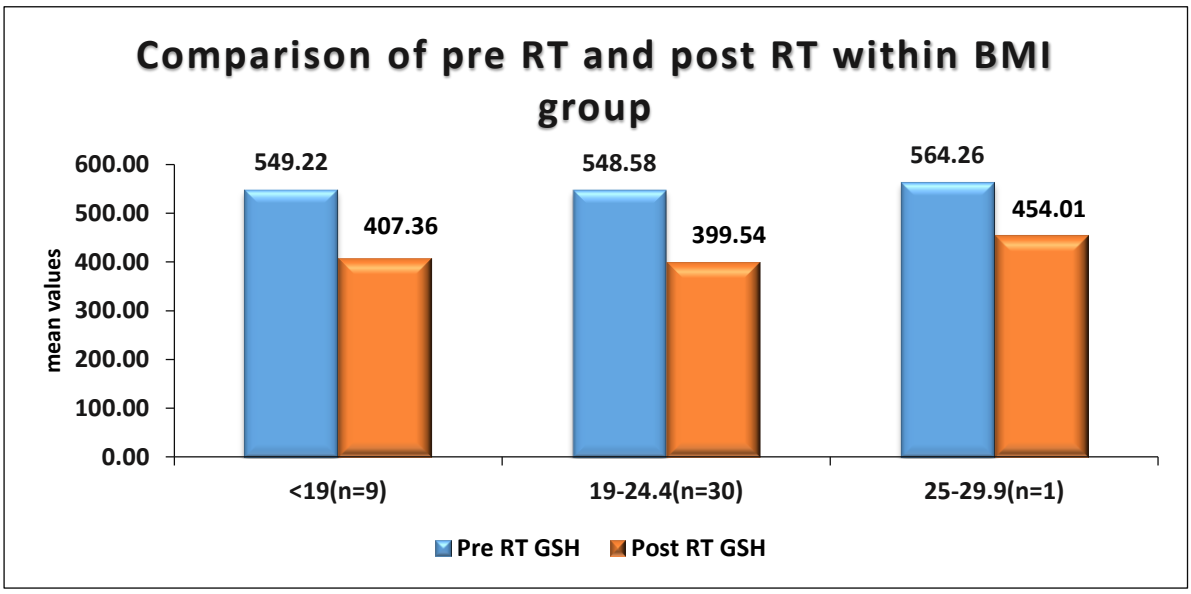
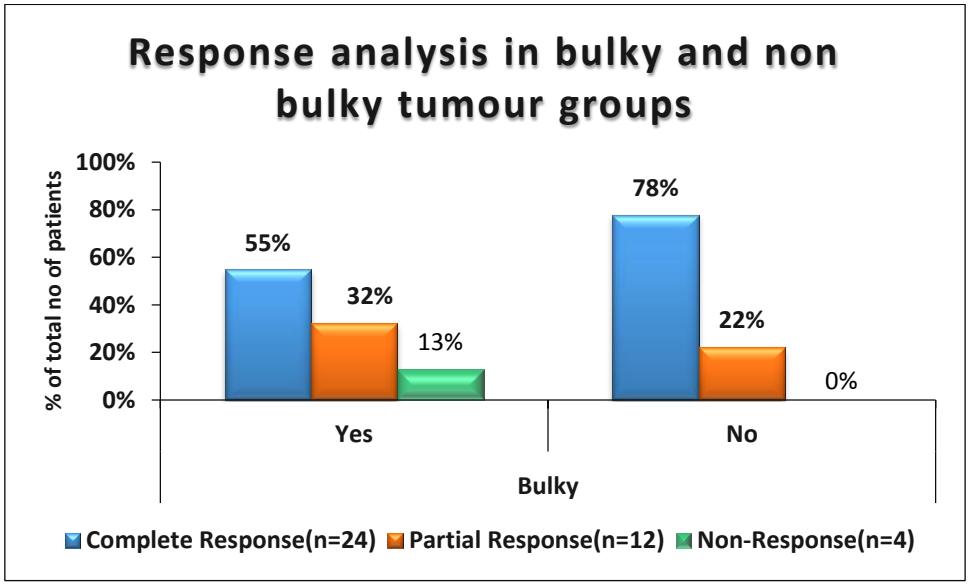


Figure 21.2:- Comparison of pre RT and post RT within BMI group

GSH value showed significant fall in BMI less than 19kg/m2 and in 19-24.4kg/m2 with no difference between these two groups. BMI group 25-29.9 kg/m2 could not be analysed since the number of patients in this group was too small for any type of statistical analysis (table 24 and figure 21.1, 21.2).

Table 25:- Response analysis in bulky and non bulky tumour groups

Bulky	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Yes	17 (54.84%)	10 (32.26%)	4 (12.90%)	31 (100.00%)	0.367
No	7 (77.78%)	2 (22.22%)	0 (0.00%)	9 (100.00%)	



Graph 22:- Response analysis in bulky and non bulky tumour groups

Bulky and non bulky tumour groups were evenly matched in response rates as shown in table 25 and Graph 22.

Table 26 :- Comparison of pre RT, post RT and mean fall in GSH in bulky and non bulky tumour groups

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
Yes(n=31)	554.95 ± 83.31	562.48(485.585 - 624.999)	423.74 ± 88.65	399.52(363.631 - 475.204)	131.18 ± 83.26	117.23(87.544 - 165.255)	<.0001
No(n=9)	529.01 ± 88.36	562.35(458.384 - 579.581)	330.06 ± 115.74	338.03(240.070 - 447.606)	198.96 ± 75.66	201.6(147.740 - 233.721)	<.0001
P value	0.422		0.013		0.035		

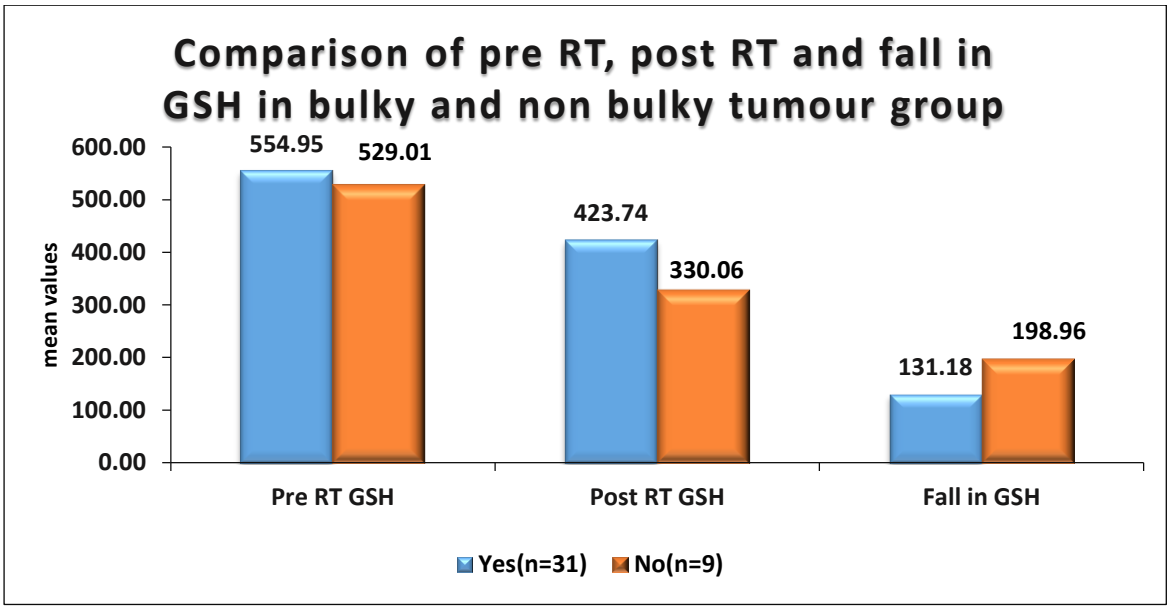
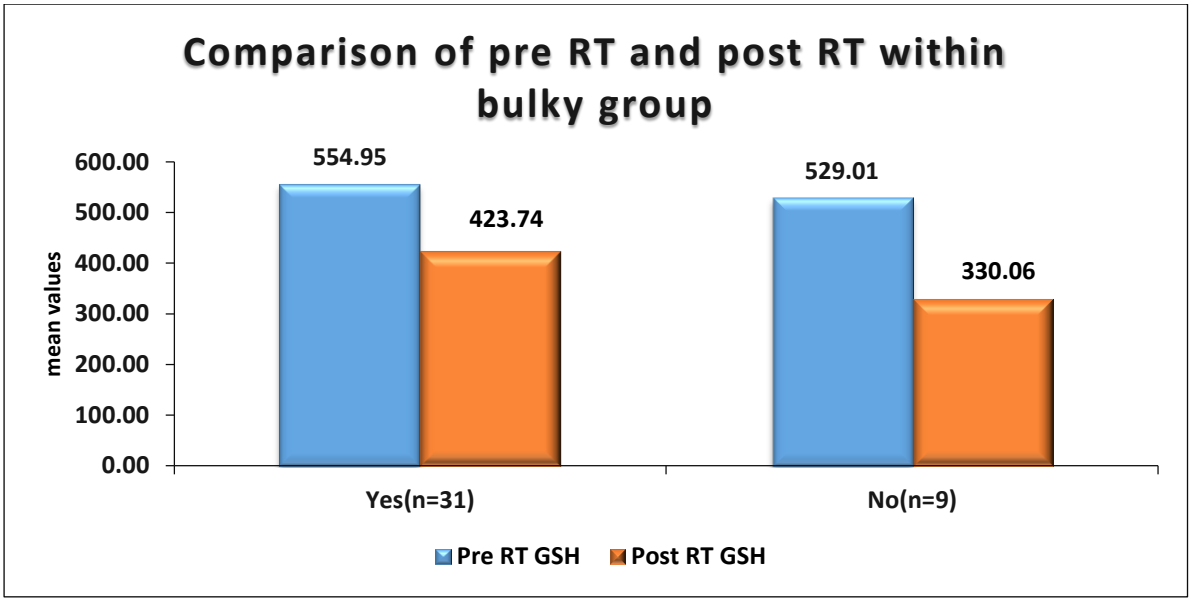


Figure 23.1:- Comparison of pre RT, post RT and mean fall in GSH in bulky and non bulky tumour group.

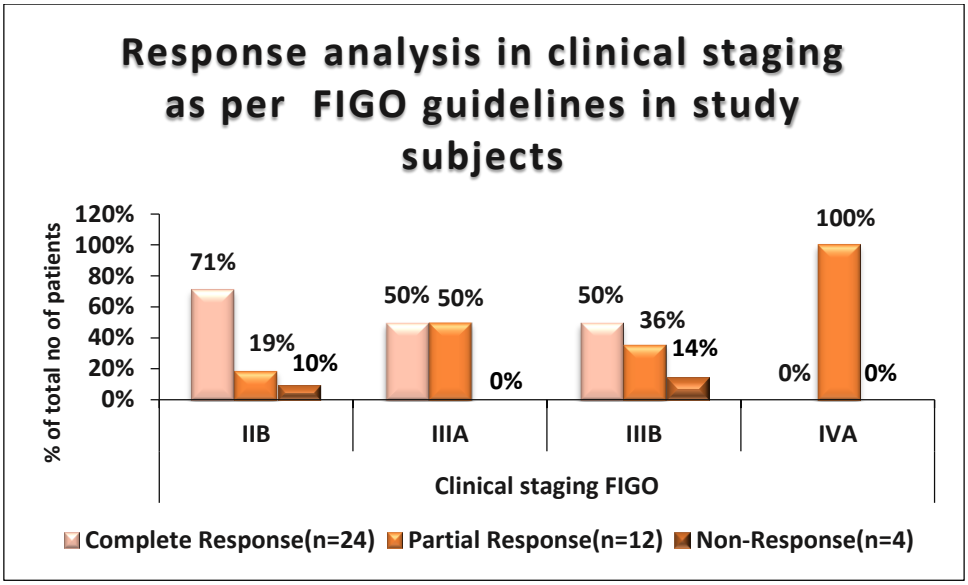


Graph 23.2:- Comparison of pre RT and post RT within bulky group.

There was a statistically significant fall in GSH values both in bulky and non bulky tumours. This implies that fall in GSH was not influenced by bulk of the tumour as shown in table 26 and Graphs 23.1, 23.2. Hence the bulk of the tumour neither influenced the response rate nor fall in GSH values.

Table 27 :- Response analysis in clinical staging as per FIGO guidelines in study subjects.

Clinical staging FIGO	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
IIB	15 (71.43%)	4 (19.05%)	2 (9.52%)	21 (100.00%)	0.509
IIIA	2 (50.00%)	2 (50.00%)	0 (0.00%)	4 (100.00%)	
IIIB	7 (50.00%)	5 (35.71%)	2 (14.29%)	14 (100.00%)	
IVA	0 (0.00%)	1 (100.00%)	0 (0.00%)	1 (100.00%)	

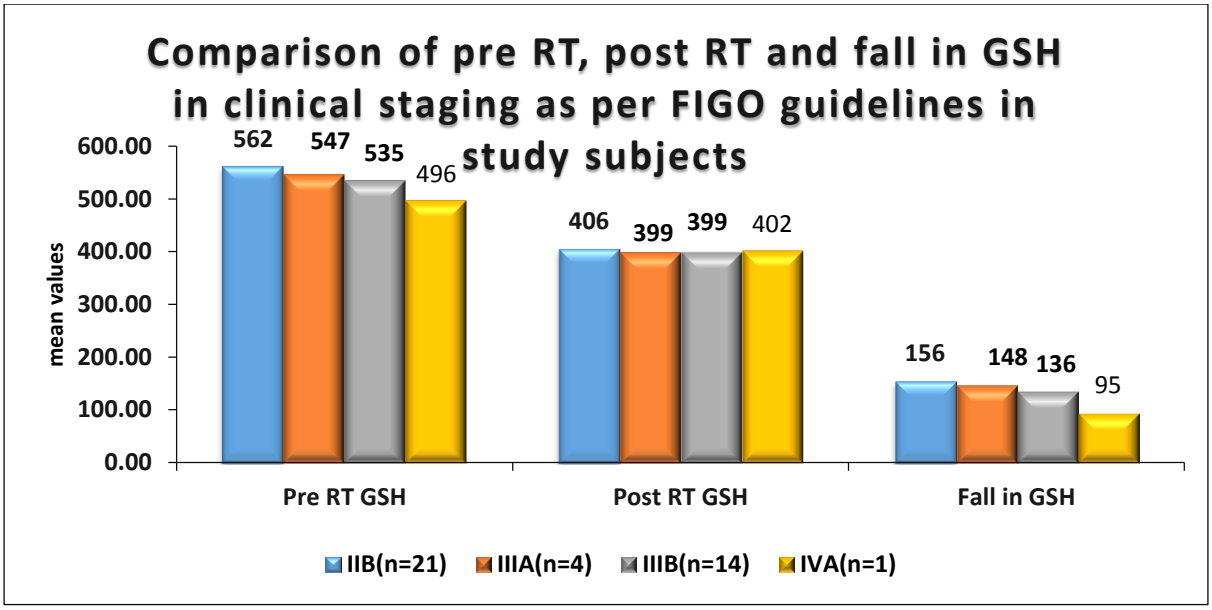


Graph 24:- Response analysis in clinical staging as per FIGO guidelines in study subjects.

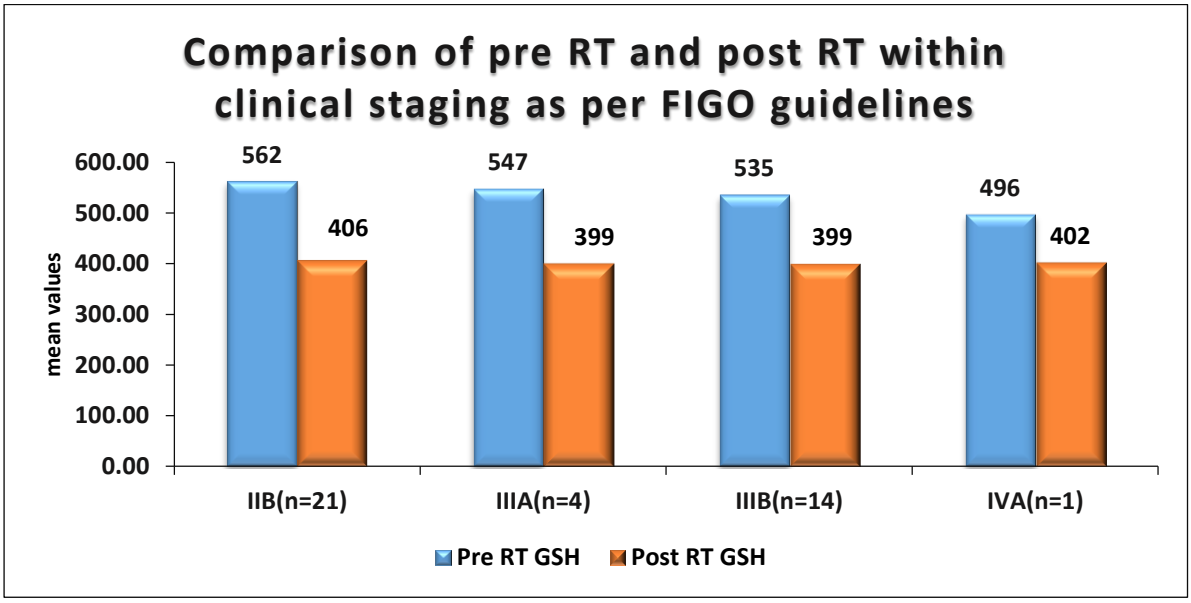
Only two patients were non responders in clinical staging IIB and IIIB whereas there was no non responder in clinical staging IIIA and IVA and the difference did not reach statistical significance(P=0.509) as shown in table 27 and Graph 24.

Table 28 :- Comparison of Pre RT, post RT and mean fall in GSH in clinical staging as per FIGO guidelines in study subjects.

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
IIB(n=21)	561.56 ± 95.32	564.26(478.963 - 625.414)	405.92 ± 118.21	397.11(335.157 - 480.837)	155.64 ± 94.59	155.24(105.081 - 185.785)	<.0001
IIIA(n=4)	547.21 ± 71.93	530.36(491.914 - 602.510)	399.34 ± 48.11	415.23(365.410 - 433.266)	147.87 ± 48.17	140.56(112.798 - 182.950)	0.009
IIIB(n=14)	534.76 ± 72.96	562.42(456.254 - 564.587)	398.81 ± 94.98	375.3(342.254 - 475.512)	135.92 ± 84.97	111.64(76.524 - 201.600)	<.0001
IVA(n=1)	496.25 ± 0	496.25(496.254 - 496.254)	401.62 ± 0	401.62(401.623 - 401.623)	94.63 ± 0	94.63(94.631 - 94.631)	-
P value	0.752		0.998		0.855		



Graph 25.1:- Comparison of Pre RT, post RT and mean fall in GSH in clinical staging as per FIGO guidelines in study subjects.

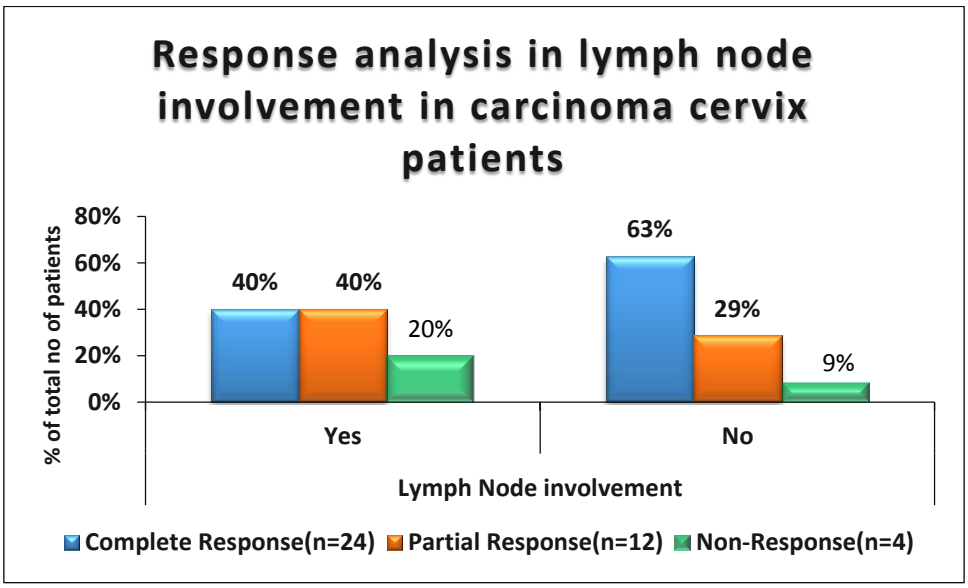


Graph 25.2:- Comparison of pre RT and post RT within clinical staging as per FIGO guidelines in study subjects

GSH values showed significant fall in clinical staging IIB, IIIA and IIIB with no difference in pre RT GSH, post RT GSH and fall in GSH between them. Clinical staging IVA could not be analysed since the number of patients in this group was too small for any type of statistical analysis as shown in table 28 and Graphs 25.1, 25.2.

Table 29 :- Response analysis in lymph node involvement in carcinoma cervix patients

Lymph Node involvement	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Yes	2 (40.00%)	2 (40.00%)	1 (20.00%)	5 (100.00%)	0.565
No	22 (62.86%)	10 (28.57%)	3 (8.57%)	35 (100.00%)	

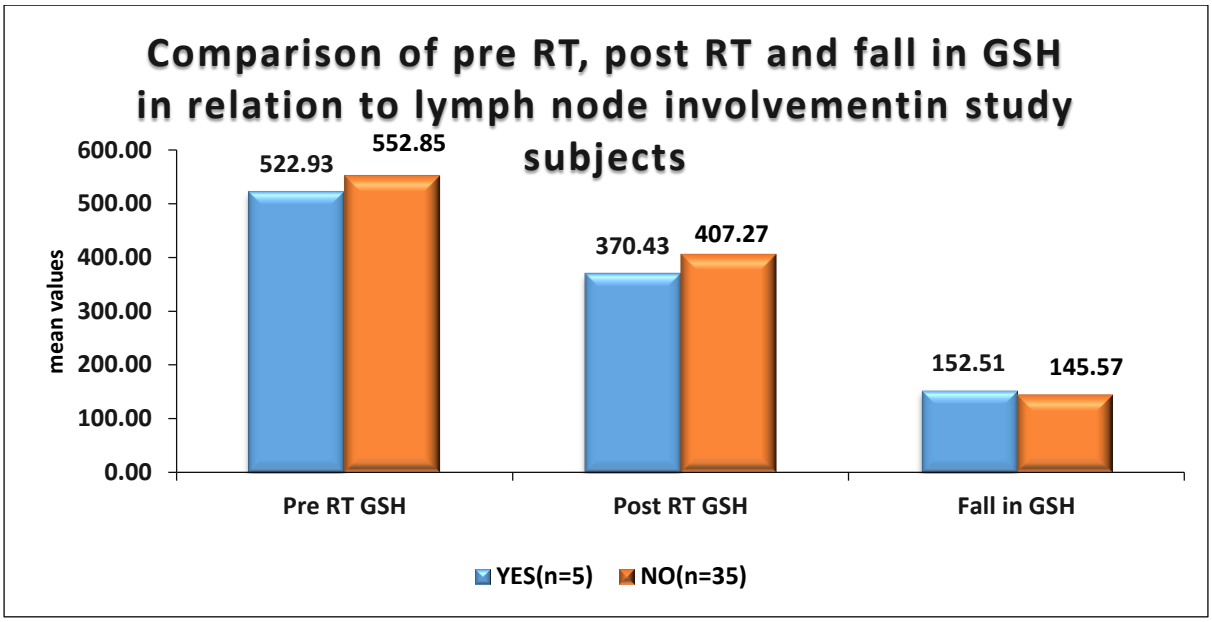


Graph 26:- Response analysis in lymph node involvement in carcinoma cervix patients

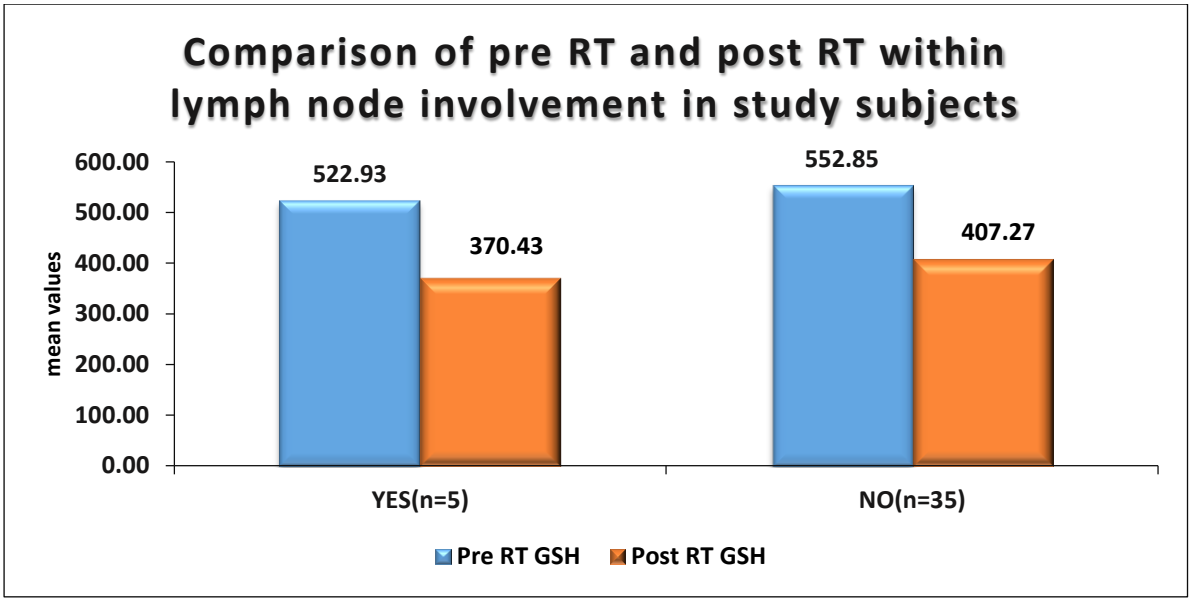
The difference in response rates between involvement and non involvement of lymph node were not statistically significant as shown in table 29and Graph 26.

Table 30:- Comparison of Pre RT, post RT and mean fall in GSH in lymph node involvement

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
YES(n=5)	522.93 ± 84.34	498.26(476.156 - 582.397)	370.43 ± 76.9	399.52(338.169 - 411.225)	152.51 ± 110.82	98.74(82.223 - 231.840)	0.037
NO(n=35)	552.85 ± 84.54	562.48(468.156 - 624.999)	407.27 ± 104.96	397.11(339.085 - 474.216)	145.57 ± 83.39	126.85(92.870 - 184.994)	<.0001
P value	0.464		0.456		0.868		



Graph 27.1:- Comparison of Pre RT, post RT and mean fall in GSH in relation to lymph node involvement in study subjects.

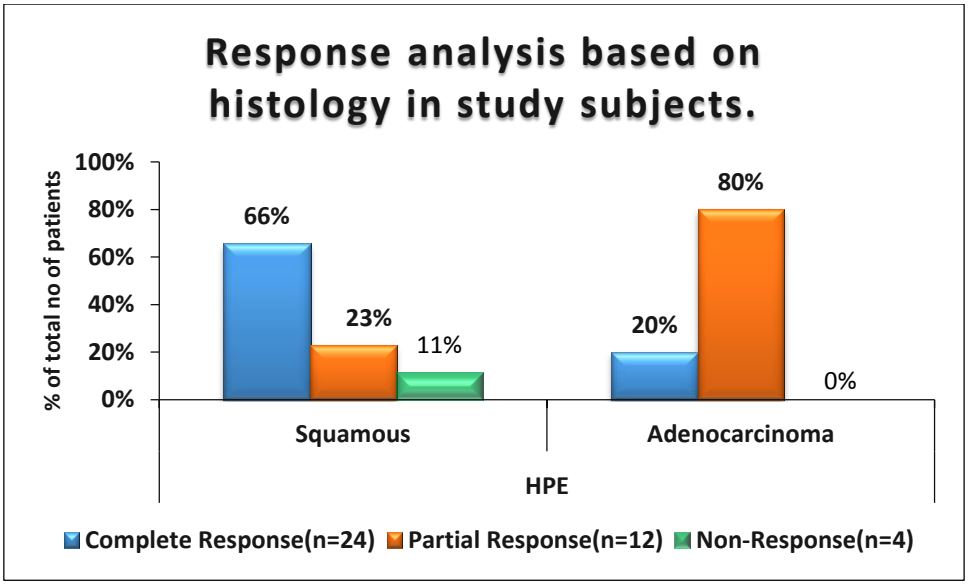


Graph 27.2:- Comparison of pre RT and post RT within lymph node involvement in study subjects.

GSH values showed significant fall in both groups with no significant difference between involvement and non involvement of lymph node as shown in table 30 and Graphs 27.1, 27.2.

Table 31:- Response analysis based on histology in study subjects.

HPE	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Squamous	23 (65.71%)	8 (22.86%)	4 (11.43%)	35 (100.00%)	0.032
Adenocarcinoma	1 (20.00%)	4 (80.00%)	0 (0.00%)	5 (100.00%)	

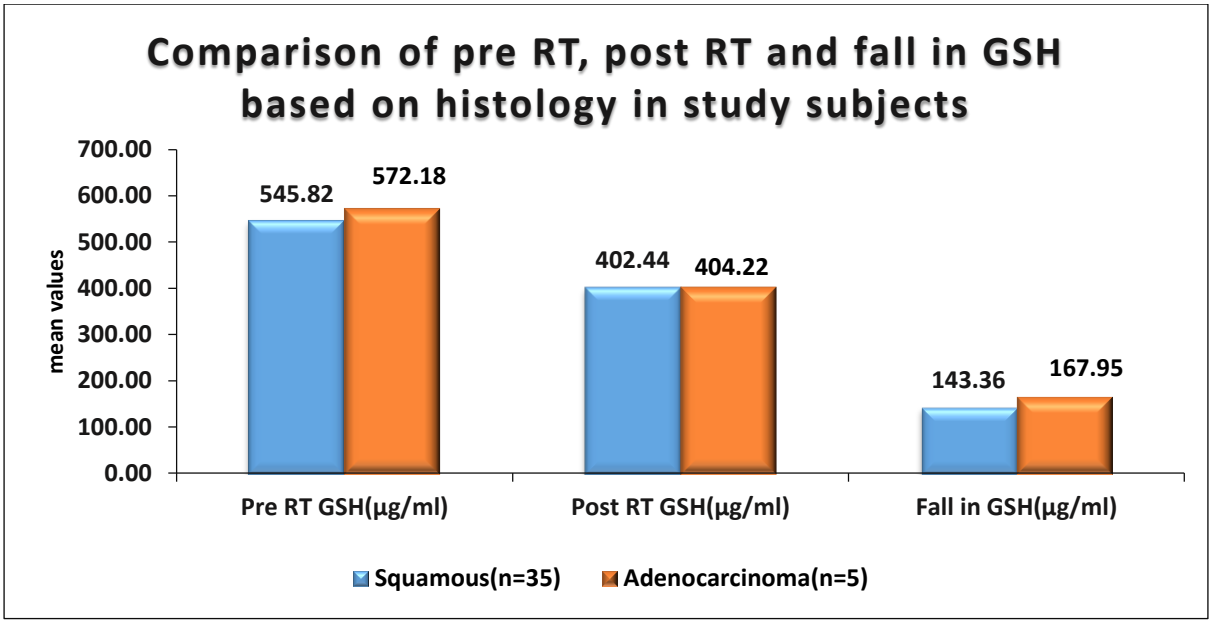


Graph 28:- Response analysis based on histology in study subjects.

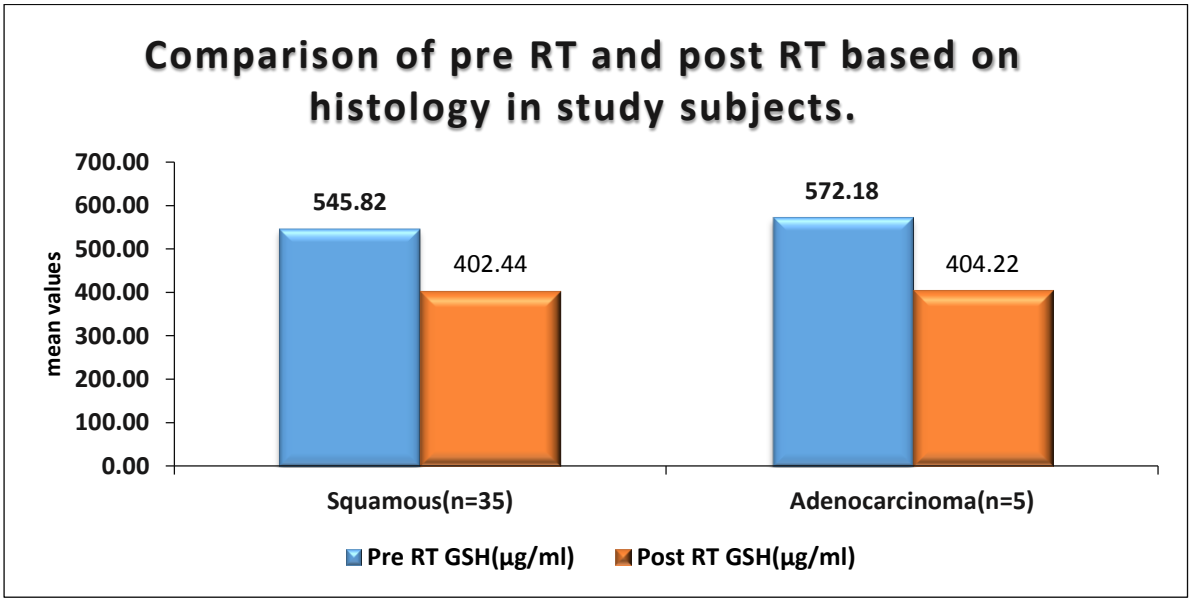
Complete response rate was significantly higher in patients reported with squamous as compared to patients reported with adenocarcinoma(P=0.032) as shown in table 31 and Graph 28.

Table 32:- Comparison of pre RT, post RT and mean fall in GSH in squamous and adenocarcinoma

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
Squamous(n=35)	545.82 ± 81.57	562.45(468.156 - 622.813)	402.44 ± 105.43	397.11(339.085 - 466.248)	143.36 ± 76.64	129.1(89.839 - 186.105)	<.0001
Adenocarcinoma(n=5)	572.18 ± 107.34	562.48(485.354 - 649.999)	404.22 ± 80.92	401.62(347.013 - 462.031)	167.95 ± 144.42	112.89(93.364 - 194.287)	0.06
P value	0.519		0.971		0.555		



Graph 29.1:- Comparison of pre RT, post RT and mean fall in GSH in squamous and adenocarcinoma



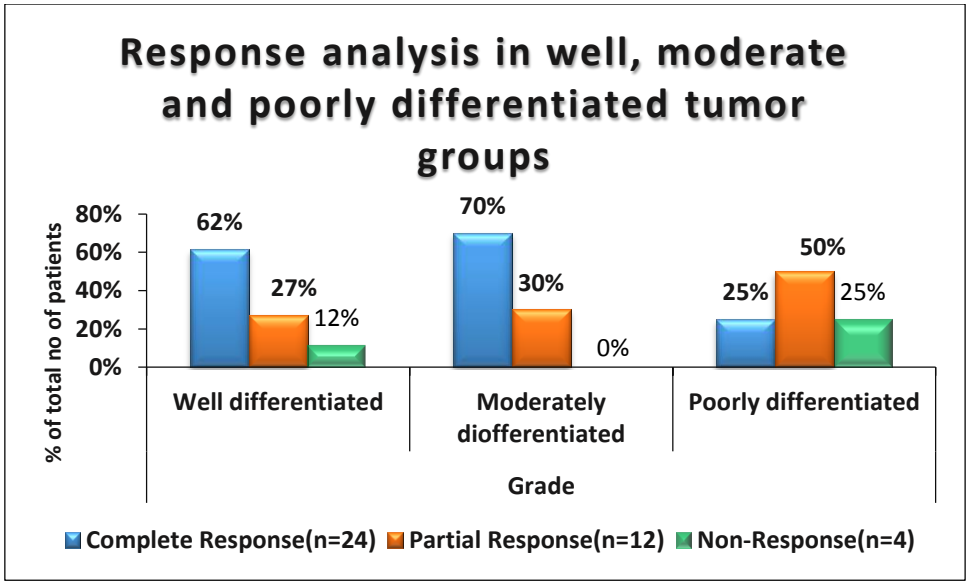
Graph 29.2:- Comparison of pre RT and post RT within HPE group

No significant difference was seen in pre RT GSH, post RT GSH and fall in GSH between squamous and adenocarcinoma as shown in table 32 and Graph 29.1.

Significant fall in GSH values was seen only in patients with squamous whereas patients reported with adenocarcinoma did not show any significant fall as shown in table 32 and Graph 29.2.

Table 33 :- Response analysis based on grade of tumour

Grade	Response			Total	P value
	Complete Response(n=24)	Partial Response(n=12)	Non-Response(n=4)		
Well differentiated	16 (61.54%)	7 (26.92%)	3 (11.54%)	26 (100.00%)	0.467
Moderately diofferentiated	7 (70.00%)	3 (30.00%)	0 (0.00%)	10 (100.00%)	
Poorly differentiated	1 (25.00%)	2 (50.00%)	1 (25.00%)	4 (100.00%)	

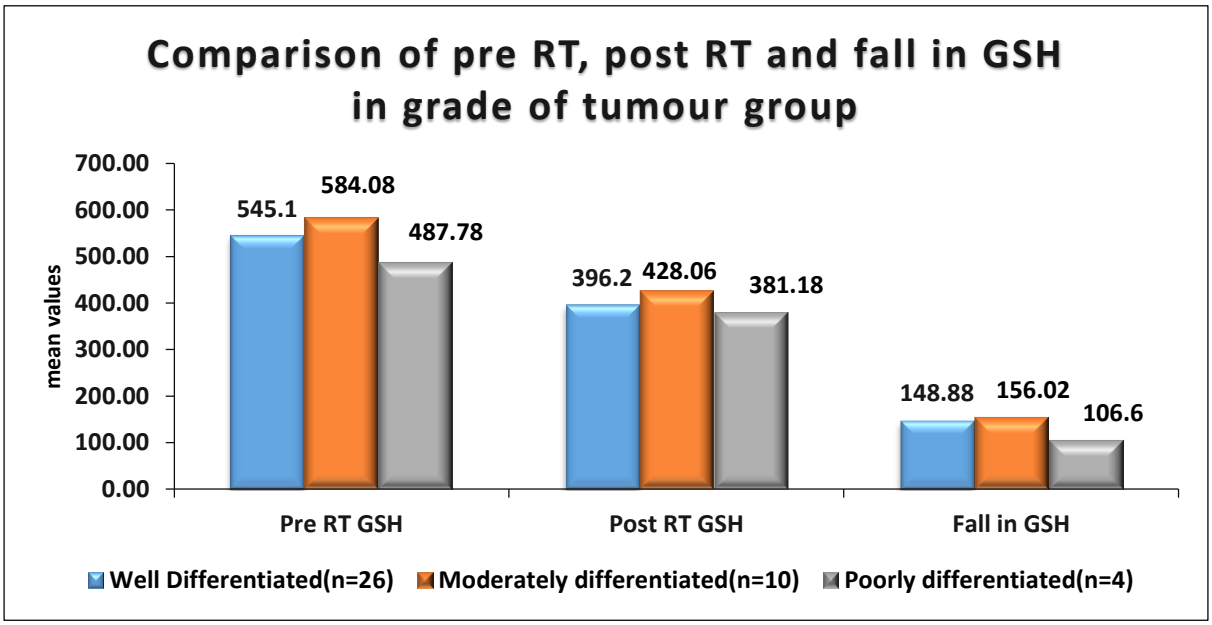


Graph 30:- Response analysis within grades of tumour.

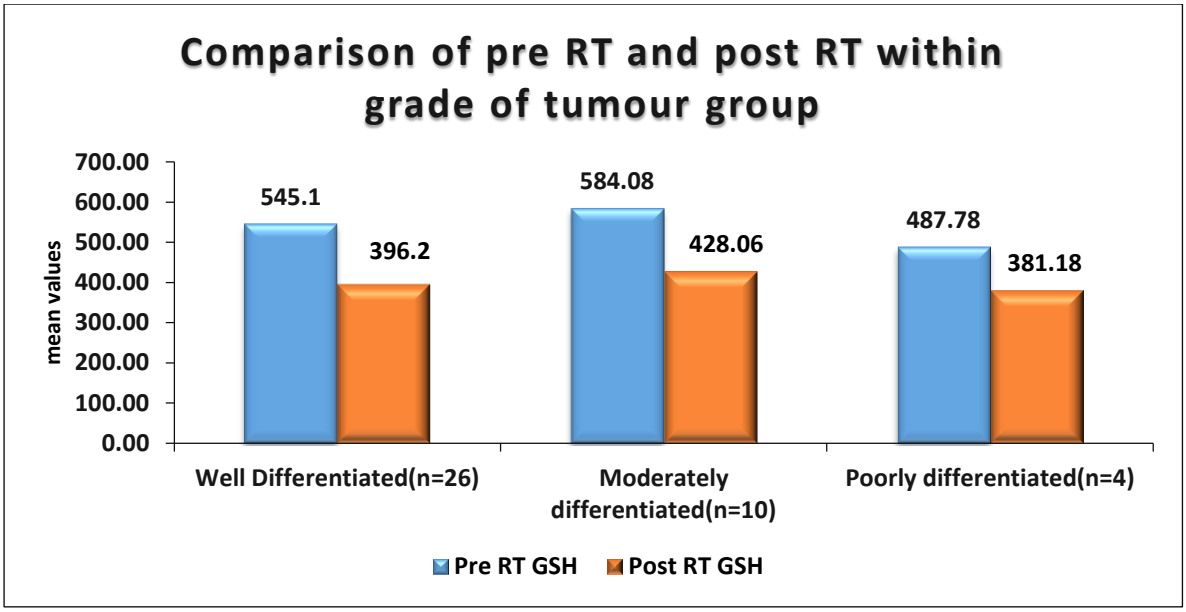
Response rates in group of patients having well differentiated, moderately differentiated and poorly differentiated were very similar with no significant difference (P=0.467) as shown in table 33 and Graph 30.

Table 34:- Comparison of pre RT, post RT and mean fall in GSH within grade of tumour group

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
Well Differentiated(n=26)	545.1 ± 79.06	562.4(485.652 - 585.652)	396.2 ± 104.05	398.25(331.297 - 443.334)	148.88 ± 92.1	126.24(98.741 - 186.265)	<.0001
Moderately differentiated (n=10)	584.08 ± 85.31	594.75(541.568 - 625.562)	428.06 ± 94.4	456.3(362.523 - 512.365)	156.02 ± 74.95	160.25(110.398 - 183.102)	0.0001
Poorly differentiated (n=4)	487.78 ± 92.91	455.51(433.994 - 541.565)	381.18 ± 120.97	368.06(306.293 - 456.063)	106.6 ± 72.48	84.92(63.985 - 149.216)	0.06
P value	0.141		0.648		0.615		



Graph 31.1:- Comparison of pre RT, post RT and mean fall in GSH within grades of tumour group



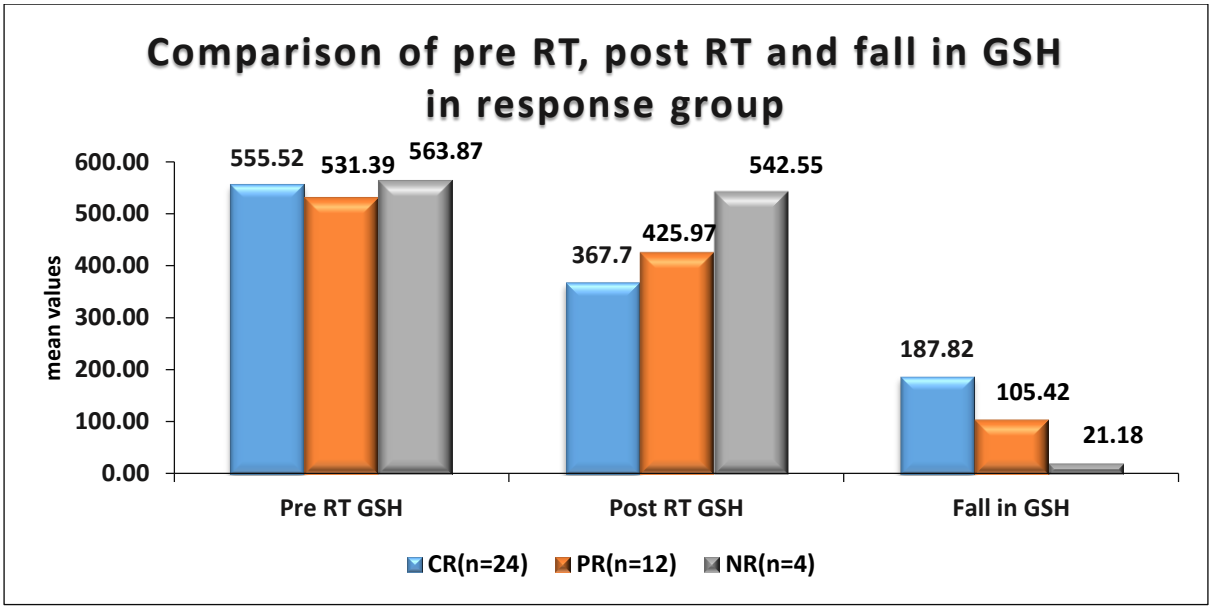
Graph 31.2:- Comparison of pre RT and post RT within grades of tumour group

GSH values showed similar trend in well differentiated, moderately differentiated and poorly differentiated with no statistically significant difference between them as shown in table 34 and Graph 31.1.

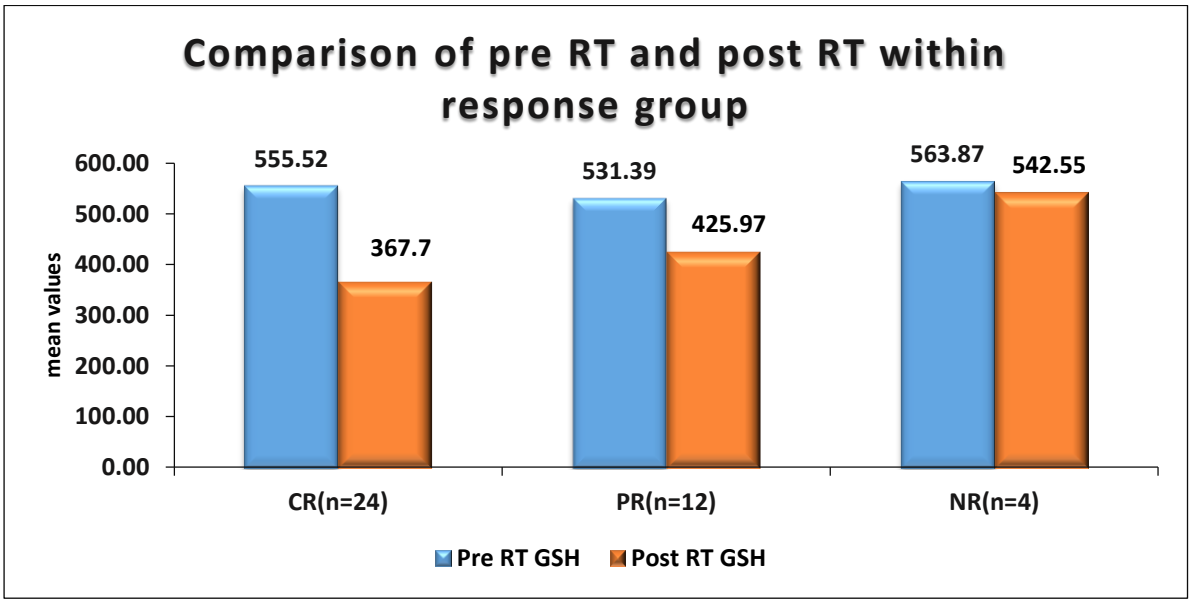
Significant fall in GSH was seen in only well differentiated and moderately differentiated but not in poorly differentiated grade as shown in table 34 and Graph 31.2.

Table 35:- Comparison of pre RT, post RT and mean fall in GSH in response status

Group	Pre RT GSH(µg/ml)		Post RT GSH(µg/ml)		Fall in GSH(µg/ml)		P value pre vs post
	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	Mean ± Stdev	Median(IQR)	
CR(n=24)	555.52 ± 88.7	563.45(485.607 - 625.345)	367.7 ± 94.23	379.9(299.058 - 441.147)	187.82 ± 81.15	178.15(127.366 - 211.599)	<.0001
PR(n=12)	531.39 ± 70.69	530.31(457.503 - 593.360)	425.97 ± 64.66	418.61(364.172 - 472.919)	105.42 ± 20.83	100.77(88.216 - 115.060)	<.0001
NR(n=4)	563.87 ± 106.2	590.6(489.258 - 638.485)	542.55 ± 115.14	593.89(476.702 - 608.407)	21.18 ± 42.8	40.23(-3.560 - 45.922)	0.393
P value	0.683		0.002		<.0001		



Graph 32.1:- Comparison of pre RT, post RT and mean fall in GSH in response status



Graph 32.2:- Comparison of pre RT and post RT within response group

Mean pre and post RT GSH values in the group of patients showing complete response was 555.52 ± 88.7 and was 367.7 ± 94.23 respectively. Hence the mean fall in GSH in this group was 187.82 ± 81.15. Mean pre and post RT GSH values in the group of patients showing partial response was 531.39 ± 70.69 and was 425.97 ± 64.66 respectively. Hence the mean fall in GSH in this group was 105.42 ± 20.83. Mean pre RT, post RT GSH and fall in GSH values in the group of patients showing

no response was 563.87 ± 106.2, 542.55 ± 115.14 and 21.18 ± 42.8 respectively. Statistical difference was seen in fall in GSH between complete response, partial response and non-response as shown in table 36 and Graph 32.1.

Table 35 shows the comparison of pre and post RT GSH levels. Paired t-test was used for the analysis since data was normally distributed. In complete responder, there was a statistically significant fall in GSH values after initiation of RT compare to baseline GSH values (P<.0001). In partial responders too there was a significant fall in GSH values after initiation of RT (P<.0001) but in non-responders, though there was a small fall in GSH values, it was not statistically significant (P=0.393) as shown in table 36 and Graph 32.2.

Discussion

DISCUSSION :

The present study was conducted to determine the clinically diagnosed cervical cancer patients response to chemotherapy and radiotherapy in terms of checking the serum glutathione level .So that, it can be served as response predictive marker for the assessment of patients response to treatment. .Patients clinical response was evaluated 3 months after treatment .

Clinical Response:

Our study results revealed that 60% of patients (n=40) as complete responder , 30% of patients as partial responder and 10% accounts to non responders. These results are supported by studies conducted by Manjunath[64] et al , Vidyasagar et al [61] .

The research work carried out by Manjunath and his co-workers reported classification of patient response to the similar treatment as complete(68%) , partial(26%) and non response (6%) patients .Similarly in another study conducted by Vidyasagar et al indicated complete response with 72% , partial response 14% and non response with 14%.

Yet another study by Jadhav et al[53] also indicates 33% of complete response , partial response (26%) and non response group with 40%. The reason for the varying results in Jadhav et al study could be because stage IIB patients received lesser fractions of external beam radiotherapy 35Gy in 16 fractions and these patients also did not receive any concurrent chemotherapy.

	COMPLETE RESPONSE	PARTIAL RESPONSE	NON RESPONSE
PRESENT STUDY	60 %	30 %	10%
Manjunath et al[64]	68%	26%	6%
VIDYASAGAR et al	72%	14%	14%
Jadhav et al	33%	26%	40%

Table 36 : Comparison of Response in present study and previous studies.

Glutathione values:

Intracellular Glutathione concentration makes modulation of cellular response to cytotoxic agents such as chemo and radiotherapy. Normally serum GSH and cellular GSH are similar in amounts. However , in tumour cells GSH appear to be high. Intra-tumoural variation of GSH is noticed in different tumours .Accordingly in the current study group 60% complete responders showed significant fall in GSH than partial and non responders. In patients with complete response the fall in GSH level was 187.82 ± 81.15mcg/ml, 105.42 ± 20.83 mcg/ml in partial responders and non responders it was 21.18 ± 42.8mcg/ml.

In both complete and partial responders there was a significant fall in post radiotherapy glutathione values. In complete responders there was a comparatively higher fall in glutathione than the partial responders. Miniscule fall in Glutathione values was seen in non responders but this fall was not significant.

In a similar study conducted by Vidyasagar et al[61], baseline glutathione values were compared with two samples one taken after 2 fractions of radiotherapy and I cycle of chemotherapy and the other one taken after 5 fractions of radiotherapy and one cycle of chemotherapy . In both samples collected post treatment there was a significant fall in serum glutathione levels seen in complete and partial responders and minimal fall in non responders which was non significant.

In another similar study by Manjunath et al [64], two samples of blood was collected , one pre-treatment and the other one 24 hrs post 2 fractions of radiotherapy and 1 cycle of chemotherapy. The results were same as in the above study and our present study were only complete and partial responders showed a significant fall.

Jadhav et al in his study, collected 2 blood samples one before the treatment and the other after 1 fraction of radiotherapy and also collected tumour tissue to assess for the fall in glutathione . he concluded that there was a fall in glutathione seen in both blood and tissue samples collected after 1 fraction of radiotherapy , and this fall was significantly higher in complete responders compared to partial and non responders.

His study also showed that blood glutathione levels can be taken as a prognostic indicator in the same way as blood and tumour glutathione values showed similar trends.

Demecri et al[63] in his study, collected one blood sample pre-treatment and the other after completion of radiotherapy. He reported that there was no significant fall in glutathione levels pre and post radiotherapy, but there was significant difference in fall in post radiotherapy values between complete, partial and non responders. This varying result might have been because second sample of blood was drawn after completion of radiotherapy and since the oxidative stress and role of anti-oxidants begin initially as soon as the treatment is started and both are nullified once the treatment is stopped as there is minimal or no tumour tissue left. Similarly there is a significant difference between the post radiotherapy values between the three responders as this depends on the size of tumour tissue left behind.

AGE:

In our present study among the age groups 41-50 yrs, there were 65% complete, 22% partial and 13 % non-responders seen. In age group between 51-60 yrs, the complete, partial and non responders were 54% , 38%, 18 %.hence there were higher number of complete responders seen between 41-60 yrs. In age groups below 40yrs and above 60yrs there were equal number of complete and partial responders and no non responders. The fall in glutathione levels were also significantly higher in age group between 41-60yrs and there was no significant fall seen in age groups below 40yrs and above 60yrs.

In another study by Manjunath et al there was difference in response rates between </= 50 years and >50years age groups. In 63% of Younger age group there was complete response and where as 81% of older than 50years group showed complete response. Both pre and post RT GSH values were higher in younger age group but fall in GSH values were more in older age group. But none of these differences were statistically significant.

In a analysis done by Demirci et al , he considered 60 yrs as the cut off and there was no significant fall in glutathione .These results are similar to study done by Manjunath et al.

GRADE OF TUMOUR :

In our present study response rates in group of patients having well differentiated , moderately differentiated and poorly differentiated were very similar with no significant difference .GSH values showed similar trend in all three grades with no statistical difference between them. Significant fall in GSH was seen in only well and moderately differentiated but not in poorly differentiated tumours.

According to Manjunath et al in well and moderately differentiated tumours response rates were similar .GSH values too showed similar trends in well and moderately differentiated tumours with no statistically significant difference between them . Hence the findings of this study are also very similar to our present study.

HISTOLOGY:

Complete response rate was significantly higher in patients reported with squamous as compared to patients with adenocarcinoma. No significant difference was seen in pre RT GSH and fall in GSH between squamous and adenocarcinoma, however significant fall in GSH values was seen only squamous whereas patients with adenocarcinoma did not show any significant fall.

According to a Manjunath et al, poor response was in patients with adeno-squamous carcinoma and the fall in GSH in these patients were not significant .these results are similar to results as in our study and this might be due to the meagre fall in GSH in these patients which might be responsible for their poor response.

TUMOUR BULK:

In present study bulky and non bulky tumour groups were evenly matched in response rates. There was significant fall in GSH values both in bulky and non bulky tumours. Hence in our study bulk of the tumour neither influenced the response rate nor fall in GSH values.

In similar study by Manjunath et al also had similar results were bulk of the tumour did not correlate with the response of tumour nor fall in GSH.

Body Mass Index:

In a study conducted by Nora et al results showed that underweight patients (BMI <18.5 kg.m2) with cervical carcinoma have increased complications and decreased survival rates compared to normal weight and obese patients[68].

In our study Response rate was comparable in BMI <18.4, 18.5-24.9, 25.0 – 29.9 kg/m^2. GSH value showed significant fall in BMI less than $19kg/m^2$ and in 19-$24.4kg/m^2$ with no difference between these two groups. BMI group 25-29.9 kg/m^2 could not be analysed since the number of patients in this group was too small for any type of statistical analysis. The fall in GSH in underweight patients might be because the liver in these patients might not be able to replenish GSH compared to the same in obese patients.

LIMITATIONS

The limitation of the present study are

- Small sample size
- Short follow up period and
- Lack of control group.

Summary

SUMMARY

Present study is a prospective hospital based longitudinal study conducted on 40 patients diagnosed with cervical carcinoma and undergoing chemo radiotherapy at R L Jalappa Hospital, Tamaka , Kolar.

- Patients received external beam radiotherapy along with 5 cycles of weekly chemotherapy followed by brachytherapy, 15days after the completion of EBRT.

- Two blood samples were collected from patients before treatment and after 2 fractions of RT and one cycle of chemotherapy. These samples were centrifuged and serum glutathione estimation was done.

- Pre and post Chemo-Radiotherapy, Glutathione values and also the fall the in GSH between the two samples were recorded.

- Response assessment of Patients was done after 3months of completion of treatment Using WHO guidelines.

- Fall in serum Glutathione values were correlated with tumour Response to therapy.

- Majority of patients involved the study were in the age group of 41-50yrs . (57.5%).

- Among the study subjects 52.5% of patients belonged to FIGO stage IIB . In around 87.5% of patients histopathological examination showed squamous cell carcinoma.

- Well differentiated tumours were higher in number in our study subjects (65%) , whereas moderately differentiated and poorly differentiated tumours were see in 10% and 4% patients respectively.

- After completion of treatment, Complete response was seen in 60% of patients, Partial response in 12 % and 4 % of patients were non responders.

- Complete response was in higher in age groups 41-50yrs and 51-60 yrs. There was also significant fall in GSH noted in the above age groups .($P<0.0001$ and $P<0.0001$)

- The response rates were similar in premenopausal and post-menopausal patients, but the fall in GSH was significant in postmenopausal women ($p<0.0001$).

- Response rates were comparable in underweight, normal weight and obese patients. GSH value showed significant fall in BMI <18.5 kg/m2 and in BMI 18.5- 24.9 kg/m2 with no difference between the two groups ($P<0.0004$ and $P<0.0001$).

- In bulky and non bulky tumours, response rates were similar .There was significant fall in GSH seen in both the tumours ($P<0.0001$). Hence, the bulk neither influenced response rate nor fall in GSH.

- There was no significant difference in response rate in relation to FIGO staging (P value 0.509) . Fall in GSH values were significant in stage IIB(P <0.0001) , IIIA (P value 0.009) , IIIB ($P<0.0001$) with no difference between them .

- Complete response was significantly higher in patients with squamous cell carcinoma as compared to patients with adenocarcinoma ($P=0.032$) and also significant fall in GSH value was seen only in squamous cell carcinoma

(P<0.0001), whereas patients reported with adenocarcinoma did not show any significant fall (P=0.06).

- In well differentiated, moderately and poorly differentiated tumours the response rate was similar. With no significant difference (P=0.467).Significant fall in GSH was seen in only well and moderately differentiated tumours(P<0.0001, P=0.0001) and not in poorly differentiated tumours (P=0.06).

- Statistical difference was seen in fall in GSH between complete, partial and Non-responders.

- In complete responders, there was significant fall in GSH values after initiation of radiotherapy (P<0.0001).In partial responders too there was significant fall in GSH (P<0.0001) but in non responder, though there was small fall in GSH values, it was not statistically significant (P=0.393).

Conclusion

CONCLUSION

The serum glutathione concentration in carcinoma cervix patients before treatment, and its fall after two fractions of radiotherapy and one cycle of chemotherapy significantly helps to predict response to treatment. Complete responders had a significant fall in serum glutathione levels post treatment. Hence, post treatment serum glutathione levels are effective prognostic markers for carcinoma cervix. However, further studies with larger sample size and longer follow up are required to assess long term response and sub group analysis.

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Annexures

CASE PROFORMA

NAME: IP NO:

AGE: DOA:

OCCUPATION: DOD:

ADDRESS:

EDUCATION:

HUSBANDS OCCUPATION:

SOCIOECONOMIC STATUS:

CHIEF COMPLAINTS:

HISTORY OF PRESENT ILLNESS:

MENSTRUAL HISTORY :

LAST MENSTRUAL PERIOD:
AGE OF MENARCHE:

PAST MENSTRUAL CYCLES:

OBSTETRIC HISTORY:

MARRIED LIFE : CONSANGUINITY:

PAST HISTORY:

HTN/DM/BA/TB/BLOOD DYSCRASIAS/EPILEPSY/THYROID DISORDER/CARDIAC DISEASE/ALLERGY

H/O blood transfusions:

H/O Surgeries or hospitalization:

PERSONAL HISTORY:

Sleep and appetite:

Diet:
Bowel and bladder:

FAMILY HISTORY:

DRUG HISTORY:

GENERAL EXAMINATION:
General condition: Fair/ moderate/ Poor

Built: Nourishment:

Ht: cms Wt: kgs BMI:

Pallor: Icterus:
Cyanosis: Clubbing:
Lymphadenopathy: Edema:

VITALS:
Pulse rate:
Respiratory rate:
Blood pressure
Temperature:

SYSTEMIC EXAMINATION:
Cardiovascular system:
Respiratory system:
Central nervous system:

Per abdomen:

LOCAL EXAMINATION:

Per Speculum:

Per vaginum:

Per rectal:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:
Blood group and Rh typing:

CBC: HB: HIV:
PCV: HbsAG:
RBC: VDRL:
WBC:
PLT:
RBS:
Blood Urea:
Serum Creatinine:
Serum Sodium:
Serum Potassium:
Urine analysis: Albumin-
Sugar-
Microscopy-

ULTRASOUND ABDOMEN AND PELVIS:
MRI/CT SCAN:
CERVICAL BIOSY REPORT:

SERUM GLUTATHIONE LEVELS:
PRE RADIOTHERAPY:(DAY 0)
POST RADIOTHERAPY:(DAY 3)

CONDITION AT DISCHARGE:

SRI DEVARAJ URS MEDICAL COLLEGE & RESEARCH CENTRE, TAMAKA, KOLAR

PATIENT CONSENT FORM

Case no:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I have understood that I have the right to refuse consent or withdraw it at any time during the study and this will not affect my treatment in any way. I consent voluntarily to participate in this study

"PROGNOSTIC SIGNIFICANCE OF GLUTAHIONE LEVELS IN CERVICAL CANCER PATIENTS UNDERGOING RADIOTHERAPY."

Name of Participant_____

Signature/ thumb print of Participant _____

Date _____

Statement by the researcher/person taking consent:

I have accurately read out the information sheet to the potential participant and to the best of my ability made sure that the participant understands that the following will be done:

2 ml venous blood sample taken for serum glutathione estimation.

I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Name and Address of Principal Investigator:

Dr. Arpitha Shruthi A.
R.L Jalappa Hospital
Tamaka, Kolar.

ತಿಳುವಳಿಕೆಯ ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ:– "Prognostic significance of glutathione levels in cancer cervix undergoing chemotherapy"

ಶ್ರೀ/ಶ್ರೀಮತಿ ಆದ ನಾನು ಈ ಮೇಲಿನ ಸಂಶೋಧನ ವಿಷಯದ ಬಗ್ಗೆ ನನಗೆ ಅರ್ಥವಾಗುವ ರೀತಿಯಲ್ಲಿ ನನ್ನದೇ ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಸಿರುತ್ತಾರೆ. ಈ ಸಂಶೋಧನಾ ವಿಷಯದಲ್ಲಿ ನಾನು ಒಬ್ಬ ವಿಷಯಿಯಾಗಿ ಭಾಗವಹಿಸಲು ನನ್ನ ಸಂಪೂರ್ಣವಾಗಿ ಒಪ್ಪಿಗೆ ಇರುತ್ತದೆ. ಈ ಸಂಶೋಧನಾ ಉದ್ದೇಶವನ್ನು ಪೂರ್ಣವಾಗಿ ಅರಿತಿರುತ್ತೇನೆ. ಈ ಸಂಶೋಧನೆಗೆ ನನ್ನಿಂದ ಯಾವುದೇ ಆರ್ಥಿಕತೆಯ ಅವಶ್ಯಕತೆ ಇರುವುದಿಲ್ಲ. ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನ ಸಹಕಾರವನ್ನು ಹಿಂಪಡೆದು ಈ ಸಂಶೋಧನೆಯಿಂದ ಹೊರಹೋಗುವ ಹಕ್ಕನ್ನು ಹೊಂದಿರುತ್ತೇನೆ. ಇದರಿಂದ ನನ್ನ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ತೊಂದರೆಯಾಗುವುದಿಲ್ಲ. ಮುಖ್ಯವಾಗಿ ನನ್ನಿಂದ ಪಡೆದ ಈ ಮಾಹಿತಿಯು ಸಂಶೋಧನೆಗೆ ಮಾತ್ರ ಸೀಮಿತವಾಗಿರುತ್ತದೆ. ಮತ್ತು ಈ ಮಾಹಿತಿಯು ಎಲ್ಲೂ ಸೋರಿಕೆಯಾಗದಂತೆ ಎಚ್ಚರಿಕೆ ವಹಿಸುವುದಾಗಿ ತಿಳಿವಳಿಕೆ ನೀಡಿರುತ್ತಾರೆಂದು ನಾನು ದೃಢಪಡಿಸಿಕೊಂಡು ಒಪ್ಪಿಗೆ ನೀಡಿರುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ/ ಸಾಕ್ಷಿ ಸಹಿ. ಸಂಶೋದಕನ ಸಹಿ

ಬೆರಳಚ್ಚು.

PATIENT INFORMATION SHEET

Study title: **Prognostic significance of glutathione levels in cervical cancer patients undergoing radiotherapy.**

Study location: R L Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

Patients who are of clinically diagnosed cases of carcinoma cervix admitted to OBG department of R L Jalappa hospital attached to Sri Devaraj Urs medical college are recruited in the study after obtaining patient information consent.

Patients will be classified based on FIGO guidelines and treatment will be started.

2 ml of venous blood is collected from the study subjects for serum glutathione estimation , 1st sample before treatment and second sample on day 3 of treatment.

Details-

Please read the following information and discuss with your family members. You can ask any question regarding the study. If you agree to participate in the study we will collect information (as per proforma) from you or from a person responsible for you or both. Relevant history will be taken. This information collected will be used only for dissertation and publication.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. This study has been reviewed by the Institutional Ethics Committee and you are free to contact the member of the Institutional Ethics Committee. There is no compulsion to agree to this study. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

For further information contact

Dr. Arpitha Shruthi

Post graduate, Department of obstetrics and Gynaecology

R L Jalappa hospital, Kolar .

KEY TO MASTER CHART

- IP NO. - IN PATIENT HOSPITAL NUMBER
- AGE GROUP:

1. 1 : 31-40 YEARS
2. 2 : 41-50YEARS
3. 3 : 51-60 YEARS
4. 4 : 61-70 YEARS

- SOCIOECONOMIC STATUS

1. 1 : BG PRASAD CLASS I
2. 2 : BG PRASAD CLASS II
3. 3 : BG PRASAD CLASS III
4. 4 : BG PRASAD CLASS IV
5. 5 : BG PRASAD CLASS V

- PARITY

1. 1 : PARA 1
2. 2 : PARA 2
3. 3 : PARA 3
4. 4 : PARA 4

- AGE OF COITUS

1. 1 : <20 YEARS
2. 2 : 20-29 YEARS
3. 3 : > 30 YEARS

- AGE OF MENARCHE

1. 1 : 10-12 YEARS
2. 2 :13-14 YEARS
3. 3 :> 14 YEARS

- MENSTRUAL STATUS

1. 1 : PREMENOPAUSAL
2. 2 : POSTMENOPAUSAL

- INTERMENSTRUAL BLEEDING

1. 1 : YES
2. 2 : NO

- POST COITAL BLEEDING

1. 1 : YES
2. 2 : NO

- POST MENOPAUSAL BLEEDING

1. 1 : YES

2. 2: NO

- BODY MASS INDEX

1. 1: <18.5 kg/m2
2. 2 : 18.5-24.5 kg/m2
3. 3: > 24.5 kg/m2
4. 4 : > 30kg/m2

- BULKY TUMOUR

1. 1: YES
2. 2: NO

- CLINICAL STAGING

1. 1 :IIB
2. 2 :IIIA
3. 3:IIIB
4. 4 : IVA

- LYMPH NODE:

1. 1 : YES
2. 2 : NO

- HISTOPATHOLOGICAL EXAMINATION:

1. 1 :SQUAMOUS CELL CARCINOMA
2. 2 : ADENOCARCINOMA

- GRADE OF TUMOUR :

1. 1 : WELL DIFFERENTIATED
2. 2 : MODERATELY DIFFERENTIATED
3. 3: POORLY DIFFERENTIATED

- RESPONSE TO TREATMENT

1. 1 : COMPLETE RESPONSE
2. 2 : PARTIAL RESPONSE
3. 3: NON RESPONSE

- PRE RADIOTHERAPY GLUTATHIONE
- POST RADIOTHEARPY GLUTATHIONE
- FALL IN GLUTATHIONE

SL.NO.	IP. No	AGE	SOCIOECONOMIC STATUS	PARITY	AGE OF MARRIAGE	AGE OF MENARCHE	MENSTRUAL STATUS	INTERMENTRUAL BLEEDING	POST COITAL BLEEDING	POST MENOPAUSAL BLEEDING	BMI	BULKY	CLINICAL STAGING FIGO	LYMPH NODE INVOLVEMENT	HPE	GRADE	RESPONSE	PRE RT GSH	POST RT GSH	FALL IN GSH
1	446789	2	4	3	1	1	2	2	1	1	2	1	1	2	1	1	1	568.964	443.334	125.63
2	443723	2	4	4	1	1	2	2	2	1	2	2	3	1	1	1	1	641.632	440.032	201.6
3	428994	3	4	3	1	1	2	2	1	1	2	1	1	2	1	2	1	701.623	536.362	165.261
4	439974	2	4	4	1	2	1	1	1	2	2	1	2	2	1	1	1	642.569	430.934	211.635
5	431196	2	4	5	1	2	2	2	1	1	2	1	1	2	1	1	3	562.654	605.231	-42.577
6	440783	3	3	4	1	1	2	2	2	1	1	2	1	2	1	2	2	625.562	470.327	155.235
7	424939	2	4	5	2	2	1	1	1	2	2	1	3	1	1	3	3	415.862	370.862	45
8	462541	1	4	4	2	1	1	2	2	2	1	1	1	2	2	1	2	452.652	363.09	89.562
9	481312	3	4	5	2	1	2	2	2	1	2	1	1	2	1	1	1	485.652	326.542	159.11
10	428184	2	4	3	2	2	2	2	2	1	2	2	3	1	1	2	1	562.652	240.09	322.562
11	460944	2	5	4	1	1	2	2	1	1	2	1	3	2	1	1	3	618.546	582.542	35.458
12	475273	2	4	4	1	1	2	2	2	1	2	1	2	2	1	1	2	562.452	435.598	126.854
13	464217	2	4	3	1	1	2	2	1	1	1	2	1	2	1	1	1	456.852	156.652	300.2
14	423340	3	4	2	1	1	2	2	2	1	1	1	3	2	1	2	2	452.652	342.254	110.398
15	436204	2	5	2	1	2	2	2	1	1	2	2	1	2	1	3	1	458.895	247.333	211.562
16	443171	2	5	2	2	2	2	2	2	1	2	1	1	2	1	1	1	521.365	397.111	124.254
17	410264	2	4	4	2	2	2	2	2	1	2	1	3	2	1	2	1	456.254	379.73	76.524
18	380605	2	4	3	2	2	1	1	1	2	2	1	3	2	1	1	1	564.587	299.333	265.254
19	419720	2	3	2	3	2	2	2	2	1	2	1	1	2	1	3	2	452.125	365.254	86.871
20	436203	2	4	4	1	3	1	2	1	2	3	1	1	2	1	1	1	564.265	454.011	110.254
21	380256	3	4	4	1	2	2	2	2	1	1	2	1	2	1	1	1	523.654	338.029	185.625
22	582537	4	4	4	1	2	2	2	2	1	1	1	3	2	2	2	2	625.254	512.365	112.889
23	386528	2	5	3	1	2	2	2	2	1	2	1	1	2	2	1	1	724.235	298.783	425.452
24	410328	2	4	4	1	2	1	1	1	2	2	1	4	1	2	1	2	496.254	401.623	94.631
25	412553	2	4	3	2	1	2	2	2	1	2	1	1	2	1	1	1	585.652	399.387	186.265
26	422195	3	5	3	1	1	2	2	2	1	2	2	3	2	1	2	1	564.254	362.523	201.731

27	422634	2	4	3	1	2	2	2	2	1	2	1	2	2	1	1	1	485.562	331.297	154.265
28	424401	3	4	4	1	1	2	2	1	1	1	1	1	2	1	2	1	685.562	512.365	173.197
29	390419	3	4	3	1	2	2	2	2	1	1	1	3	2	1	1	2	462.354	359.562	102.792
30	430698	3	4	3	2	1	2	2	2	1	2	1	2	1	1	1	2	498.265	399.524	98.741
31	433042	2	3	3	1	2	1	1	1	2	2	1	1	2	1	2	1	625.365	442.263	183.102
32	435418	2	5	3	2	1	2	2	2	1	2	2	3	2	1	1	2	562.354	475.512	86.842
33	455884	1	4	3	1	1	2	2	1	1	2	1	1	2	1	1	1	625.325	380.072	245.253
34	436116	2	4	2	1	2	1	2	1	2	2	1	3	2	1	2	1	541.568	482.268	59.292
35	438544	2	4	2	1	2	1	1	1	2	2	1	3	2	2	1	2	562.485	445.253	117.232
36	435198	3	4	2	1	1	2	2	2	1	2	1	1	2	1	3	2	624.235	541.265	82.97
37	437181	3	5	4	1	2	2	2	1	1	2	1	1	2	1	1	1	524.365	395.263	129.102
38	439981	3	4	4	1	1	2	2	2	1	2	2	1	2	1	1	1	365.265	240.011	125.254
39	432314	3	4	3	1	2	2	2	2	1	1	1	1	2	1	1	3	658.425	611.582	46.843
40	443215	4	5	3	1	1	2	2	1	1	2	1	3	2	1	1	1	456.254	291.019	165.235