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ORIGINAL ARTICLE

A Randomised Clinical Trial to Compare the Efficacy of Hyperbaric Oxygen Therapy with Neoadjuvant Chemotherapy with Neoadjuvant Chemotherapy Alone for Carcinoma Breast: a Pilot Study

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Abstract
Hypoxia plays a major role in cell survival, angiogenesis, glycolytic metabolism and metastasis in breast cancer. Hyperbaric oxygen therapy (HBOT) has a well-established effect for attenuating the effects of hypoxia by enhancing the effects of chemotherapy drugs and increasing oxygen delivery to the tissues. The hypoxic environment encourages cancer cells to acquire stemness and become resistant to chemotherapeutic drugs. We have used HBOT as an adjunct to neoadjuvant chemotherapy in breast cancer patients with the objective of reducing tumour hypoxia and improving the response to chemotherapy. A randomised controlled trial was conducted in patients with breast cancer. The patients were randomly allocated into the study group receiving neoadjuvant chemotherapy (NAC) with HBOT and the control group who received NAC only. The NAC consisted of 3 cycles of intravenous injection of cyclophosphamide, 5-fluorouracil and epirubicin. The HBOT was administered along with the chemotherapy in 3 sessions in each cycle at graded pressures in each sessions. Ultrasound evaluation of patients was carried out and breast tumour size was measured after every cycle of HBOT. All the patients were operated after 3 cycles of chemotherapy and modified radical mastectomy was carried out. The response of therapy was recorded in both the groups. Patients receiving HBOT achieved significantly higher percentage reduction of tumour volume (43.1%) and the largest dimension (80.21%) ($p < 0.0001$) compared with those receiving chemotherapy alone. Trial registry: CTRI/2019/03/018258

Keywords Breast carcinoma · Hyperbaric oxygen therapy · Neoadjuvant therapy · Breast carcinoma · Complete pathological remission · Margins of excision

Introduction

The carcinoma of the female breast continues to be a major cause of mortality and morbidity worldwide [1, 2]. Incidence of breast cancer is rising, particularly among younger females. Radical breast surgeries are associated with significant psychological trauma in females, with poor results in terms of metastasis and recurrence, even so for the chemotherapy and radiotherapy resistant tumours. Neoadjuvant chemotherapy can reduce the tumour bulk or downstage the tumour but the results have not been satisfactory [3, 4].

Hypoxia plays a major role in cell survival, angiogenesis, glycolytic metabolism and metastasis in breast cancer. Hypoxia has also been shown to increase genetic instability, activate invasive growth and generate a state of stemness in tumour cells [5, 6].

Hyperbaric oxygen therapy [HBOT] is defined as the administration of 100% inhaled oxygen to patients at increased atmospheric pressure. It has a well-established effect of enhancing the amount of dissolved oxygen in plasma and thereby increasing the oxygen delivery to the tissues. It can help in enhancing the effect of chemotherapy in drug-resistant tumours [5, 6].

A pilot study in breast cancer patients combining HBOT with chemotherapy demonstrated a decrease in oedema and angiogenesis within the tumour, but with no promising results in terms

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of reduction in tumour cell volume and disease-free survival, likely as the study was conducted in metastatic cancers and was done as a 10-day continuous therapy with no lag time [7].

Another study done on mouse mammary cell carcinomas with HBOT and chemotherapy concluded that HBOT inhibited benign and malignant proliferation of mammary cells but did not facilitate cell death [8]. A study on DMBA-induced mammary tumours comparing HBOT with HBOT plus 5-fluorouracil (5-FU) showed that the combined therapy resulted in decreased size of the tumour but extent of reduction in size was not statistically significantly different from that of the control group [8]. In vitro studies on breast cancer cells have shown anti-proliferative and anti-angiogenic effect of HBOT [9].

HBOT in patients with carcinoma cervix as adjunct to radiotherapy has demonstrated improved local control and survival [10]. A combination of chemotherapeutic drugs (doxorubicin) and HBOT used in metastatic lung cancer in rat models showed enhanced tumoricidal effect of doxorubicin [11]. Combining chemotherapy with HBOT has increased the tumoricidal property of chemotherapy and improved the quality of life and disease-free survival of the patient [12].

We embarked upon a randomised controlled trial to evaluate the benefit of HBOT along with neoadjuvant chemotherapy in achieving reduction in tumour size in patients with locally advanced carcinoma of breast.

Materials and Methods

Trial Design

This was a randomised controlled clinical trial done in patients having locally advanced breast cancer [13].

The patients with histologically confirmed invasive breast carcinoma with age range 30 to 70 years were included in the trial after signing an informed written consent. Patients with single primary breast cancer with locally advanced breast cancer with no distant metastasis were included. The study was approved by the institutional review board of Government medical college and Sir T hospital. The study period was 2 years from August 2017 to August 2019.

The CONSORT flow chart is shown in supplementary file.

Randomisation and Interventions

Randomisation was done by computer-derived random numbers by primary investigator.

A sonography examination of the breast and axilla was carried out by an expert sonologist and the dimensions of the breast mass were recorded.

The patients included in the control group were offered only neoadjuvant chemotherapy of 3 cycles at 21 days interval.

The patients included in the HBOT group were given neoadjuvant chemotherapy of 3 cycles at the interval of 21 days with HBOT of 3 cycles (3 sittings per cycle).

The HBOT was administered on the day before the chemotherapy (1 h at 1.75ATA), on the day of chemotherapy (1 h at 2.0ATA) and the day after chemotherapy (1 h at 2.5ATA), followed by 21 days interval.

The neoadjuvant chemotherapy regimen is as follows [14]:

- Inj cyclophosphamide 600 mg/m2 IV
- Inj epirubicin 100 mg/m2 IV
- Inj 5-Fu 600 mg/m2 IV

Three cycles of neoadjuvant chemotherapy was given at an interval of 21 days each.

The neo-adjuvant hyperbaric therapy is as follows:

- The patient was rested supine inside a hyperbaric chamber.
- Each cycle of chemotherapy was accompanied by 3 sittings of hyperbaric therapy.
- Each sitting lasted for 1 h with graded increase in pressure every sitting.
- The first sitting had pressure of 1.75 ATA, second had 2.0 ATA and the third was at 2.5 ATA.
- This was performed to maximise the benefit of HBOT to the patients without untoward adverse effects.

The measurement of outcome variables:

Volume of the breast lump (V) [9]:

The volume of the lump in cubic centimetre was calculated. The lump was considered an ellipsoid.

The volume was calculated as an ellipsoid using the following formula:

$$V = a \times b \times c \times 0.52$$

where a = largest dimension, b = dimension at right angles to a and c = average of a and $b = (a + b)/2$.

Largest dimension (LD):

It is the largest dimension of the breast mass observed on ultrasonography.

The data on the volume of breast mass and largest diameter were compared by using Mann-Whitney test as the data was not normally distributed.

Outcome and Assessment

At the end of the neoadjuvant therapy, they were subjected to ultrasonography to assess the largest diameter and dimensions of the breast lump.

The volume of the lump was calculated by the above formula and was compared with the volume status at the baseline.

The response to chemotherapy was assessed by RECIST 1.1 criteria, where complete response (CR); primary tumour disappearance, partial response (PR), 30% or greater decrease in largest dimension of primary tumour, progressive disease (PD), 20% or more increase in largest dimension of primary tumour, and stable disease (SD); tumours did not show sufficient increase or decrease in tumour size [15].

The modified radical mastectomy was carried out after 3 cycles of neoadjuvant treatment and mastectomy specimens and lymph nodes were assessed by Miller-Payne criteria.

The primary outcome was to evaluate and compare the difference in the largest dimension and volume after the end of neoadjuvant therapy in both groups.

The secondary outcome was to evaluate and compare the downstaging of breast cancer in both groups.

Statistical Analysis

The primary end point was to assess the difference in the volume of the breast lump (V) before and after the neoadjuvant therapy and for the difference in the largest dimension of the breast lump before and after the neoadjuvant therapy.

The data were not distributed normally but, hence, were evaluated by the Mann-Whitney test.

The difference was considered statistically significant at $p = 0.05$.

The median largest dimension of breast lump in the HBO group was 4.0 cm (ranging from 2.0 to 7.6 cm) and the median largest dimension of breast lump in the control group was 4.1 cm (ranging from 2.2 to 7 cm) before the start of neoadjuvant therapy.

The median largest dimension of breast lump in the HBOT group after treatment was 2.0 cm while that in the control group was 3.3 cm.

According to the RECIST criteria 1.1, the overall partial response (PR) was seen in the HBOT group in 87% of patients with 13% of patients in the HBOT group showing complete response (CR).

The control group displayed 6% patients with progressive disease (PD) and 6% patients with partial response (PR), while in others, 88% patients were stable disease.

The HBOT group achieved a reduction in largest dimension of 1.85 cm (43.4% reduction) compared with the control group with a reduction in largest dimension of 0.73 (12.54% reduction). The two-tailed p value for the largest dimension reduction in the HBOT group compared with the control equals 0.0001 which is highly significant.

The mean volume (V) of tumour size before therapy in the HBOT group was 50.72 cc (ranging from 1.9 to 177 cc) and the mean volume (V) of tumour size before therapy in the control group was 36.85 cc (ranging from 5.5 to 125 cc). The HBOT group achieved a significantly greater reduction in the volume compared with the control group ($p = 0.0001$) displaying a volume reduction of 38.44 cc (80.21% change) and the control group displaying a volume reduction of 13.33 cc (23.65% change).

In the HBOT group, 13 (86.66%) out of 15 patients had downstaging after undergoing neoadjuvant therapy and HBOT. In the control group 4 (26.66%) out of 15 patients underwent downstaging after only NAC. The difference was statistically significant with $p < 0.05$.

Outcomes and Results

The HBOT group showed overall favourable response to therapy compared with the control group. Two patients in the HBOT group displayed complete pathological response while the rest of the breast lumps had significant partial response (PR) with consistent decrease in size of the breast lump.

The control group had variable response to chemotherapy with one patient showing progressive disease while one patient showing partial response and all the others had stable disease.

There was 80.21% decrease in the volume of lump measured by ultrasonography in the HBOT group and 23.65% in the control group, measured by V formula. The change in the largest dimension in the HBOT group was 43.4% while it was 12.54% in the control group.

There was downstaging in 86.66% of patients in the HBOT group compared with 26.66% of patients in the control group.

Trial was halted to analyse and evaluate the results and reform the errors for further trials.

Adverse Effects

The patients included in the HBOT group did not show any serious or life-threatening adverse effect specifically related to HBOT. Patients mainly complained of earache while inside the hyperbaric chamber with no permanent hearing defects. No other complication was seen in the study.

The patients receiving neoadjuvant chemotherapy (both HBOT and control group) had side effects of gastritis, vomiting, generalised weakness, alopecia, oral ulcers and

thrombophlebitis with ulcer. These patients were managed with vitamin supplements, proton pump inhibitors (PPIs) and other supportive treatment with reassurance.

Discussion

The solid tumours like breast carcinoma get adapted in a hypoxic environment by stabilisation of hypoxia-inducible factor-1 (HIF-1) that releases vascular endothelial growth factor (VEGF) and other factors to enhance angiogenesis and growth of the tumour. They also act by p53 dysregulation to prevent apoptosis and increase aggressiveness of the tumour. The aggressiveness is also contributed by release of reactive oxygen species that cause genomic instability. Due to hypoxia, the tumour cells switch to anaerobic glycolysis which facilitates rapid tumour progression. The oxygen deficiency also prevents chemotherapeutic drugs from reaching the tumour cells in a poorly perfused micro-environment [5]. The administration of higher dose of chemotherapeutic drugs to compensate this may lead to severe toxicity.

Hyperbaric oxygen therapy interferes in each of these adaptations by providing the cells 100% of oxygen under pressure. There is an improvement in oxygenation of the cells and an increase in drug delivery [5, 16–18].

The hyperoxia induced by HBOT may cause induction of apoptosis of tumour cells.

The neoadjuvant therapy has been previously used in early breast cancer and is associated with clinical response seen in NSABP B-27 [3]. The neoadjuvant therapy has also been used as a standard of care for locally advanced breast cancers. The neoadjuvant chemotherapy has been used fulfilling multiple purposes like downstaging primary advanced tumours to facilitate cure or to allow breast conservation.

The neoadjuvant chemotherapy regimens most commonly used are anthracycline and taxane-based therapy as well as cyclophosphamide, 5-FU and epirubicin (CEF) therapy. As previous literature supports the enhancement of chemotherapeutic effect of 5-FU when used in combination with HBOT, we preferred to include 5-FU-based chemotherapy regime [8, 19].

In animal studies, HBOT enhanced the uptake of chemotherapeutic drugs within the tumour tissue [20].

As there have been no similar previous clinical trials of hyperbaric oxygen therapy along with chemotherapy in breast cancer patients, we decided to administer hyperbaric oxygen therapy in a graded increasing pressure over 3 days with 1.75 ATA on day 1, 2.00 ATA on day 2 and 2.5 ATA on day 3 to avoid potential side effects of sudden exposure to high pressure. The exposure was for 60 min, as used in other diseases.

The increased atmospheric pressure at 100% oxygen was used in combination with chemotherapy that maximised its benefits. The maximal cytostatic agent uptake increases immediately after single HBO treatment, most probably due to transient increase in the oxygenation in the tumour tissue. This effect lasts up to 24 to 48 h.

Hence, we have designed this particular schedule of increasing pressure of HBOT over 3 days with chemotherapy administered on the second day of this cycle.

We sequentially measured the size of the breast lump before and after each cycle. The patients who had an increase in size of the lump were removed from the trial and treated as per conventional treatment. There were two such patients in the chemotherapy-only control group who showed an increase in size. All the patients in the HBOT group showed tumour regression. This could possibly be due to chemo-sensitisation and chemo-enhancing effect of hyperbaric oxygen therapy.

The tumour measurement was done by ultrasonography in three dimensions by a single expert sonologist who was blinded regarding the therapy received by the patient to avoid inter-observer error and any measurement bias.

We have found a significant overall decrease in size of breast lump of patients receiving HBOT along with chemotherapy.

There was 80.21% decrease in the volume of lump measured by ultrasonography in the HBOT group and 23.65% in the control group. The change in the largest dimension in the HBOT group was 43.4% while it was 12.54% in the control group.

Most of the change in the size of breast lump was noticed after the second cycle. Two patients in Group A had complete pathological response.

There is further scope of downstaging the tumour to achieve a more cosmetic outcome. Further studies can be done to achieve pathological complete response in early-stage tumours. This regimen could be continued up to 6 cycles in well responders who have achieved partial (> 30% regression) or complete pathological response in 3 cycles.

Hyperbaric oxygen therapy along with neoadjuvant chemotherapy has promising results in the treatment of breast carcinoma. Giving 3 cycles of neoadjuvant chemotherapy along with HBOT of 3 sessions with graded pressure at 21 days interval caused a significant reduction of breast tumour size and margin free status with downstaging of the tumour. The results of the combination therapy are significantly better than only neoadjuvant chemotherapy.

This study is performed as a pilot study for initial analysis of results to further apply to a larger population. Further subgroup analysis may be required for hormone receptor status. The benefit of other adjuvant modalities like radiotherapy or hormone therapy may be added. The favourable results showing downstaging may also be included for breast conservation therapy.

The only drawback of HBOT is the cost of HBOT chamber which can be overcome by downstaging of tumour and the

ultimate benefit to the patient in terms of survival benefit and life expectancy.

Application of this combination neoadjuvant therapy regime can help in the conversion of mastectomy to breast conversion for patients who are looking out for a better cosmetic outcome.

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Compliance with Ethical Standards

Conflict of interest There is no conflict of interest in this study.

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