Detection of T cell in Hodgkin’s Lymphoma before and after of BCG Administration

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Abstract
BCG is presently most studied immunostimulating agent which is able to stimulate delayed type of hypersensitivity (DTH) reaction by activating macrophages (Florentin et al. 1976) we have taken diagnosed cases of HD (Hodgkin disease), patient from cancer hospital and research institute Gwalior. The studies are carried under the supervision of cancer specialist and clinician for correlation of Results.

The beneficial effect was evaluated by observing improved clinical responses with regression of tumor and increase of life span of these patients. These patients were given BCG immunotherapy along with definitive therapy in various combinations. Therefore this study has been under taken with the aim and objectives. 1. To study of immunostimulatory effect of BCG in cases of Hodgkins lymphoma. 2. To evaluate immunotherapy of HD in correlation with clinical stages of disease.

Key words: (HI) Hodgkin’s lymphoma; BCG (Bacillus calmette Guerin).

Introduction
BCG is (1921) vaccine used for immunisation of person for protection from tuberculosis. Lymphoma is considered as the malignant tumour of immune system, which develops in the lymphoid system of an individual.

In lymphoma lymphonodes are enlarged. This enlargement is usually painless discreate and firm, and patient has tiredness, loss of weight, fever and sweating. The disease starts when certain type of lymphoid cells begin to divide and multiply abnormally in the lymphnodes. Such cells may then spread from one lymphnode to another throughout the body. The lymphoma can be broadly viewed of two types such as Hodgkins disease (HD) and Non Hodgkins lymphoma (NHL) Hodgkins disease is a lymphoproliferative disorder associated with abnomalities in cellular immune function.

Aisenberg (1973) Cosidered hodgkins disease as a disorder of T lymphocyte function. Histologically in HD,Reed-Steinberg cells (Giant cells) are present. Cell mediated immunity (cmi) is the immunity in which T cells and macrophages play an important role against the infection.

For the inducion of CMI, living organism is necessary. When T cells are sensitised by living organism they produce different products called “lymphokines.” These product are responsible for the manifestation of CMI. In HD generally cell mediated immunity is suppressed. Mukhopadhay et al (1987) evaluated T lymphocyte disfunction in HD patients and reported T cells disfuction irrespective of the stage and grade of diseases. As it is evident from literature that HD is disfunction of cell mediated immune response.
where by predominant cell type affected is T cell.

BCG stimulates the immune response both specifically as well as none specifically through T cells and macrophages respectively.

T cell produce lymphokines which are regulatory proteins whose main function appears to be growth and functional activation of reticuloendothelial system. Activated macrophages are known to stimulate lymphocytes, natural killer cells (NK cells) and these participate in the induction of immune response (Best et al 1974 and Henney et al 1978).

After BCG vaccination macrophages are activated which are able to potentiate delayed type of hypersensitivity reaction (Floretin et al. 1976)

Macrophages can be activated none specifically by BCG and once activated can effect the non specific destruction of neoplastic cells in cancer patients (Freedman 1979). Macrophages are large (15-20 um) diameter motile cells distinguished by intense phagocytic activity having abundant lysosomes (cytoplasmic vesicles) filled with proteases, nucleases, lipases, phosphatases, lysozyme etc. Macrophages secrete proteolytic enzymes and various soluble factors that act on T cells. They are derived from bone marrow promonocytes which give rise to circulating blood monocyte. The second important cell type of CMI is T lymphocyte produced in the thymus and are called thymic lymphocyte or T cells (T dependant).

Third cell type whose main function in destruction of cancer cells is natural killer cells (NK cells) which are large lymphocyte containing Azurophilic granules in the cytoplasm and therefore they are called large granular lymphocytes (LGL). NK cells are capable of non specific killing in transformed target cells and tumor rejection. NK cells are present in the spleen and peripheral blood. In man they play important role in tumor immunity.

Mechanism of action of BCG

Sparks and breading (1974) described the mechanism of BCG induced tumor regression in following four steps.

1. Local non tumors specific delayed hypersensitivity type immunological reaction.
2. Systemic tumor specific immunoresponse.
3. Generalized non specific stimulation of entire RE system.
4. A local non immunological direct antitumor effect.

Cytotoxic T lymphocyte (CTLS or T killer) lyse target cells. CTLSR are elicited in vivo by “stimulator” cell i.e. any cell that carries new surface antigen (neo antigen)

Mechanism of lysis by BCG

CTLS have surface lyt-2 and lyt-3 glycoprotein, but little on non lymphocyte 1=1 (because lyt-2 and lyt-3 are always co expressed) therefore called ly2, 3 cells.

Contact between CTL and target cells is regulated for this lysis.

Material and methods

Studies pertaining to changes of immune status in the cancer patients of Hodgkin's lymphoma in patients of cancer hospital and Research institute Gwalior and Jaya Arogya hospital Gwalior. Diagnosed cases of Hodgkin’s disease

Before therapy -35 cases
After therapy -35 cases

A record of clinical history of HD patients including age, sex, clinical staging of disease, personal habits like alcoholic, tobacco, chewing, smoking etc. Were taken from record section. Each patient was given 0.1ml of heat stable freeze dried, live BCG vaccine. Blood has collected after 21-28 days. Patients of HD were further divided according to stage wise stage I, stage II, stage III, & stage IV,

Farther study of cellular immune response, two parameters was used.
1. Total and differential leukocyte count.
2. T lymphocyte count.
Lymphocyte Separation

Five ml of venous blood was collected in heparinised tube and diluted with equal volume of RPMI 1640 medium. This diluted blood was layered over 5ml of Ficell solution in a sterile centrifuge tube and spun at 1750 rpm for 120 min.

Leucocyte rich interface was collected and washed thrice with RPMI 1640 at 3000 rpm for 30 min each. The washed cells were suspended in 1ml of RPMI 1640 containing 10% foetal calf serum (FCS).

We used, RPMI 1640 medium and collected sheep RBC, and phosphate buffer saline (PBS).

Determination of T cells by e-(weir-1978).

Two ml of 50% suspension in Alsevies solution of washed SRBC was once again washed with normal saline and resuspended in PBS. To one ml of this solution 4 volumes of AET solution (.402g of s2- Amino ethylisothiouronium hydrobromide (sigma USA) dissolved in 10ml of distilled water and the PH was adjusted to 9.0 with 4 N NaOH was added. Mixed well and incubated at 37°C for 15 minutes. The cells were washed three times in normal saline, once in PBS and resuspended in 7ml of BPS and 2ml of foetal cold serum to give a final 10% suspension of E (AET) solution. This solution could be stored at 4°C for up to 1 week.

To.05ml of lymphocyte suspension (2x10⁶ cell/ ml) fetal calf serum was added giving approximately 8 red cells per one lymphocyte and mixed thoroughly.

This was incubated over night at 4°C for maximum rosette formation. The pellet was resuspended gently with the help of Pasteur pipette and slide was made. Slides were air-dried and fixed in methanol for 15-30 minutes. The staining was done with May- Grunwald Gilemsa stain. All lymphocytes having three or more cells bound on to it were counted as rosette. Total and differential leukocyte count: - this count was done by usual method.

Statistical analysis:- Data were analyzed using various statistical formulae and expressed as mean + SE (x+ SE) statistical significance was determined using paired and un paired “t” test.

Results

The study was carried out in 35 HD patients the observations were made by comparing mean values of each parameter i.e TLC DLC, &rosetting.

After BCG therapy stage II HD cases, total cell count was not much affected after BCG therapy. Stage III patients indicated no changes, while stage IV patient cell count was significantly increased as compared to control. Pooled data of total peripheral leucocyte count did not show much changes before and after BCG therapy in HD cases. Polymorph of HD patients showed significant changes after BCG therapy (p<0.01) in HD patient of stage II and III. Lymphocyte count is also increases after BCG therapy.

Discussion

The present study was undertaken to delineate the immunostimulatory effect of BCG therapy in HD patients. In this lymphomas “Reed Steinberg” cells are seen. The Parameters are total leucocyte count, differential leucocyte count (polymorphs, monocytes, lymphocytes, & eosinophils) and absolute lymphocyte count, percentage of erythrocyte rosetting T cells. Studies were carried out stage wise also. In this study we observed an altered total leucocyte count in stage II and III. While stage IV patients were found to have increased leucocyte count.

Posner et al 1981 considered the total cell count to be an important parameter but could not be useful only when analysed in stage wise grouped patients. In stage II cases indicated significant increase in differential leucocytes count which recovered on BCG therapy.

Impaired cell mediated immunity is present in HD patients (Miller(1962) where lymphocytopenia is not considered to be responsible, there appear to be some specific alteration in the type of lymphocyte. Pooled data also reported similar decrease in “E” resotting, T cells and beneficial effects of BCG therapy. In stage II, III and IV of HD a decrease of lymphocyte count was observed and BCG was
Diagram: The Lytic cyclic in the attack of a cytokine T lymphocyte (CTL) on a target cell

After therapy stage IV patients show no significant values. In HD patients eosinophils were also altered after therapy.

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<th>Before</th>
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<tr>
<td>HD</td>
<td>Numbered= 35</td>
<td>29.4+ 1.3</td>
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<tr>
<td>HD</td>
<td>Numbered. 35</td>
<td>2.77+ 0.29</td>
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After therapy stage IV patients of HD showed beneficial effect on monocyte count.

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<tr>
<td>HD</td>
<td>Numbered 5) stage IV</td>
<td>1.6+0.4</td>
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Absolute lymphocyte count.

In stage III (HD patients) cell count increases significantly. After BCG therapy absolute count was increased significantly about near normal count (P<0.01).

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<tr>
<td>HD</td>
<td>(Numbered-35)</td>
<td>1818.62+116</td>
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StageIII

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<tbody>
<tr>
<td>HD</td>
<td>Numbered=13</td>
<td>1784.4+167.3</td>
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StageII

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Pooled values indicated significant decrease percentage of E rosetting T cell, in HD cases P(0.001). It helped restoration of near normal.

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<td>Numbered 35</td>
<td>56.5+1.9</td>
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In HD cases significant decrease in E - rosetting cells followed by beneficial increase after BCG vaccination. It was visible in stage II, III and IV cases. In stage II effect was marginal (P<0.05) but in stage III it was significant (P<0.01) when complained before and after therapy.

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<td>Stage II</td>
<td>54.17+3.2</td>
<td>63.41+ 0.81</td>
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<tr>
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<td>66. +1.1</td>
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<tr>
<td>Stage IV</td>
<td>54.4+6.5</td>
<td>57.2+6.6</td>
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<td>HD 5</td>
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The lytic cycle in the attack of a cytotoxin T lymphocyte (ctl) on a target cell.

found to be increasing the lymphocyte count in these patients.

(1) Stage IV shows significant- changes.
(2) Specific lymphocyte count increases after therapy in stage wise as well as in pooled data.
(3) Stage III HD cases indicated significant decrease in eosinophil count. BCG was found to restore this count near normal values indicating beneficial effects.
(4) Monocyte count in stage IV cases was decreased, where BCG vaccination indicated marginal change.
(5) Absolute lymphocyte count decreased significantly in HD cases, where BCG vaccination showed beneficial changes.
(6) Erosetting T cells decreased significantly in HD patient where BCG vaccination showed beneficial effect. The analysis of pooled data and stage wise data support usefulness of BCG in these patients.

References


9. Sparks FC, Breeding JH. Tumor regression and enhancement resulting from immunotherapy with Bacillus Calmette Guerin and neuraminidase. Cancer Res 1974; 34: 3262-3269