

Original Research Article

Immunological monitoring of brain tumour patients

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ABSTRACT

Background: Patient suffering from CNS tumours are among the best suited as regards the study of their immunologic status is concerned because these tumours rarely metastasize and general condition of patient is not much affected. Extensive research has been done on immunological response in neoplasms of other organs, but immunology of CNS tumours studied mainly during last five decades. It is now realized that immunologic reactions may be important in the development and growth of the CNS tumours. Although there is evidence that immunotherapy is helpful in control of some solid tumours but adequate knowledge of the immunology of glial tumours to guide the rational treatment is not yet available.

Methods: This study was conducted on 60 cases that included 20 controls and 40 patients of primary intracranial brain tumors admitted to neurosurgical services of University Hospital, Banaras Hindu University, Varanasi during the period of January 1987 to January 1988.

Results: The study revealed, medulloblastoma and glioblastoma or anaplastic astrocytoma show more marked suppression of cell mediated immunity than astrocytoma grade +II and other malignant tumour subgroups. In case of humoral immune response, antigen of brain tumours elicit an Ig M response rather IgG response which is commonly elicited in other neoplasia.

Conclusions: The results regarding Ig M, and Ig G levels are in agreement with most of the other studies. It appears that antigen of brain tumours elicits an Ig M response rather IgG response which is commonly elicited in other neoplasia.

Keywords: Brain tumor, Glioblastoma multiforme, Medulloblastoma

INTRODUCTION

Patient suffering from CNS tumours are among the best suited as regards the study of their immunologic status is concerned because these tumours rarely metastasize and general condition of patient is not much affected. Extensive research has been done on immunological response in neoplasms of other organs, but immunology of CNS tumours studied mainly during last five decades. It is now realized that immunologic reactions may be

important in the development and growth of the CNS tumours. Although there is evidence that immunotherapy is helpful in control of some solid tumours but adequate knowledge of the immunology of glial tumours to guide the rational treatment is not yet available. CNS tumours constitute about 3-5 percent of total solid tumours in humans.

The concept of immunologic privilege and existence of blood brain barrier makes brain tumours different from other neoplasms of the body.¹⁻³ Malignant brain tumours

have a short survival time there by preclude any long term follow up.

Most of the workers have reported that immunological activity, chiefly cellular immunity is suppressed in brain tumour patients, whereas the immunological status of those patients showing good recovery is usually normal.⁴⁻¹⁰ Monoclonal antibodies are a new biological probe for the evaluation of primary brain tumours. Since their development they have virtually revolutionized immunology.

Although at an early stage of development, their application to neuroimmunology and neuro-oncology has already resulted in a new awareness of the complex relationship that exist within CNS. With creative use, their specificity and reproductivity can provide the means to qualitatively and quantitatively define the phenotypic heterogeneity of malignant gliomas.¹¹

Recently experimental studies are being carried out to detect human glioma associated antigens by rat monoclonal antibody, raised against synergeic rat glioma cells.¹² The results of surgery, radiotherapy and chemotherapy are depressing hence there is a need to explore other possible and probable modes of treatment. Research on immunobiology may suggest ways and means of improving the depressed immune status of the patients with better quality of survival.

Furthermore, they may suggest the possible etiopathogenesis and may help in assessing the results of various modes of therapies. The present work comprises of a study of cell mediated and humoral immune responses in both benign and malignant brain tumour cases.

METHODS

This study was conducted on 60 cases that included 20 controls and 40 patients of primary intracranial brain tumors admitted to neurosurgical services of University Hospital, Banaras Hindu University, Varanasi during the period of January 1987 to January 1988. The control group consisted of age and sex matched patients relative or attendants who came along with the patients. The patients included in the study had not been treated by any modality and were not receiving steroids. In all cases diagnosis was confirmed by histopathological examination. A thorough clinical and neurological examination was done. Investigations were carried out in the department of pathology and radiology, Institute of Medical Sciences, Varanasi.

Investigations

Routine investigations

Hemoglobin, Total and differentiated blood counts, erythrocyte sedimentation rate, blood sugar, blood urea,

total protein, serum electrolyte and absolute lymphocyte count in peripheral blood.

Radiological investigations

Radiological investigations were done with a view to diagnose and localise the lesions and included plain X-ray skull AP lateral and special views where necessary. Angiograms (carotid or vertebral), ventriculograms, pneumocephalogram CAT scanning and X-ray chest.

Histopathological confirmation of diagnosis was carried out in all the cases.

Immunological investigations

These were done on the day of admission or within a day or two before steroid administration.

1) Cell mediated immunity

- Absolute lymphocyte count (ALC)
- Cutaneous delayed type hypersensitivity (DTH): Response to 2,4 Di nitro chlorobenzene (DNCB), Candida albicans antigen, Purified protein derivative (PPD) antigen
- T cell rosette percentage.

2) Humoral immunity

Serum immunoglobulins IgG, IgA and IgM levels were estimated by single radial diffusion technique using monospecific antisera.

Investigations for cell mediated immunity

1) Absolute lymphocyte count (ALC)

This was calculated from total and differential leucocyte counts as follows.

$$ALC = (\text{Total leucocyte count} \times \text{Lymphocyte count})/100$$

Total count was done by the Neubauer Chamber.

Differential leucocyte count was done by staining peripheral blood smears with Leishmann stain and counting hundred WBCs in oil emersion field.

2) T-Cell Rosetter count

- Material: Purified lymphocytes from peripheral blood, Sheep red blood cells (SRBC)
- Reagents: Ficoll F-P (Sigma, USA), Heparin-preservative free (Microlab, Bombay India), Hanks balanced salt solution (HBSS) (Sigma, USA), Tissue culture medium RPMI 1640 fortified with fetal calf serum, Alserver's solution, Tryptan Blue and methylene blue stains, Fuchs Rosenthal WBC counting chamber.

Procedure

Purified viable lymphocyte suspension

- 6ml of fresh heparinized blood was collected
- Blood gently layered along the wall of a centrifuge tube with a Pasteur pipette over 3ml of Ficoll Isopaque.
- The above is centrifuged at room temperature for 40 minutes at 1600 rpm. The interphase layer containing lymphocytes removed with a Pasteur pipette into another centrifuge tube.
- The above is washed twice with Hanks BSS (pH 7.2) at 1000 rpm for 10 minutes at 40°C.
- Final washing done with RPMT 1640 (pH 7.2 at 1000rpm for 10minutes at 40C and resuspended in RPMT 1640 tissue culture medium to obtain a concentration of 2*10⁶ cells per ml after testing viability with 1% Tryptan blur dye exclusion test.

Sheep red blood cells

- SRBC collected in equal volume of Alsever’s solution and kept in a refrigerator at 40°C. The cells can be used up to 10 days after collection.
- For use, Alsever’s solution pipetted off and SRBC washed twice in Hanks BSS at 1000rpm for 10minutes at 40°C. A third washing with RPMT 1640 given. One ml of packed SRBC are suspended in 10ml of RPMT 1640.
- Finally, a 0.5% suspension of SRBC in RPMT 1640 is made by further diluting the above suspension to 1:20 with RPMT 1640.

Rosetting

- 0.2ml of washed 0.5% suspension of SRBC taken and mixed with 0.2ml of purified suspension of viable lymphocytes (2*10⁶ cell/ml) and to this 0.05 ml of foetal calf serum (FCS) added.
 - The above mixture is incubated at 370C for 15 minutes
 - The mixture is then centrifuged at 700 rpm for 15 minutes and kept at 40C for 2 hours.
 - The above is then shaken gently and 0.1 ml of 25% gluteraldehyde solution is added and the tubes are left on ice for 5 minutes.
 - One drop of methylene blue (0.02 % in normal saline) added to the above to stain the nucleus of lymphocytes.
 - Rosettes counted in a minimum of 200 lymphocytes and the percentage calculated.
 - A true rosette comprises of three or more SRBC clustered around a lymphocyte.
- 3) Contact sensitization to 2:4 dinitro chloro benzene (dncb)¹³

Pure DNCB was dissolved in acetone to form a solution of 20000 µg/0.1ml. A fresh solution was prepared each time and application was completed within one hour of preparation. Sensitization was carried out by percutaneous application of 2000 µg (0.1ml) of DNCB to an area on the volar aspect of the right forearm confined by a plastic ring of 2.2 cms in diameter. The solvent was allowed to evaporate, and the site was covered with a dry gauze piece dressing and the patient instructed to keep the dressing clean and dry. The dressing was removed at 48 hours and the test site examined for evidence of an inflammatory response. The patients were challenged 10 days later with 0.1ml (100 µg/0.1 ml) of DNCB. This was also prepared and applied in the same manner but at different site. Higher dose of DNCB was avoided so as to minimise the risk of its irritant effects. The reactivity was quantitated as given in Table 1.

Table 1: Quantitation of reactivity to DNCB.

Grade	Response
0	No eyethema or induration
+	Erethema only
++	Erythema and induration
+++	Erythema, induration and miniature blisters
++++	Erythema, induration and large blisters or ulcerations

0-Indicates anergy, + and ++ Indicates a weak response (Impaired cell mediated immunity), +++ and ++++ Indicate a strong response (Adequate cell mediated immunity)

Delayed hypersensitivity response to recall antigens ppd and candida.^{14,15} The volar aspect of forearm was cleaned with spirit. 0.1ml of PPD (10 T.U./0.1ml) and 0.1 ml of *Candida albicans* (1:100 dilution of dermatophytin O) antigens were injected intradermally with a tuberculin syringe. The test sites were marked with ball point pen and left uncovered.

The patient was instructed not to scratch the area. The sites were inspected at 24 and 48 hours. The diameter of induration was recorded at 48 hours. Induration less than 10 mm was taken as negative.

Estimation of immunoglobulins by cell division method

5 ml of blood was collected by a sterile syringe. The serum was separated and kept at 40 C after adding sodium azide to prevent the bacterial contamination.¹⁶

Reagents

- Stock solution of 0.45 M Potassium phosphate buffer: pH 8.0 with 0.01 M sodium azide, K₂HPO₄ 0.45 M-950 ml of 78.3 gm / litre, KH₂PO₄ 0.45 M-50 ml of 12.5 gm/litre, 0.01 M Sodium azide 0.650 gm.
- 3% Diclo noble agar made in phosphate buffered saline (PBS) (0.034 M) potassium phosphate buffer pH 8.0, 1 part in 14.5 parts of normal saline.
- Slides cleaned and sterile.

Method

Preparation of agar solution-Difco agar 3 gm was added to 97ml of phosphate buffered saline pH 8.6. It was heated with continuous stirring till the solution became clear. Preparation of antiserum and immune-diffusion plate antisera (Behringwerke AG Marburg, Hoechst) was diluted in the PBS as shown in Table 1 and the equal quantity of the diluted antisera (3.2ml) was mixed in 3% agar solution (3.2ml) to give a total of 6.4ml solution. The solution was heated in a water bath at 560 till it became clear. Then it was poured on a slide by single fluid motion. The plate was kept overnight at 40°C. Placement of antigens in plate. The sliders were placed under a template and 5 rows of 6 wells were cut on each slide. The diameter of each well was 2mm and each well was separated by 14mm centre to centre. 0.002 ml of diluted serum was placed in each well and the last 3 wells were filed by 3 dilutions of standard sera (WHO immunoglobulin ref no 67/97).

Dilutions of standards and samples were quantitated as given in Table 2 and 3.

Table 2: Dilution of standard sera and test samples.

Antisera	Standard sera and dilutions	Test sample dilution
IgG	1:10, 1:20, 1:40	1:20
IgA	1:2, 1:4, 1:8	1:4
IgM	1:1, 1:2, 1:4	1:2

Table 3: Incubation of Immunodiffusion plates.

Anti sera	Diffusion time hours	Temperature
IgG	24	4°C
IgA	48	4°C
IgM	72	4°C

Readings and calculations

After the incubation the diameter of the diffusion ring was measured with the help of a divider against a black background. The squares of the diameter were noted, and standard curve was plotted for each class of immunoglobulin concentration and the result was calculated as under:

Protein concentration from the graph: X mg/dl, Diffusion factor: Y, Immunoglobulin concentration: X×Y mg/dl.

RESULTS

The present study comprised of a total of 60 cases of which 10 belonged to benign brain tumours (16.66%), 30 to malignant tumours (50%), 20 to sex, age and socioeconomically matched controls (33.33%). These cases reported to neurosurgery unit of university hospital, Banaras Hindu University, Varanasi from January 1987 to January 1988. Maximum cases were in malignant brain

tumours, followed by control group. The most common tumor in benign group was meningioma (40%) followed by acoustic neuroma (30%).

The astrocytoma (Grade I+II) was the commonest tumor (43.33%) followed by glioblastoma/anaplastic astrocytoma (23.33%) and medulloblastoma (10%). Rest of the varieties contributed to single tumor each except oligodendroglioma which contributed (6.66%). The most common age group affected by benign and malignant tumors was 31-40 years (30% of cases). No benign tumor was seen below the age of 10 years, whereas the tumors were equally distributed (20%) in each decade. Males dominated in both benign (60%) and malignant groups (63.33%) in comparison to females. However, same was observed in controls (70% males). This clearly shows that little significance can be given to the preponderance of brain tumors in males as more of male patients reported to the hospital in comparison to females.

For sensitizing dose, 70% of benign and 40% of malignant tumor cases showed a positive response in contrast to 90% of controls. While for challenge dose positive cutaneous DTH response was seen in 80% of benign and 70% of malignant tumor group in comparison to 95% of controls. For sensitization dose, 100% medulloblastoma cases showed energy, followed by glioblastoma (57.14%), other malignant tumors (57.14%) and astrocytoma grade I+II (53.84%). For challenge dose, again medulloblastoma shown total energy (100%) followed by glioblastoma (28.57%), astrocytoma grade I+II (23.76%) and other malignant tumors (14.285%). In contrast to 25% of controls, 50% benign and 60% malignant tumor cases shown negative response to PPD antigen. So, it is evident that response to recall PPD antigen was suppressed in both benign and malignant tumor groups in comparison to controls. In contrast to negative response in 30% of controls, 60% of benign tumor cases and 66.66% of malignant tumor cases failed to respond to candida recall antigen. The difference between benign and malignant groups was not significant. When considered together the response to PPD and candida antigens, 30% of benign and 40% of malignant tumor cases showed anergy in contrast to absence of anergy in controls. When negative response to recall antigen PPD and candida was considered together it was seen that 33.33% medulloblastoma, 38.46% astrocytoma grade I+II, 85.71% glioblastoma and none in other malignant tumor subgroup shown negative response in contrast to none (0%) case in controls. It was observed that taking in consideration, response to all the three antigens; 2:4 DNCB, candida and PPD, 1(10%) case of benign and 4(13.33%) cases of malignant tumor group showed negative response in contrast to none (0%) of the controls. T cell rosette count was depressed significantly in both benign and malignant tumor groups in comparison to controls. T-cell rosette count was highly significantly depressed (p<0.001) both in astrocytoma grade I+II and glioblastoma/anaplastic astrocytoma compared to controls. It was depressed (p<0.01) both in

medulloblastoma and other malignant tumor group, but to a lesser extent (Figure 1).

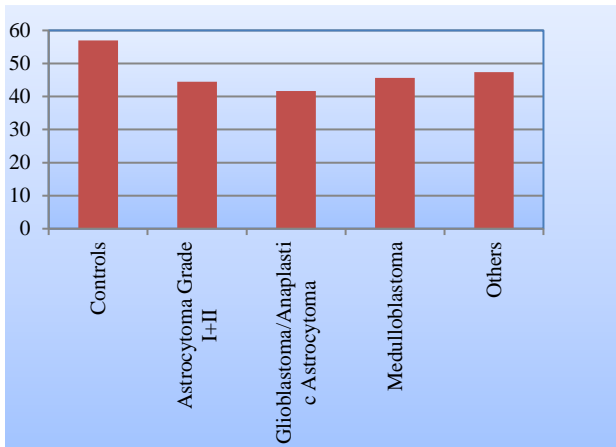


Figure 1: T-cell rosette count in malignant brain tumour subgroups.

In malignant tumor cases, absolute lymphocyte count was highly significantly reduced in comparison to both benign brain tumor cases and controls.

Serum IgG levels did not differ significantly in controls, benign brain tumor cases and malignant tumor cases.

Serum IgA levels were significantly depressed in the benign and malignant brain tumor groups in comparison to controls. Serum IgM levels were significantly increased both in benign and malignant tumor cases in comparison to controls. There was no significant difference between benign and malignant groups.

ALC was highly significantly reduced in the malignant tumor group, where as T cell rosette count was depressed in both benign and malignant brain tumor cases ($p < 0.05$). Malignant group showed positive DTH response to 2:4DNCB in 70% in comparison of 80% benign and 95% controls. Similarly, for recall antigen PPD and candida malignant group showed positive response in 40% and 33.33% respectively. It is obvious from above observation that cell mediated immunity was overall depressed in both benign and malignant brain tumor cases. The depression was more marked in malignant tumor cases in comparison to benign tumor cases (Table 4).

Table 4: Cell mediated immunity in brain tumor cases and controls.

	No of cases	ALC (/cu.mm) ±SD	T cell rosette count % ± SD	Positive response to DNCB No. %	Positive response to PPD No. %	Positive response to candida No. %
Control	20	2658.3±507.438	57±6.121	19(95)	15(75)	14(70)
Benign	10	2449.6±870.177 P<0.3	47.1±8.06 P<0.05	8(80)	5(50)	10(40)
Malignant	30	1578.33±418.511 P<0.001	44.63±7.416 P<0.05	21(70)	12(40)	10(33.33)

Table 5: Humoral immunity in brain tumor cases and controls.

	No of cases	IgG levels (mg/dl) ± SD	IgA levels (mg/dl) ±SD	IgM levels (mg/dl) ±SD
Control	20	1443±179.856	246.6±58.244	154.5±35.758
Benign	10	1460±222.111	196±72.295, P<0.01	228±59.217, P<0.001
Malignant	30	1303.33±343.394	200.33±73.036, P<0.01	202.66±59.765, P<0.001

Comparing cell mediated immunity in various malignant subgroups it was observed that except for DNCB response all other parameters were maximum affected in glioblastoma/anaplastic astrocytoma subgroup. Astrocytoma grade I+II ranks second in the list. Medulloblastoma subgroup except for total anergy to DNCB comes next and other malignant tumors in the last in order for depression of cell mediated immunity. (Table 6). Analysis of serum immunoglobulin levels revealed

significantly low serum IgA levels in astrocytoma grade I+II and medulloblastoma. This was associated with significantly high serum IgM levels in these tumor subgroups. In all other malignant brain tumor subgroups, serum immunoglobulin levels did not show any significant change. It is difficult to assign any significance to fall in serum IgA levels, though rise in IgM level can easily be explained by antigenic stimulus provided by tumor antigens (Table 7).

Table 6: Cell mediated immunity in malignant tumor subgroups and controls.

	No of cases	ALC (/cu.mm) ± SD	T cell rosette count % ± SD	Negative response to DNCB, No.%	Negative response to recall antigen PPD and candida
Controls	20	2658.3±507.438	57±6.121	1(5)	0(0)
Astrocytoma Grade I+II	13	1530.714±281.335 P<0.001	44.46±7.03 P<0.001	3(23.76)	5(38.56)
Glioblastoma/Anaplastic Astrocytoma	7	1704.615±458.108 P<0.001	41.71±7.60 P<0.001	2(28.57)	6(85.71)
Medulloblastoma	3	1337.33±83.362 P<0.001	45.66±4.04 P<0.01	3(100)	1(33.33)
Others	7	1494.714±520.494 P<0.001	47.42±9.16 P<0.01	1(14.28)	0(0)

Table 7: Humoral immunity in malignant brain tumor subgroups and controls.

	No of cases	IgG(mg/dl)	IgA(mg/dl)	IgM(mg/dl)
Controls	20	1443±179.856	246.5±58.244	154.5±35.758
Astrocytoma Grade I+II	13	1346.1538±349.67	177.692±48.158, P<0.01	227.692±65.083, P<0.001
Glioblastoma/Anaplastic Astrocytoma	7	1328.571±391.42	224.285±124.88, P>0.68	182.857±58.227, P>0.1
Medulloblastoma	3	1216.666±425.245	163.33±25.166, P<0.05	226.66±50.332, P<0.01
Others	7	1150±276.8874	234.285±39.09, P>0.7	165.714±29.358, P>0.5

DISCUSSION

The present study was undertaken to evaluate general cell mediated and humoral response of the patient suffering from benign and malignant brain tumours. Our observations showed that suppression of various parameters of cell mediated immunity both in vivo and invitro more or less paralleled the grade of malignancy.

Part I

Response to 2:4 DNCB in brain tumour cases

Thirty percent of malignant brain tumour cases, and 20% of benign brain tumour cases showed anergy to the challenged dose of 2: 4 DNCB compared to only 5% cases in the control group, showing there by marked depression of cell mediated immunity in the brain tumour group. Analysing the data in the malignant tumour subgroups, all patients of medulloblastoma (100%) were anergic, where as 28.57% of glioblastoma / anaplastic astrocytoma were anergic and anergy was seen only in 23.76% cases of astrocytoma grade I+II and in 14.28% cases of other malignant tumour subgroups.

This clearly brings out that the response to 2:4 DNCB directly correlated with the grade of malignancy. Maximum suppression being in medulloblastoma, followed by glioblastoma / anaplastic astrocytoma, next being astrocytoma grade I+II, and least being in the other malignant tumours. Brooks As early as 1972 tested 21

brain tumour cases and control of these 9 were benign and 12 were malignant brain tumour patients⁴. In their study, marked suppression to 2:4 DNCB response was observed in the brain tumour cases, where in the malignant group 25% cases, in benign group 22.22% cases and in the control group 100% cases could be sensitized.

In another study on 20 primary glial tumours noted that only 35 % of the primary glial tumours gave a positive response to 2:4 DNCB as compared to 100% controls.¹⁷ Sobue studying malignant brain tumours, observed good correlation between suppression of response to 2:4 DNCB and malignancy.¹⁸ Young in a study on glioblastoma cases, noted that lymphocytes from 50% of the cases showed depression of the cell mediated immunity invitro, whereas the lymphocytes from benign astrocytoma failed to reveal any such depression.⁸ However, in our study cutaneous DTH response to 2:4 DNCB was suppressed (anergy) in 23.76 % cases of astrocytoma grade I+II compared to 5% cases in controls, showing thereby that cutaneous DTH response invitro is a more sensitive test to detect immune response.

Mahaley studied immuno biology of primary intracranial tumours and analysing the results of immunocompetence of brain tumour patients observed that some but not all patients with brain tumours have at the time of surgical diagnosis a significant degree of anergy of the cell mediated immune system which is proportionate to the degree of anaplasia of the neoplasm.¹⁹ Whereas in a similar study from our laboratory could not demonstrate

clear cut suppression of cell mediated immunity in significant number of brain tumour cases by response to 2:4 DNCB.¹⁰

However, the present study clearly brings out that 2:4 DNCB testing correlates well with the degree of malignancy in general and the degree of negative response directly correlates with the grade of anaplasia of the tumour.

Response to recall antigens

Anergy to recall antigen PPD and candida also correlated directly with condition of patient. Anergy was seen in 40% of cases in malignant tumour group, 30% of the benign, where it was absent in the control group. On further analysing the data in the malignant tumour subgroups, anergy to PPD and candida, correlated well with grade of anaplasia. It was 85.71% in glioblastoma or anaplastic astrocytoma, 38.46% in astrocytoma grade I+II and 33.33% in medulloblastoma, where as it was absent in other malignant tumour subgroup.

However, results of Recall antigen test differed from the response to 2:4 DNCB where 100% anergy was noted in medulloblastoma and 28.57% in the glioblastoma or anaplastic astrocytoma and in 14.28% cases of other malignant tumour subgroup. It appears that DNCB sensitization test is a more sensitive denominator of cell mediated immunity than Recall antigen test. It is difficult to assign significance to the variations seen with regard to glioblastoma and medulloblastoma. Brooks studied 20 primary glial tumours and found that response to PPD was positive in 5% glial tumour cases as opposed to 50% of controls.¹⁷

Mahaley and Brooks observed that PPD skin test was positive in 19% glioblastoma, 6% of anaplastic glioma, 24% of meningioma and 50% of control cases. Responders to two or more of 4 skin tests were 31% in glioblastoma, 50% in other anaplastic glioma and in 59% of meningioma cases when test preoperatively.¹⁹

In a study on 40 brain tumour cases reported positive response to PPD in 55% of benign tumour cases and 50% of malignant tumour cases, where it was positive in 80% of controls.¹⁰ However when results of both PPD and candida were analysed together, these authors found anergy in 25% benign tumour cases and in 20% of malignant tumour cases in contrast, in none of the controls.

Absolute lymphocyte count

Absolute lymphocyte count normally ranges between 2000 – 4000 cells / cubic mm. In the present study, mean control value was 2658.3±507.438. In the benign tumour group the values were within normal range but were significantly depressed in the malignant tumour groups. (p<0.001). Further analysis of data in the malignant

subgroups revealed highly significant reduction (p<0.001) in all the subgroups, ie astrocytoma grade I+II, glioblastoma or anaplastic astrocytoma, medulloblastoma and other malignant tumours. Thus, in the present study, ALC testing appeared to be a good indicator of the suppression of, cell mediated immunity in malignant brain tumour cases.

Mahaley also observed suppression of ALC (<1000 /cubic mm) in glioblastoma patients. The most significant lymphopenia was apparent in patients with glioblastoma multiforme, whereas patients with other tumour types tended to maintain higher although subnormal counts. Brooks have emphasised that long-term assessment of sequential estimation of ALC in primary brain tumour cases, preoperatively, postoperatively and during follow up, are of great value in predicting recurrence before even clinical presentation.⁸ Ramachandran from this laboratory also reported depression in ALC values in the malignant brain tumour cases.⁹

In a study of 40 primary brain tumours found a significant depression in the ALC value in both benign and malignant tumour groups, compared to controls.¹⁰

T cell rosette count

T cell rosette count was depressed significantly in both benign and malignant tumours (p value <0.05) cases (Figure 1). Further analysis of the data in the malignant subgroups, revealed a highly significant reduction (p<0.001) in T cell rosette count in astrocytoma grade I+II, and glioblastoma or anaplastic astrocytoma. However, in medulloblastoma and other malignant tumour subgroups though there was reduction in T cell count, it was less significant (p<0.01).

In the study by Gerosa, T cell mediated immunity and E A Rosette (T cell rosette) forming cells and mitogen induced blastogenesis test turn out to be markedly depressed in cases of primary supratentorial gliomas.⁵ Other workers have also found marked suppression of T cell rosette count in gliomas.^{4,8,10}

In the present study, all the parameters of cell mediated immunity such as cutaneous DTH response to contact sensitising agent 2:4 DNCB, cutaneous DTH response to Recall antigens, T cell rosette count and Absolute lymphocyte count in peripheral blood, were found to be significantly suppressed in malignant brain tumour cases compared to the controls. In the benign brain tumours also all these parameters except the ALC count were suppressed but to a lesser extent than in the malignant tumours. Analysing the data in malignant subgroups, it was observed that in the astrocytoma grade I+II anergy to 2:4 DNCB was present in 23.76% cases, to recall antigens was present in 38.46% cases, T cell rosette count was highly significantly depressed (p value <0.001) and absolute lymphocyte count was also highly significantly depressed (p value <0.001).

Glioblastoma or anaplastic astrocytoma revealed anergy to 2:4 DNCB in 28.57% cases, anergy to Recall antigens in 85.71% cases, highly significant suppression of T cell rosette count (p value < 0.001) and equally significant suppression of ALC (p value < 0.001). Medulloblastoma revealed 100% anergy to 2:4 DNCB, 33.33% anergy to recall antigens, significant fall in T cell rosette count (P value < 0.01) but highly significant fall in ALC (p value < 0.001). Other malignant tumour subgroups which included, oligodendroglioma, ependymoma, ganglioglioma, malignant neurilemmoma and meningioma sarcoma showed anergy to 2:4 DNCB in 14.28% cases, failed to show anergy to Recall antigens, T cell rosette count was moderately depressed (p value < 0.01) but ALC was highly significantly suppressed (p value < 0.001).

With these results it is difficult to attribute any direct correlation between the suppression of individual parameters of cell mediated immunity and anaplasticity of the tumour. However, medulloblastoma and glioblastoma or anaplastic astrocytoma do show more marked suppression of cell mediated immunity than astrocytoma grade +II and other malignant tumour subgroups.

Part II

Humoral Immunity estimation in various brain tumours and controls. In this study, serum IgG, IgM and IgA levels were estimated in 30 malignant, 10 benign brain tumour patients and in 20 sex, age and socio economically matched controls. In the present study IgG values were within normal range both in benign and malignant brain tumour cases.

In the study of Mahaley, it was found that Ig G levels were within normal range and there was no significant difference among value of Ig G levels in glioblastoma, other anaplastic glioma and meningioma.²⁰

Yokoyama noticed no decrease in serum immunoglobulins in brain tumour patients, but Tokomaru reported decrease in Ig G and Ig A levels in brain tumour cases but not significant.²¹ Shimizu also reported a decrease in serum immunoglobulin level in brain tumour patients.²² But earlier work done Sharma also revealed no significant difference in the value of Ig G in brain tumour cases and controls.¹⁰

It is interesting note that serum Ig G levels remain unaffected in presence of brain tumours both benign and malignant. Normally Ig G levels raise in response to any antigenic stimulus. Absence of raise in Ig G perhaps indicates that the tumour antigens in brain tumour cases be such as to stimulate only IgM response. It is well known that certain parasitic antigens like malaria and kala azar elicit only Ig M response.

Regarding serum Ig A levels, there was significant fall of levels both in benign and malignant tumour groups in

contrast to controls. But there was no significant difference between benign and malignant cases. Further, comparing various malignant subgroups with controls, Ig A levels were found to be depressed significantly in astrocytoma I+II (p value < 0.01) and medulloblastoma (p value < 0.05). The depression in other subgroups were not significant statistically. It is difficult to assign any reason to this observation.

Mahaley did not find any change in serum Ig A levels in brain tumour cases, whereas Tokomaru and Shimizu reported a decrease in serum IgA levels in brain tumour cases. Yokoyama observed depressed Ig A level in 1 out of 39 patients of brain tumour.²⁰⁻²²

In our study, Ig M levels were raised significantly in both benign and malignant tumour cases (p value < 0.001) in contrast to controls. But there was no significant difference between benign and malignant group. On further analysis of malignant subgroups, it was seen that IgM levels were significantly raised in astrocytoma grade I + II and medulloblastoma in comparison to controls.

Raised Ig M levels were also reported by Mahaley in glioblastoma patients. In a study by Yokoyama there was no significant change in the values of Ig M levels.^{20,21} Sharma also found elevated Ig M levels, significantly in both benign and malignant tumour cases.¹⁰ Tokomaru also found raised Ig M levels in meningioma's. In study on 40 brain tumour patients (20 benign and 20 malignant) Tiwari observed a significant raise in the IgM levels in benign brain tumour cases.

So, present results regarding Ig M, and Ig G levels are in agreement with most of the other studies. It appears that antigen of brain tumours elicit an Ig M response rather IgG response which is commonly elicited in other neoplasia.

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