EVALUATION OF CUTANEOUS DTH RESPONSE TO 2:4 DNCB, RECALL ANTIGENS-CANDIDA AND PPD AND SERUM IMMUNOGLOBULINS IN MALIGNANT AND BENIGN OVARIAN TUMOURS

by

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SUMMARY

Forty-five patients of ovarian neoplasia (11 of malignant ovarian tumour and 34 of benign neoplasia) were investigated alongwith sixteen control cases for assessment of cell-mediated immunity by cutaneous DTH response to recall antigens and 2 : 4 DNCB. It was observed that response to PPD and 2 : 4 DNCB was significantly suppressed in ovarian neoplasia and it correlated well with the clinical stages of the ovarian malignancies. Furthermore, there was definite enhancement of response to 2 : 4 DNCB in two-third cases of malignant ovarian tumour after six weeks of surgical operation. It was propounded that evaluation of cell-mediated immune-response could be employed as a useful prognostic tool in following up patients undergoing surgical resection. Patients not eliciting improvement in cutaneous DTH response after surgery alone probably need radiation or chemotherapy or a combination of both.

Introduction

The ovary is the third most common site for primary malignancy of genital tract. Women of menopausal age group show highest incidence of ovarian neoplasia. Frequency of ovarian tumours recorded at the hospital based cancer registry of department of pathology from 1963 to 1973 is 8.02%.

Nalick *et al* (1974) reported highly significant relationship between cellular immunity and clinical course of patients suffering from gynaecological malignancies. Philips *et al* (1971) observed that tumour specific antigens were present both in carcinoma cervix and adenocarcinoma of ovary which elicited both humoral and cell-mediated immune response. Khoo and Mackay (1974); Michel (1978) reported impaired response to 2 : 4 DNCB and recall antigens in majority of patients with ovarian malignancies. Piver *et al* (1981) studied cutaneous DTH response in patients of ovarian malignancies treated

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with chemotherapy and immunotherapy but failed to demonstrate a correlation between response to chemotherapy and immunocompetence as demonstrated by delayed cutaneous hypersensitivity reaction.

The present study was undertaken to evaluate non-tumour specific cell-mediated-immunity and humoral immunity in patients suffering from ovarian neoplasia, both benign and malignant.

Material and Methods

The present study included 61 cases admitted to the obstetrics and Gynaecology ward of university hospital, B.H.U., Varanasi. Eleven patients with malignant ovarian tumours (2 in stage I, 3 in stage II, 2 in stage III and 4 in stage IV) and 34 patients with benign ovarian tumours (9 with pseudomucinous cystadenoma, 8 with serons cyst adenoma, 7 with teratoma, 3 each with cystic ovary and infarcted cyst, 2 with granulosa-theca cell tumour and one each with Brenner tumour and fibroma), all histologically proved. There were 16 control cases. Staging of carcinomaovary was carried out according to the International Federation of Gynaecology and Obstetrics adopted in 1964.

Routine investigations included a thorough history taking, clinical examination, haematological investigations, Serum protein studies and detailed histopathological study of surgically removed tumours.

Immunological investigations included study of CMI by evaluating :

(a) Cutaneous DTH response to —
  (i) 2 : 4 Dinitrochlorobenzene (DNCB) by the method adopted from Eliber *et al.*
  (ii) Recall antigens — PPD and Candida albicans by the method adopted from Nalick *et al.*
(b) Absolute lymphocyte count study of humoral immunity by quantitative estimation of serum IgG, IgM and IgA (Fahey 1968).

Observation

The present study comprised of 11 patients with malignant ovarian tumour and 34 with benign ovarian tumour and 16 age and sex matched control cases. The mean age of patients with malignant ovarian tumour was 43 years and 37 years for benign tumour patients.

Evaluation of CMI by Cutaneous DTH response to recall antigens-PPD and Candida albicans :

Diameter size of positive response to PPD in control cases ranged from 5 mm to 25 mm, whereas both in benign and malignant ovarian tumour groups, it varied from 3 mm to 10 mm. There was statistically significant reduction in the diameter of skin response in the malignant ovarian tumour group as compared to control ($P < 0.01$). A highly significant reduction ($P < .001$) in the mean diameter of positive response was noted in the benign tumour group vs. control group. But, the response did not statistically differ between malignant and beningn subgroup of ovarian tumours ($P < 0.06$) (Table I). However, evaluating response to candida albicans showed no significant alteration between control group and ovarian tumours (both benign and malignant) ($P < 0.2$ and < 0.7 respectively).

Evaluation of CMI by Cutaneous DTH response to 2 : 4 DNCB

Revealed that anergy was observed in 27 : 27% cases with malignant ovarian tumour but it was absent in benign neoplasia group and in control group. Detailed analysis of the malignant ovarian group showed 100% positive response in patients having malignant tumour in stage I and II and anergy was present in patients having

TABLE I

Cutaneous DTH Response to Recall Antigen PPD in Ovarian Tumours and Control

Classification		Range	Mean	S.D.	t	P
Malignant Ovarian tumours	11	3-10 mm	8.18 mm	1.96	3.9578	<0.01
Stage I	2	7-10	8.5	2.12	—	—
Stage II	3	4-8	6.0	2.00	—	—
Stage III	2	6-7	6.5	0.71	—	—
Stage IV	4	3-7	5.0	1.83	—	—
Benign Ovarian tumours	34	3-10	5.74	1.73	4.6870	<0.001
Cystic Ovary	3	5-9	6.33	2.98	—	—
Pseudomucinous Cystadenoma	9	4-9	6.22	1.48	4.38	<0.01
Serus Cystadenoma	8	3-7	5.38	1.19	4.76	<0.001
Granulosa and Theca Cell tumour	2	4-5	4.53	0.71	—	—
Teratoma	7	2-7	5.43	2.15	4.37	<0.001
Infarcted cyst	3	4-10	6.46	3.46	2.73	<0.02
Brenner's tumour	1	6	—	—	—	—
Fibroma	1	5	—	—	—	—
Control	16	5-25	12.8	5.95	—	—
Malignant vs. Benign Ovarian tumours	—	—	—	—	0.664	<0.6

TABLE II

Cutaneous DTH Response to DNCB in Patients With Ovarian Tumours and Control

Classification	Response to the challenge doses of DNCB after 48 hours (per cent positivity)					
	+	++	+++	++++	Anergy	Total + %
Malignant ovarian tumour	27.27	18.18	9.09	18.18	27.27	72.73
Stage I	—	—	—	100%	—	100
Stage II	33.33	33.33	33.33	—	—	100
Stage III	—	50	—	—	50	50
Stage IV	50	—	—	—	50	50
Bengin ovarian tumour	35.29	41.18	23.53	—	—	100
Cystic Ovary	—	66.67	33.33	—	—	100
Pseudomicinous Cystadenoma	—	77.78	22.22	—	—	100
Serous Cystadenoma	75	—	25	—	—	100
Granulosa and Theca Cell tumour	—	50	50	—	—	100
Teratoma	57.14	28.57	14.28	—	—	100
Infarcted Cyst	33.33	66.67	—	—	—	100
Brenner's tumour	100	—	—	—	—	100
Fibroma	—		100	—	—	100
Control	62.50	18.75	18.75	—	—	100

malignant tumour in stage III and IV (Table II). Comparative study of cutaneous DTH response to challange dose of DNCB in patients of malignant ovarian tumour before and six weeks after operation revealed that two-third cases showed enhanced response to challange dose of 2 : 4 DNCB whereas in benign tumour group, response to the challange dose of 2 : 4 DNCB after operation was not much altered (Table III).

Comparative Study of Immunoglobulins levels before and six Week after operation :

Elicited increased IgG and IgM level in two-third cases and IgA level in One-third cases of malignant ovarian tumour after operation. In the benign neoplasia group five-eighth cases showed rise in IgG and four-eighth cases showed rise in IgM and IgA level after operation (Table IV).

Discussion

Immunoglobulin quantitation after surgical resection showed a definite rise in levels in the patients with ovarian neoplasia probably indicating a release of anti-tumour antibody in circulation due to marked decrease in tumour load. It is well known that patient suffering from neoplasia produce tumour specific antibodies and in most cases of solid tumours these antibodies are injurious to the patients as they serve as enhancing antibodies.

Evaluation of cell medicated immunity revealed impaired response to PPD and 2 : 4 DNCB in ovarian malignancies

TABLE III

Comparative Study of Cutaneous DTH Response to Challenge Dose of DNCB in Patients of Ovarian Tumours Before and 6 Weeks After Operation

Classification	Serum No.	Histological	Before operation	After operation (6 weeks)
Malignant neoplasms	7180	Anaplastic Carcinoma (Stage III)	—	—
	6913	Endometroid Ovarian Carcinoma (Stage II)	+	++
	7147	Papillary Adeno-carcinoma (Stage IV)	+	++
Benign neoplasms	7536	Carpora Albicantes	++	++
	6895	Pseudomucinous cystadenoma	++	+++
	7077	Papillary mucinous cystadenoma	++	++
	7148	Teratoma	+++	+
	7286	Teratoma	+	+
	7231	Infarcted cyst	+	+
	7080	Fibroma	+++	++
	6736	Granulosa Cell Tumour	+++	+++

TABLE IV

Comparative Study of Serum Immunoglobulin IgG, IgA and IgM Alteration Before and After 6 Weeks of Surgical Treatment

Classification		Histological diagnosis	IgG before operation	IgG after operation	IgA before operation	IgA after operation	IgM before operation	IgM after operation
Malignant Neoplasms	7080	Anaplastic Ca (Stage III)	1600	1600	100	240	350	80
	6913	Endometroid Ovarian Ca (Stage II)	700	1600	200	110	80	300
	7147	Papillary adeno-carcinoma (Stage IV)	1600	2600	200	120	100	160
Benign Neoplasms	7536	Corpora albicantes	1200	1400	380	300	140	120
	6895	Pseudomucinous Cystadenoma	1400	1900	400	190	190	320
	7077	Papillarymucinous Cystadenoma	1350	1250	180	300	300	140
	7148	Teratoma	1000	2400	350	320	120	180
	7286	Teratoma	700	1250	280	300	90	140
	7231	Infarcted Cyst	1150	1200	250	200	160	160
	7080	Fibroma	1600	1500	140	200	320	140
	6736	Granulosa Cell Tumour	1400	1250	200	360	100	200

(Tables I and II). This is in complete agreement to the study conducted by Hughes and Mackey who observed suppression of tuberculin response in ovarian tumour compared to control group. Nalick *et al* also noted suppressed response to PPD in ovarian malignancies . Khoo (1974, 1978) observed increased incidence of anegry and impaired activity to 2 : 4 DNCB and PPD in patients with ovarian malignancies as compared to the control group and furthermore, they also observed progressive increase in the incidence of anergy and impaired response with worsening of clinical staging of tumour. They propounded that impairement of cell-mediated-immunity was an unfavourable factor in host tumour interaction. However, Michael and Levi (1971) observed that lymphocylotoxicity test did not always correspond to the clinical stage of the patient. Our follow-up study showed a good correlation between cutaneous DTH response to 2 : 4 DNCB and clinical staging of ovarian malignancies (Table II). Capacity to respond to the challange dose of DNCB improved in two-third cases of malignant ovarian tumour after operation (Table III). The present study therefore, conclude that evaluation of cell-mediated non-tumour specific immune response to recall antigens and 2 : 4 DNCB in patients with ovarian tumour correlates well with the clinical stage of the disease and could be employed as a useful prognostic tool in following up patients undergoing surgical resection. Patients not showing much improvement in cutaneous DTH response after surgery alone probably need radiation or chemotherapy or a combination of both.

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