IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 6 Ver. V (June. 2017), PP 111-115
www.iosrjournals.org

Role of Immunohistochemical Evaluation of D2- 40 in Differenciating Seminoma from Embryonal Carcinoma

*Dr.Debashis Roy Burman ,**Dr.Debkumar Ray
**Associate Professor ,Department Of Laboratory Oncology (Oncopathology) , Calcutta Medical College, Kolkata*
***Associate Professor, Department Of Biochemistry, Burdwan Medical College, Burdwan*

Abstract: *Differentiation of seminoma from embryonal carcinoma is essential because difference in treatment protocol. In most cases seminomas can be easily distinguished from embryonal carcinoma. In some cases, because of confusing histomorphology, such differentiation become difficult in routine Haematoxylene and eosin stained tissue section alone, and necessitates use of immunohistochemical analysis. Researchers used various immunohistochemical markers including Keratin antibodies ,combination of CD30,CD117(c-kit) to this end. D2-40 is a monoclonal antibody that reacts with an oncofetal membrane antigen that is present in fetal germ cell as well as neoplastic germ cell tumour of testis. Various Testicular Germ Cell neoplasm was evaluated with D2-40 and results werecompared with that of CD30,CD117(c-kit) Total 63 testicular germ cell tumours were evaluated.23 pure seminomas,3 pure embryonal carcinomas,3 pure yolk sac tumours,3 teratoms and 28 Mixed Germ Cell Tumours were among them. All cases of pure seminoma and seminomatous component of Mixed Germ Cell Tumours were stained with D2-40. 8 embryonal carcinoma component of Mixed Germ Cell Tumoursalso expressed positivity with D2-40. Whereas a uniform membrane pattern of staining was observed in seminomas, D2-40 positivity in embryonal carcinomas was focal and was concentrated at the luminal surface of the neoplastic cells. 22 pure seminoma and 13 seminomatous component of Mixed Germ Cell Tumours was positive for CD117(c-kit).2 embryonal carcinoma component of Mixed Germ Cell Tumour also revealed positive reaction .CD 117(c-kit) was positive in2 embryonal carcinomas. CD 30 was positive in 2 pure embryonal carcinoma and 20 embryonal carcinoma compoment of Mixed Germ Cell Tumour.CD 30 positivity was observed in seminomatous component in 2 out of total 37 cases.*
D2-40 is highly sensitive marker for seminoma and can be effective in distinguishing seminoma from embryonal carcinomas because differential expressions in these two tumor type.
Keywords: *Embryonal carcinoma, Seminoma,D2-40,CD117(c-kit),CD 30*

I. Introduction

Germ cell tumours are the most common malignant neoplasm of the testis.Seminoma constitutes 35-50% of germ cell Tumours(1) . Usually seminomoutus cell are easily recognized with routine haematoxylene and eosin stains. However, some seminomas revealed more than usual nuclear pleomorphism ,presence of large bizarre nuclei bearing cell and markedly less lymphocytic infiltration ,creating confusion with solid pattern of embryonal caricinoma(1,4,5).The distinction between seminoma and embryonal caricinoma is very important because of difference of treatment protocol.Seminoma confined to the testis is treated by Orciechtomy and radiation therapy to pelvic lymph nodes.After Orchiectomy, retroperitoneal lymph node dissection or chemotherapy is needed in embryonal carcinoma(2). CD 30 is a member of the tumour necrosis factor(TNF) superfamilly and is expressed on the surface cell of EC but very rarely in seminomas(3,6,16).Proto-oncogene c-kit encodes a surface membranetyrosine kinase receptor that is required in normalspermatogenesis. CD117(c-Kit) is regularly expressed in seminoma but rarely in non-seminomatous germ-cell tumors. KIT signal transduction appears to be an important pathway for carcinogenesis of seminoma (13).

Evidence suggests CD 30 and CD 117(c-kit) are useful markers in this regard.CD 30 is expressed by majority of embryonal carcinoma and is generally negative in seminoma(6-9), while CD 117(c-kit) is regularly expressed in seminomas and only rarely positive in embryonal carcinoma(9,10,13-15).According to them Immununohistochemical evaluation of combined expression of CD 30 and CD 117(c-kit) is the mainstay in differentiation between seminoma and embryonal carcinoma. However CD 30 immunoreactivity is expressed in a number of pure seminomas and seminomatous component of Mixed Germ Cell Tumours(4,9,16,17,19) and CD 117(c-kit) positivity in seminomas has been noted to be variable and weak,(20) somewhat limiting the diagnostic ability of these particular markers.

D2-40 is a monoclonal antibody that reacts with an oncofetal membrane antigen known as the M2A antigen(21) which is present in fetal germ cell of the testis as well as lymphatic endothelial cell and mesothelial cell.(22-24). In the context of germ cell neoplasia, the distribution of M2A antigen has been shown to be largely

restricted to Intratubular Germ Cell Neoplasia and Seminoma, with limited to absent expression in nonseminomatous Germ cell tumour(21-26).The selective expression of M2A antigen in seminoma suggests potential use of D2 -40 antibody in distinguishing this particular tumors from embryonal carcinoma. In the present study D2 40 expression - in morphologically and immunohistochemicaly (with CD 30 and CD 117(c-kit) diagnosed cases of seminoma and embryonal carcinoma- is studied to determine its role in precise differentiation of this two tumours.

II. Materials And Methods

Formalin fixed, paraffin embedded tissue sectionsfrom 23 pure seminomas, 3 pure embryonal Carcinomas , 3 Pure Yolk Sac Tumours,3 Teratomas and 28 Malignant Mixed Germ Cell tumours bearing and seminoma component (Table1)… were selected from the files of the department of pathology of Burdwan Medical College Hematoxylin and eosin-stained sections from all cases were reviewed to confirm the diagnosis. The testicular germ cell tumors were classified according to the World Health Organization criteria(27).Immunohistochemical analysis was conducted on 5micrometer thick paraffin sections. After deparaffinisation(in xylene),and rehydration (in graded alcohol),immunohistochemistry was performed using three step process based on streptavidin-biotin complex. The primary antibodies used are CD 30 (1:40,Ber H2: Dako,Glostrup ,Denmark), CD 117(1:50,Dako.),

D2-40 (clone D2-40, dilution 1:2, Signet Laboratories, Inc., Dedham, MA, USA), Pressure cooker pretreatment in citrate buffer6.0) was performed for 1 minute 30 seconds. Antigen retrieval for D2 40 was performed by similarly heating slides in EDTA buffer (pH 8.0) .Endogenous peroxide activity was suppressed by first incubating the specimen in 3% hydrogen peroxide. Negative control was made of sections without primary antibody. Mast cell were used as internal positive control for CD 117(c-kit).Lymphatic endothelial cell were used as similar internal positive control for D2-40.,Anaplastic large cell Lymphoma as positive control for CD 30and Mesothlioma for D2 40.The sections were counterstained with haematoxylene and eosin. Immunohistochemistry was evaluated by pathologist by determining percentage of positively staining cell as follows;0,no staining;+,1-10% of staining cells: ++,11-50 % of staining cell; +++ > 50% of staining cells.

Table1

Type of Testicular germ cell tumour Studied								
Pure Germ Cell Tumour(Total- 35)				Malignant Mixed Germ Cell tumour(total -28)				
Seminoma	Embryonal Carcinoma(EC)	Yolk Sac Tumour	Teratoma	Seminoma component	Embryonal Carcinoma(EC) component	Yolk Sac Tumour component	Teratoma component	Choriocarcinoma component
23	3	3	6	14	22	10	6	2

III. Result

Result of the seminomatous tumours 22 of total 23 pure seminomas showed positivity to CD 117(c-kit).Staining was often intense and diffuse with a membranous pattern(Figure 1).13 out of 14 of seminomatous component of Malignant Mixed Germ Cell Tumors were also stained with to CD 117(c-kit).2 (5.4 %) of tumours were non responsive toCD 117(c-kit).CD 30 though was negative in majority (35 out of 37 cases)of seminoma, 2 (5.4 %) cases showed focal positivity in less than 10 % of tumour cell.one of such positive case was of pure seminoma type where as other was member of seminomatous component of malignant Mixed Germ Cell Tumors category (Table. 2 ,3).

Of embryonal carcinoma 22 (88%),of total 25 cases showed positive reaction with CD 30. Two (2) out of total 3 cases of pure embryonal carcinoma and 20 out of 22 cases of embryonal carcinoma component of Malignant Mixed Germ Cell Tumors were positive for CD 30.The staining was membranous and diffuse in the great majority, though weak and focal positivity was seen in 5 of them.Weak focal positivity with CD 30 was seen embryonal carcinoma component of 2 Malignant Mixed Germ Cell Tumors(Table.2,3). All cases of pure seminoma and the seminomatous components of mixed germ cell tumors reacted with the D2-40 antibody. D2-40 immunoreactivity in seminomas was characterized by strong and diffuse membrane staining of the neoplastic cells (Figure 1). All three cases of pure embryonal carcinoma were negative for D2-40, while the embryonal carcinoma component of mixed germ cell tumors were positive for D2-40 in 8of 14 cases. In contrast to the strong membrane staining observed in seminomas, D2-40 immunoreactivity in the embryonal carcinomas was typically weak, focal, and distributed along the apical or luminal surfaces of the neoplastic cells in 7 cases (Figure 2).In one case, moderate degree intensity inimmunohistochemical positivity was expressed . Other germ cell tumor components including yolk sac tumor, teratoma, and choriocarcinoma were immunohistochemically negative for D2-40, CD 117(c-kit) and CD30.

Table 2

CD117(c-kit), CD 30 andD2-40 immunoreactivity in testicular Germ cell Tumour			
Tumour Type	Number of Positive cases		
	CD 117(c-kit)	CD 30	D2-40
Pure Germ Cell Tumour			
Seminoma	22/23	1/23	23/23
Embryonal Carcinoma	0/3	2/3	0/3
Yolk Sac Tumour	0/3	0/3	0/3
Teratoma	0/6	0/6	0/6
Malignant Mixed Germ Cell tumour			
Seminoma component	13/14	1/14	14/14
Embryonal Carcinoma component	2/22	20/22	8/22
Yolk Sac Tumour component	0/6	0/6	0/6
Teratoma component	0/8	0/8	0/8
Choriocarcinoma component	0/2	0/2	0/2

Table3:

Results of Immunohistochemical staining				
		CD 117(c-kit)	CD 30	D2-40
Seminoma				
	negative	2 (5.4 %)	35 (94.6 %)	0
	+	2 (5.4 %)	2 (5.4 %)	0
	++	3 (8.1 %)	0	2 (5.4%)
	+++	30 (81.1 %)	0	35 (94.6 %)
Embryonal Carcinoma(EC)				
	negative	23 (92 %)	3 (12 %)	17 (68 %)
	+	2 (8 %)	1 (4 %)	7 (28%)
	++	0	4 (16 %)	1 (4 %)
	+++	0	17 (68 %)	0
Yolk Sac Tumour		0	0	0
Teratoma		0	0	0
Choriocarcinoma		0	0	0

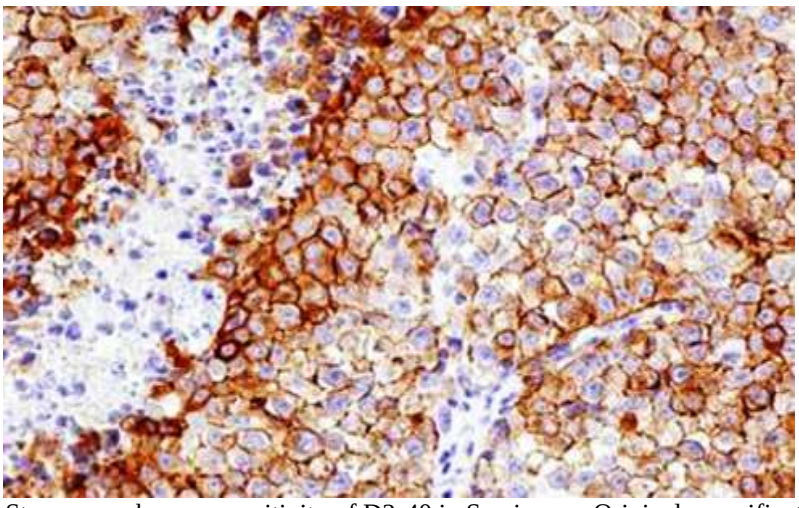

Figure1: Strong membranous positivity of D2-40 in Seminoma .Original magnification x400

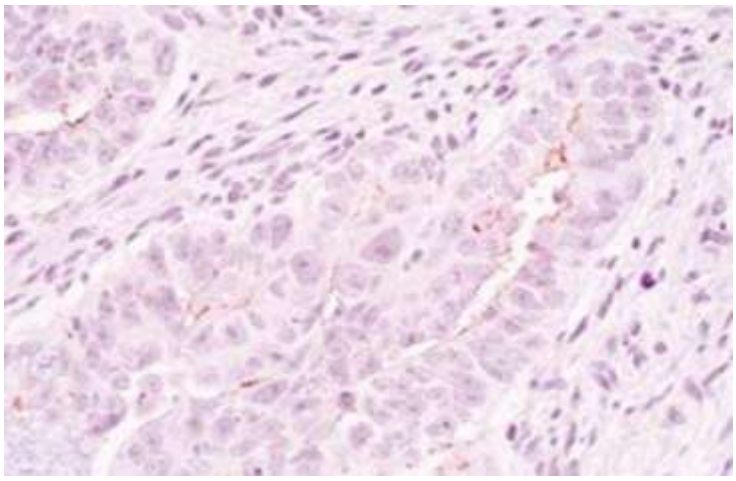

Figure2 : Weak and Focal positivity of D2-40 at luminal surface of Embryonal Carcinoma.Original magnification x400

IV. Discussion

Differentiation between seminoma and embryonal carcinoma is very important in consideration of the therapeutic approach. Though light microscopic examination remains the gold standard for the diagnosis, in some cases the differential diagnosis between seminoma with more than usual pleomorphic features and the solid pattern of embryonal carcinoma may be difficult (1,2).Therefore, Immunohistochemical evaluation is necessary for precise differentiation. Cytokeratin is generally more expressed in embryonal carcinoma than in seminoma. However, in alarge series study of cytokeratin expression in seminomas, 30 % of these tumors reacted with various antikeratin antibodies (19). The stainingwas often focal (less than 10% of cells stained), mainlywith CK7. In another study, 73% seminomas expressed cytokeratins, and constantly positive with CK8 and CK18 (18). In embryonal carcinoma ,cytokeratins are often diffusely expressed but rare tumors may be entirely negative(19).

In 1988, a study about CD30 expression in non-hematopietic tissues showed that CD30 was expressed by embryonal carcinoma and not by seminomas (6). Further studies confirmed that CD30was a valuable marker of embryonal carcinoma(7). But, again, some embryonal carcinomas were found negative and seminomas were focally positive with CD30 (3,16). In one tumor, the seminomatous cells were stained both with cytokeratin andCD30 (16).CD 117(c-kit) was regularly expressed in seminomatous cells (11-13).Izquierdo et al reported a diffuse membranouspositivity of c-kit in all of 28 seminomas. In 32% of nonseminomatous germ cell tumors of the series, a focalcytoplasmic staining in occasional cells was observedwithout membranous reactivity(13).in another study,It was showed that all classical seminomas tested intheir work were CD 117(c-kit)positive (22/22) but that somespermatocytic seminomas (7/17) were also stained(12).

It was demonstrated that no embryonal carcinoma showed negative CD30 expression along with simultaneous positive CD 117(c-kit) expressionand no seminoma had a phenotype of CD 30 negative along with simultaneous CD117(c-kit) positivity.Researchers opined that a phenotype of CD30 negative along with CD117 (c-kit) positive was conclusive of seminoma and that a phenotype of CD30 positive along withCD117(c-kit) negative was very suggestive of embryonal carcinoma (9). D2-40 is a monoclonal antibody which recognizes 40 kDa O-glycosylated sialoglycoprotein with a simple mucin type carbohydrate structure known as M2A antigen(16).Normally this antigen is present in fetal germ cells, lymphatic endothelium, and mesothelial cells.(22-24).Previously, immunohistochemical investigation using monoclonal antibody demonstrated M2A antigen in all seminomas and seminomatous components of mixed germ cell tumors by the researchers. Such experiment was done on frozen tissue sections and all nonseminomatous germ cell tumors studied were negative to D2-40 in their study.(22,23).

Utilizing the D2-40antibody on paraffin-embedded tissue samples, we have observed positive immunoreactivity in all cases of seminoma .Our findings is similar to and in agreement with the distribution of the M2A antigen established by previous studies.(22,23).We found D2-40 immunoreactivity in eight of 25(32%) samples of embryonal carcinoma, Such findings varied from the results of these aforementioned reports. We found focal D2-40 positivity in luminal aspect of embryonal carcinoma. Such immunopostivity was distinctly different from diffuse membranous expression as seen in seminoma. These finding is comparable to that obtained by other investigators using the D2-40antibody.(20,21)and is in concordance with Mark`s et al. who reported D2-40 immunoreactivity in 98% of seminomas, and were also able to demonstrate positivity for this antibody in 69% of embryonal carcinomas. Similar to the present study, they noted D2-40 positivity in embryonal carcinomas in focal pattern and was concentrated at the luminal surface of the neoplastic cells,

whereas a uniform membrane pattern of staining was observed in seminomas.(21)The data obtained in the current investigationsuggest D2-40 may serve as a useful immunohistochemical marker for the identification ofseminoma. D2-40 was commonly expressed inseminomas, with sensitivity higherthan that of CD 117(c-kit).Because of expression of D2-40 in a subset of embryonal carcinomas, it may be considered less specific marker for seminoma than CD 117(c-kit).However importance of pattern of expression of D2-40 is profound -as positivity in seminomas was distinctly different from that observed in embryonal carcinomas. Reactivity for D2-40 in seminomas was typically diffuse and membranous, while in embryonal carcinomas, positivity was always focal and limited to the apical or luminal surface of the cells In summary, D2-40 shows higher sensitivity in seminoma and though sometimes expressed in Embryonalcarcinomas –pattern of expression is different from that of seminoma and thus can be useful aid in differentiation between these two tumours in difficult scenario.

Referance

[1]. Ulbright TM, Amin MB, Young RH (1999) Tumors of the Testis, Adnexa, Spermatic Cord, and Scrotum. AFIP, 3nd ser, fas 25,Wasington ,DC

[2]. Parkinson MC, Harland SJ, Harnden P, et al. The role of the histopathologist in the management of testicular germ cell tumour in adults. Histopathology 2001;38: 183–194.

[3]. Pera MF, Bennett W, Cerretti DP (1998) CD30 and its ligand: pos- sible role in regulation of teratoma stem cells. APMIS 106:169–172

[4]. Tickoo SK, Hutchinson B, Bacik J, et al. Testicular seminoma: a clinicopathologic and mmunohistochemical study of 105 cases with special reference to seminomas with atypical features. Int J SurgPathol 2002;10:23–32.

[5]. Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differentialdiagnosis, newly appreciated, and controversial issues. Mod Pathol 2005;18:S61–S79.

[6]. Pallesen G, Hamilton-Dutoit SJ. Ki-1 (CD30) antigen is regularly expressed by tumor cells of embryonalcarcinoma. Am J Pathol 1988;133:446–450.

[7]. Ferreiro JA. Ber-H2 expression in testicular germ cell tumors. Hum Pathol 1994;25:522–524.

[8]. Durkop H, Foss HD, Eitelbach F, et al. Expression of the CD30 antigen in non-lymphoid tissues and cells.JPathol 2000;190:613–618.

[9]. Leroy X, Augusto D, Leteurtre E, et al. CD30 and CD117 (c-kit) used in combination are useful for distinguishing embryonal carcinoma from seminoma. J HistochemCytochem 2002;50:283–285.

[10]. Tsuura Y, Hiraki H, Watanabe K, et al. Preferential localization of c-kit product in tissue mast cells, basalcells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human:immunohistochemical study on formalin-fixed, paraffin embedded tissues. Virchows Arch 1994;424:135–141.

[11]. Bokemeyer C, Kuczyk MA, Dunn T, Serth J, Hartmann K, Jonasson J, Pietsch T, Jonas U, Schmoll HJ (1996) Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours. J Cancer Res ClinOncol 122:301-306

[12]. Kraggerud SM, Berner A, Bryne M, Pettersen EO, Fossa SD (1999) Spermatocytic seminoma as compared to classical seminoma: an immunohistochemical and DNA flow cytometric study. APMIS 107:297¬302

[13]. Izquierdo MA, Van der Valk P, Van Ark-Otte J, et al.Differential expression of the c-kit proto-oncogene in germ cell tumours. J Pathol 1995;177:253–258.

[14]. Strohmeyer T, Reese D, Press M, et al. Expression of the c-kit proto-oncogene and its ligand stem cell factor(SCF) in normal and malignant human testicular tissue. J Urol 1995;153:511–515.

[15]. Tian Q, FriersonJr HF, Krystal GW, et al. Activating c-kit gene mutations in human germ cell tumors. Am J Pathol 1999;154:1643–1647.

[16]. Hittmair A, Rogatsch H, Hobisch A, et al. CD30 expression in seminoma. Hum Pathol 1996;27:1166–1171.

[17]. Suster S, Moran CA, Dominguez-Malagon H, et al. Germ cell tumors of the mediastinum and testis: acomparativeimmunohistochemical study of 120 cases.HumPathol 1998;29:737–742.

[18]. Fogel M, Lifschitz–Mercer B, Moll R, Kushnir I, Jacob N, Wadherr R, Livoff A, Franke WW, Czernobilsky B (1990) Heterogeneity of intermediate filament expression in human testicular semino-mas. Differentiation 45:242-249

[19]. Cheville JC, Rao S, Iczkowski KA, et al. Cytokeratin expression in seminoma of the human testis. Am J ClinPathol 2000;113:583–588.

[20]. Biermann K, Klingmuller D, Koch A, et al. Diagnostic value of markers M2A, OCT3/4, AP-2gamma, PLAP and c-KIT in the detection of extragonadal seminomas. Histopathology 2006;49:290–297.

[21]. Marks A, Sutherland DR, Bailey D, et al. Characterization and distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumours. Br J Cancer 1999;80:569–578.

[22]. Bailey D, Baumal R, Law J, et al. Production of a monoclonal antibody specific for seminomas anddysgerminomas. ProcNatlAcadSci USA 1986;83:5291–5295.

[23]. Bailey D, Marks A, Stratis M, et al. Immunohistochemical staining of germ cell tumors and intratubular malignant germ cells of the testis using antibody to placental alkaline phosphatase and a monoclonal antiseminoma antibody. Mod Pathol 1991;4:167–171.

[24]. Sonne SB, Herlihy AS, Hoei-Hansen CE, et al. Identity of M2A (D2-40) antigen and gp36 (Aggrus, T1A-2,podoplanin) in human developing testis, testicular carcinoma in situ and germ-cell tumours. VirchowsArch 2006;449:200–206.

[25]. GiwercmanA, Marks A, Bailey D, et al. A monoclonal antibody as a marker for carcinoma in situ germ cells of the human adult testis. APMIS 1988;96:667–670.

[26]. Giwercman A, Cantell L, Marks A. Placental-like alkaline phosphatase as a marker of carcinoma-in-situof the testis. Comparison with monoclonal antibodies M2A and 43-9F. APMIS 1991;99:586–594.

[27]. Eble JN, Sauter G, Epstein JI, et al. World health organization classification of tumours. Pathology andGenetics of Tumours of the Urinary System and Male Genital Organs. IARC Press: Lyon, 2004.