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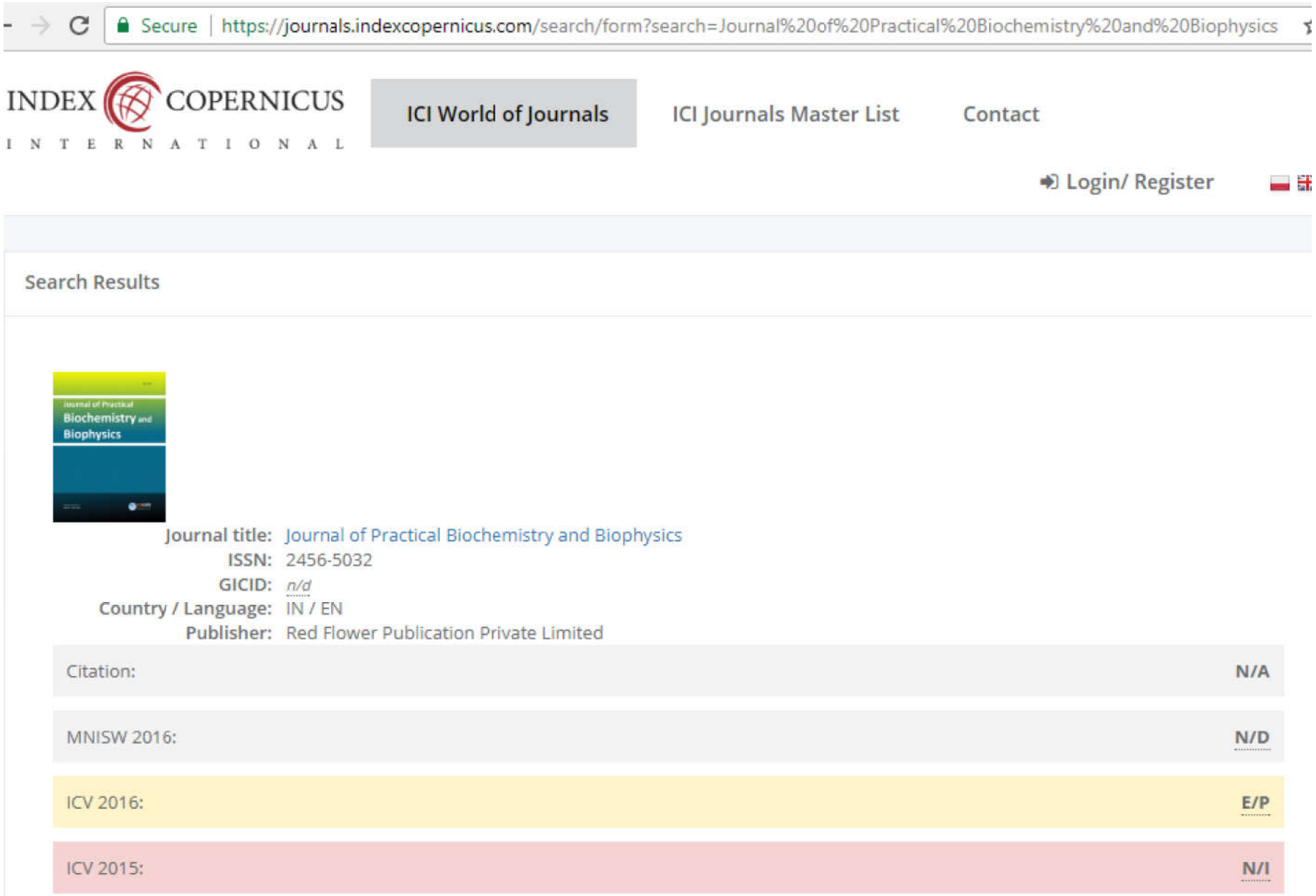
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Original Article

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Role of Lactate Dehydrogenase Activity and C-Reactive Protein in Cerebrospinal Fluid for Different Types of Meningitis

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Abstract

Background: Meningitis most common neurological disorder with high mortality rate. Cerebrospinal fluid (CSF) examination by routine tests does not always provide rapid definite information as far as causative agent of different types of meningitis. Bacterial meningitis a common problem especially in many developing countries; *Aim:* To evaluate the diagnostic and prognostic significance of Lactate Dehydrogenase (LDH) enzymes and C-reactive protein (CRP) by comparing it with the levels of serum in CSF of different types of meningitis. *Material and Methods:* A total of 150 cases, aged between 2 month and 60 years, including patients with bacterial meningitis (n=40), pyogenic meningitis (n=46), viral meningitis (n=24) and a control group (n=40), were analyzed on the basis of data from the initial clinical examinations. *Results*: Significant increase in LDH level (P<0.001) were observed in the test group when compared to the control group. The LDH activity was significantly elevated in the CSF and serum ($p < 0.001$) in cases of pyogenic (PM) as well as tuberculous meningitis (TBM). CRP was positive in almost all cases and was in the range of 0.7 to 9.7 mg/dl and values were corresponding in the serum. Bacterial meningitis is more common than non-bacterial meningitis. *Conclusion:* The enzymatic activity of LDH although significantly raised in PM compared to TBM but there was no cutoff level to differentiate them. CRP can be used as a supportive evidence of meningitis.

Keywords: Lactate Dehydrogenase; CRP; Cerebrospinal Fluid; Meningitis.

Introduction

Bacterial meningitis is a common problem during childhood, and considerable cause of mortality and morbidity especially in children [1-3].

Although many studies have acknowledged the CSF in either diagnosis or prognosis of bacterial meningitis patients [4-6], recent studies however emphasize the fact that absence or low levels of CSF (especially after 12 hours' manifestation of clinical symptoms) strongly rule out bacterial meningitis [7].

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Lactic dehydrogenase (LDH) is present in most tissues and body fluids examined, including cerebrospinal fluid (CSF) and potentially useful biomarker of bacterial meningitis.

C-reactive protein (CRP), an acute phase serum protein formed by the body in response to various non-specific stimuli such as infection, tissue necrosis or neoplasm.

However, routine diagnostic use of cerebrospinal fluid (CSF) CRP in differentiating bacterial and non-bacterial meningitis has been evaluated in very few studies [8]. The present work has been undertaken with aims to assess whether there is any significant difference in LDH activity in CSF in different types of meningitis, so that it can differentiate between pyogenic, tuberculous and viral meningitis.

Material and Methods

The present study carried out at Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, during the period from Feb 2014 to Nov 2016. Total 150 CSF samples were examined. Out of them 110 patients of all age groups and either sex of clinically suspected cases of meningitis were taken as test group. 40 control subjects of all age and either sex having no neurological, hepatic, muscular, and cardiac disorders were taken as control group. Cerebrospinal fluids were collected by the lumbar puncture with all aseptic and antiseptic precautions were taken in a clean, dry and sterile vial. CSF sample was tested for CRP by simple antigen antibody precipitation test, i.e., latex slide agglutination method with the help of commercially available kit supplied by Span Diagnostic. CSF was centrifuged at 3000 rpm for 10 minutes and estimation of LDH, was done with clear supernatant parts of CSF. LDH was estimated by UV kinetic method (using Kit) by semi-auto analyzer.

Results

The LDH level did rise quite significantly in pyogenic meningitis (Mean 230.4 IU/L Range 189-330 IU/L and p<0.0001). In control group the range of CSF-LDH was 10-44 I.U./L with a mean of 31.0±9.47 I.U./L. It was almost concluded that the estimation of CSF-LDH is of diagnostic as well as prognostic value particularly if interpreted together with clinical examination and routine cytochemical examinations. In cases of tuberculous meningitis also the CSF-LDH level was significantly high but less than that of pyogenic meningitis (Range 95-250 IU/L, p<0.0001). In tuberculous meningitis also CSF-LDH estimation is of diagnostic and prognostic importance. In viral meningitis the CSF-LDH levels was slightly higher than that of normal and significantly lower than that of tuberculous meningitis and pyogenic meningitis (Range 24-70 IU/L, mean 46.4 IU/L, S.D. 13.5 IU/L shown in table 1. In viral meningitis CSF-LDH estimation may differentiate it from that of tuberculous and pyogenic meningitis and so of diagnostic importance.

Table 1 shows that CSF-LDH, mean levels in pyogenic meningitis, tuberculous meningitis and viral meningitis were 230.4±35.8, 132.3±32.3 and 46.4±13.5 IU/L respectively, which is highly significant (P<0.0001) as compared to controls. CSF-protein, mean levels in pyogenic meningitis, tuberculous meningitis and viral meningitis were 216.5±118.3, 150.2±31.6, and 53.9±9.5 mg/100 ml respectively, which is highly significant (P<0.0001) as compared to controls. CSF-sugar, mean levels in pyogenic meningitis, tuberculous meningitis and viral meningitis were 20.6±7.6, 32.3 ±8.1, and 56.1 ± 10.8 mg/100 ml respectively, which is highly significant (P<0.0001) as compared to controls. CSF CRP was increased in 44 cases (95.65%) of pyogenic meningitis. The mean CSF CRP in cases was 2.15±1.83 mg/dl was statistically significant when compared with control (p <0.001). The mean CSF CRP in controls was 0.052±0.12 mg/dl. Serum CRP was increased in 43 cases (93.47%) of pyogenic meningitis. The mean serum CRP in cases was 1.23±1.98 mg/dl as compared to control (p <0.0001). The mean serum CRP in controls was 0.04±.19 mg/dl.

Table 1: Table showing the mean, S.D,'t' and P values of CSF LDH, protein, sugar levels in different types of meningitis

Types of meningitis	LDH IU/L				Protein mg/100 ml				Sugar mg/100 ml			
	Mean	± S.D.	't' values	P values	Mean	± S.D.	't' values	P values	Mean	± S.D.	't' values	P values
Pyogenic meningitis	230.4	35.8	29.2	0.0001	216.5	118.3	5.76	0.0001	20.6	7.6	11.2	0.0001
Tuberculous meningitis	132.1	32.3	19.0	0.0001	150.2	31.6	3.27	0.0001	32.3	8.1	18.3	0.0001
Viral meningitis	46.4	13.5	10.01	0.0001	53.9	9.5	18.6	0.0001	56.1	10.8	17.90	0.0001

Table 2: CRP value in cases and control

	CSF- CRP	Serum –CRP
Control	0.052±0.12 mg/dl	0.04±0.19 mg/dl
Meningitis	2.15±1.8 mg/dl	1.23±1.98 mg/dl

Discussion

The meningitis is one of the important causes of considerable morbidity and mortality in children's. In order to differentiate aseptic meningitis to the bacterial meningitis, numbers of studies have shown the effectiveness of rapid and definite tests using CSF variables and markers of peripheral blood for various common and uncommon laboratory measurements [9-10]. This observation is quite in accordance with the observations made earlier by M. Sharma et al [11]; Moshe Nussinovitch [12] who also observed raised LDH level in the CSF of patients of pyogenic meningitis. Some researchers have suggested a disturbance in the blood-brain barrier which enables plasma LDH to reach the CSF, or production of LDH by neoplastic tissue or by white blood cells and exogenous bacterial sources [13-15]. In viral meningitis CSF-LDH estimation may differentiate it from that of tuberculous and pyogenic meningitis and so of diagnostic importance. CSF CRP. Shimetani et al [16] also showed a substantial increase in CSF and serum CRP levels in cases of meningitis. Kumar et al [17] observed a very significant increase ($p<0.0001$) in CSF in cases of pyogenic, so CSF-LDH and CRP estimation is of importance as a diagnostic and prognostic tool as far as the dreaded disease of different types of meningitis are concerned. CSF and serum CRP was elevated in 96% of cases when compared to control. Vaishnavi et al [18] and Takhiwale et al [19] observed a similar trend with the levels of CSF CRP.

Conclusion

Bacterial meningitis is more common and frequently reported than non-bacterial meningitis. Evaluation of CSF-LDH and CRP may help to differentiates pyogenic meningitis from non-bacterial meningitis.

References

1. Tunkel, A.R. and S.W. Michael. Acute Meningitis. In: Principle and Practice of Infectious Disease, Mandell, G.L., R.G. Dollin and J.E. Bennett (Eds.). Churchill Livingstone/Elsevier, Philadelphia, 2010.p.1083-1119.
2. Roos, K.L. and K.L. Tyler. Meningitis. In: Harrison Principle of Internal Medicine, Kasper D.L., E. Braunwald, A.S. Fauci, S.L. Hauser and D.L. Longo *et al*. (Eds.). MC Graw Hill, New York, 2008.p.2471-2490.
3. Feigin, R.D. and E. Pearlman. Bacterial Meningitis beyond the Neonatal Period. In: Text Book of Pediatric Infectious Disease, Feigin, R.D., G.J. Demmler, J.D. Cherry and S.L. Kaplan, (Eds.). Saunders, Philadelphia, 2004.p.443-474.
4. Kanoh Y, Ohtani H. Levels of interleukin-6, CRP and alpha 2 macroglobulin in cerebrospinal fluid (CSF) and serum as indicator of blood-CSF barrier damage. Biochem Mol Biol Int 1997;43(2):269-78.
5. Paradowski M, Lebos M, Kuydowicz J, et al. Acute phase proteins in serum and cerebrospinal fluid in the course of bacterial meningitis. Clin Biochem 1995;28(4):459-66.
6. Sormunen P, Kallio MJT, Kilpi T, et al. C-reactive protein is useful in distinguishing gram stain-negative bacterial meningitis from viral meningitis in children. J Pediatr 1999;134(6):725-9.
7. Gendrel D, Raymond J, oste J, et al. Comparisson of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentiation of bacterial vs. Viral infections. Pediatr Infect Dis J 1999;18(10):875-81.
8. Corral CJ, Pepple JM, Moxon R, Hughes WT. C-reactive protein in cerebrospinal fluid in children with meningitis. J Pediatr 1981;99:365–9.
9. Sormunen P, Kallio MJ, Kilpi T, Peltola H. C-reactive protein is useful in distinguishing Gram stain-negative bacterial meningitis from viral meningitis in children. J Pediatr. 1999;134(6):725-9.
10. Snyder RD. Bacterial meningitis: diagnosis and treatment. Curr Neurol Neurosci Rep 2003;3(6):461-9.
11. Sharma M. and Nand N. Evaluation of Enzymes in Pyogenic and Tuberculous meningitis. JAPI, Feb. 2006;54.
12. Moshe Nussinovitch et al: Cerebrospinal fluid lactate dehydrogenase isoenzyme in children with bacterial and aseptic meningitis. Accepted June 2009.
13. Lending M, Slobody LB, Mestern J. Cerebrospinal fluid glutamic oxalacetic transaminase and lactic dehydrogenase activities in children with neurologic disorders. J Pediatr 1964;65:415–21.
14. Wroblewski, F, Decker B, Wroblewski R. The clinical implications of spinal fluid LDH activity. N Engl J Med 1958;258:635–9.
15. Aicardi J. Disease of the nervous system in childhood. Clin Dev Med 1992;1115/118:1132–5.
16. Shimetani N, Shimetani K, Mori M. Levels of three inflammation markers, C-reactive protein, serum amyloid A protein and procalcitonin, in the serum and cerebrospinal fluid of patients with meningitis. Scand J Clin Lab Invest. 2001;61(7):567-574.
17. Kumar TA, Punith K, Revankar S, Kumar VNR, Rao MY, Sudhir U. Significance of cerebrospinal fluid C-reactive protein level in pyogenic and non-pyogenic meningitis in adults. J Indian Acad Clin Med 2010;11(2):112-115.

18. Vaishnavi C, Dhand UK, Dhand R, Agnihotri N, Ganguly NK. C-reactive proteins, immunoglobulin profile and mycobacterial antigens in cerebrospinal fluid of patients with pyogenic and tuberculous meningitis. J Hyg Epidemiol Microbiol immunol 1992;36(3):317-325.

19. Tankhiwale SS, Jagtap PM, Khadse RK, Jalgaonkar SV. Bacteriological study of pyogenic meningitis with special reference to C reactive protein. Indian J Med Microbiol 2001;19(3):159-160.

Original Article

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Study of Glycosylated Hemoglobin and it's Relation with Changes in Lipid Profile in Type II Diabetic Patients

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Abstract

Introduction: Patients with type 2 diabetes (T2DM) have an increased prevalence of dyslipidemia, which contributes to their high risk of cardiovascular diseases (CVDs). Hemoglobin A1c (HbA1c) is widely used as an index of mean glycaemia, a measure of risk for the development of diabetes complications and a measure of the quality of diabetes care. This study is an attempt to determine the correlation between hemoglobin A1c (HbA1c) and serum lipid profile and to evaluate the importance of HbA1c as an indicator of dyslipidemia in patients with T2DM. *Study Subjects:* 100 non obese, non hypertensive type 2 diabetic patients attending the Diabetic OPD, IIMSR Medical College,Jalna will be enrolled in this study. After obtaining informed consent from patients, detailed history will be taken followed by investigations like fasting and post prandial blood sugar, HbA1c and lipid profile (Cholesterol, Triglycerides, HDL, LDL & VLDL). *Material &Methods*: Lipid profile and blood glucose levels will be analysed using respective biochemical kits Erba EM 200 automated biochemical analyser in central clinical laboratory, Biochemistry section of IIMSR Medical College, Jalna. Glycosylated Hemoglobin was analysed by using SDA1c Care portable analyser.

Keywords: Glycemic Control; HbA1c; Serum Lipid Profile; Type 2 Diabetes.

Introduction

Diabetes mellitus is characterized by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. Total prevalence of diabetes mellitus globally is estimated to rise from current estimate of 415 million to 642 million by 2040. The number of people with type 2 diabetes mellitus is increasing in every country and 75% of people with diabetes mellitus are living in developing countries [2]. With an increasing incidence worldwide, diabetes mellitus will be a likely leading cause of morbidity and mortality in the future [3]. Diabetes is associated with a greater risk of morbidity and mortality from cardiovascular disease (CVD). Serum lipids are frequently abnormal and are likely to contribute to the risk of coronary artery disease [4]. Worsening of glycemic control deteriorates lipid and lipoprotein abnormalities and particularly of diabetes mellitus [5]. Dyslipidemia in diabetes commonly manifests as raised low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) levels, or elevated triglyceride (TG) levels. Furthermore, data from the United Kingdom Prospective Diabetes Study suggest that both decreased HDL-C and elevated LDL-C predict CVD in diabetes. All national and international guidelines recommend aggressive management of lipids in this population [6,7]. Glycated hemoglobin (HbA1c) is routinely used as a diagnostic tool for measuring long term glycemic control. In accordance with its function as an

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indicator for the mean blood glucose level, HbA1c predicts the risk for the development of diabetic complication in diabetes patients. The UKPDS study has shown that in patients with type 2 diabetes, the risk of diabetic complications were strongly associatedwith previous hyperglycemia. Glycemic control with decreased level of HbA1c is likely to reduce the risk of complications [3]. Estimated risk of Cardio Vascular Diseases (CVD) has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic [4]. Even in nondiabetic cases with HbA1c levels within normal range, positive relationship between HbA1c andCVD has been demonstrated [8,9]. A few studies have previously tried to find the correlationbetween HbA1c levels and lipid profile. Some of these have shown that all the parameters oflipid profile have significant correlation with glycemic control [10]. On the other hand, somestudies do not report significant correlation between glycemic control and all parameters oflipid profile [11]. These controversies inspired us to take forward this study which was aimed to findout association between glycemic control (HbA1c) and serum lipid profile in non obese, non hypertensive type 2 diabetic patients attending the Diabetic OPD, IIMSR Jalna.

Aims & Objectives

To determine the impact of glycemic control on lipid profile and to know utility of HbA1c as an indirect indicator as well as predictor of dyslipidemia so that adequate preventive measures can be ensured for preventing development of dyslipidemia leading to cardiovascular diseases in type 2 diabetic patients.

Inclusion Criteria

Patients of age ≥30 years of both genders Patients with known diagnosis of type- 2 DM.

Exclusion Criteria

- Obese
- Hypertensive
- T2DM patients with concomitant diseases or conditions affecting lipid levels like chronic liver disease and hypothyroidism.
- Patients on drugs like oral contraceptive pills, steroids and diuretics.

Study Subjects

In all 100 subjects having DM – II were enrolled in the study out of which 66 were male and 34 were female100 non obese, non hypertensive type 2 diabetic patients attending the Diabetic OPD, IIMSR Medical College,Jalna were enrolled in this study. After obtaining informed consent from patients, detailed history will be taken followed by investigations like fasting and post prandial blood sugar, HbA1c and lipid profile (Cholesterol, Triglycerides, HDL, LDL & VLDL).

The patients were classified into two groups depending on their glycated hemoglobin (HbA1c); Good Glycemic Control (GGC) group having HbA1c < 7.0% and Poor Glycemic Control (PGC) group having HbA1c>7.0%. For serum lipid reference level, National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred [12].

Dyslipidemia was defined by presence of one or more than oneabnormal serum lipid concentration [13]. Statistical analysis was carried out by using student's unpaired 't' test. Pearson's correlation coefficient was also calculated to find the correlation between HbA1c and lipid parameters.

Material & Methods

Lipid profile and blood glucose levels wereanalysed using respective biochemical kits Erba EM 200 automated biochemical analyser in central clinical laboratory, Biochemistry section of IIMSR Medical College, Jalna. Glycosylated Hemoglobin was analysed by using SDA1c Care portable analyser.

Statistics

The results were evaluated by SPSS statistical package version 20 by one-way analysis of variance (ANOVA) followed by comparing with students t

Table 1: NCEP-ATP III guidelines for hypercholesterolemia/hyperlipidemia

Parameter	Normal/Reference ranges	Abnormal range in Dyslipidemia
Total Cholesterol	< 200 mg/dl	>200 mg/dl
Triglycerides	< 150 mg/dl	150 mg/dl
LDL	< 100 mg/dl	>100 mg/dl
HDL	>40 mg/dl	<40 mg/dl

Observation

Table 2: Mean values of Fasting Blood Glucose(FBG), Glycosylated hemoglobin(HbA1C) ; Lipid profile parameters of Male and Female type 2 Diabetic patients

Parameter	Males	Females	Total study subjects
FBG (mg/dl)	124.42 +- 28.64	119.21 +- 29.84	122.86 +- 29.04
HbA1c (%)	7.54 +- 1.32	6.98 +- 1.40	7.05 +- 1.60
Total Cholesterol (TC)[mg/dl]	157.62 ±30.40	146.54 ±35.31	153.79± 34.12
Triglycerides (TG) [mg/dl]	158.21 ± 49.33	148.43 ± 64.40	153.60 ± 58.62
Low Density Protein (LDL) [mg/dl]	92.83 ± 34.06	78.24 ± 29.32	85.40 ± 31.20
Very Low Density Protein (VLDL) [mg/dl]	32.66 ± 10.21	29.09 ± 12.90	32.02 ± 10.92
High Density Protein (HDL) [mg/dl]	45.79 ± 4.12	58.34 ± 3.23	51.95 ± 7.22

Table 3: Lipid parameters categorized according to patient's glycemic control (HbA1c)

Parameter	HbA1c < 7%	HbA1c > 7%	P value
FBG (mg/dl)	107.74 ± 21.68	139.61 ± 36.94	p< 0.0001
Total Cholesterol (TC)[mg/dl]	143.14 ± 26.92	205.42 ± 28.32	P < 0.001
Triglycerides (TG) [mg/dl]	146.83 ± 35.77	179.12 ± 27.7	P < 0.001
Low Density Protein (LDL) [mg/dl]	72.79 ± 22.86	87.96 ± 23.06	P< 0.001
Very Low Density Protein (VLDL) [mg/dl]	26.12 ± 9.15	34.10 ± 12.97	P = 0.0060
High Density Protein (HDL) [mg/dl]	50.46 ± 7.52	44 ± 7.5	P < 0.001

test. The results were expressed as Mean ± Standard deviation (S.D); P < 0.05 was considered significant.

Results

Our study revealed that 87% diabetic patients had deranged lipid profile(atleast one) while remaing 13% were having normal lipid parameters. This study showed slightly higher FBG and HbA1c levels in males as compared to females but difference was not significant (Table 1). When lipid profile of both males and females were compared; it showed no significant difference with the exception of HDL which was significantly more in females (Table 1).

In our study 62 patients had HbA1c levels more than 7% while remaining 38 patients witnessed HbA1c levels lesser than 7%. HbA1c levels were correlated with the lipid profile levels of diabetic patients and it was found that thosepatients having HbA1c levels > 7% had their lipid profile values significantly deranged as compared to other counterparts with HbA1c levels <7% (Table2).

Discussion

In this study, prevalence of dyslipidaemia in diabetic patients by at least oneabnormal lipid parameter was found to be 87% while 13% patients had normal lipid profile.

This was concordant to the study done by Mahanto RV et al. in which they found the prevalence of dyslipidaemia among type 2 diabetic patients was 80.0% in females and 83.33% in males [14].

Insulin impacts the liver apolipoprotein production which regulates the enzymatic activity of lipoprotein lipase and Cholesterol ester transport protein. These could be thelikely causes of dyslipidemia in Diabetes mellitus as reported by Goldberg [15].

Over andabove this, insulin deficiency also reduces the activity of hepatic lipase and several other stepsin the production of biologically active lipoprotein lipase may also be altered in DM [16].

This study also revealed positive correlation between HbA1c and Lipid parameters which stamps HbA1c as potential marker of deranged lipid parameters. Similar finding were suggested by Khan et al who also stated that severity of dyslipidaemia increases in patients with higher HbA1c value [17].

Erciyas F et al. also founded positive correlation between HbA1c and dyslipidemia [18].

Khan et al has reported that reducing the HbA1c level by 0.2% could lower the mortality by 10% [17].

Thus present study suggests the importance of HbA1c use as potential marker of lipid derangements; hence targeting good glycemic control can in turn can prevent as well as decrease the the incidences of cardiovascular diseases due to dyslipidemia.

References

1. Bennett HP, Knowler WC, Definition, Classification of Diabetes Mellitus and Glucose Homeostasis. In CR Kahn, GC Weir, GL King, AC Moses, RJ Smith and AM Jacobson editors. Joslin's Diabetes Mellitus, Philadelphia: LWW; 200,331.
2. Powers AC, Diabetes Mellitus: Complications. In DL Kasper, AS Fauci, DL Longo, SL Hauser, JL Jameson and J Loscalzo editors. Harrison's Principles of Internal Medicine. New York: McGraw-Hill Education; 2015.p.2399.
3. Diabetes: facts and figures [Internet]. International Diabetes Federation. [cited 2016Jul14]. Available from: http://www. idf.org/about-diabetes/facts-figures.
4. Jamshaid T, Qureshi A. Hyperlipidemia in Diabetics. Pac Postgrad Med J 2002;13:159-60.
5. Grundy SM. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. Am J Cardiol 2006;83:25-29.
6. U.K. Prospective Diabetes Study. Ethnicity and cardiovascular disease. The incidence of myocardial infarction in white, South Asian, and Afro-Caribbean patients with type 2 diabetes Diabetes Care. 1998; 21:1271- 7.
7. Sarat Chandra K, Bansal M, Nair T, et al. Consensus statement on management of dyslipidemia in Indian subjects. Indian Heart Journal. 2014;66 (Suppl 3):S1-S51.
8. Khaw KT, Wareham N, Bingham S, Luben R,Welch A and Day N. Association ofhemoglobin A1c with car-diovascular diseaseand mortality in adults: the Euro-peanProspective Investigation into Cancer inNorfolk. Ann Intern Med 2004;141:413-420.
9. Deeg R, Ziegenhorn J. Kinetic enzymaticmethod for automated determination oftotal cholesterol in serum. ClinChem 1983;29:1798-802.
10. Gligor Ramona et al. Relationship betweengly cosylated hemoglobin and lipidmetabolism in patients with type 2 diabetes. Studia Universitatis "Vasile Goldi", Seria tiinþeleVieþii 2011;21(2):313-318.
11. Zhe Yan, Yang Liu, Hui Huang. Association ofglycosylated hemoglobin level with lipid ratio and individual lipids in type 2 diabeticpatients. Asian Pacific Journal of Tropical Medicine 2012; 469-471.
12. http://www.nhlbi.nih.gov/guidelines/cholesterol/index.htm as visited on 19/8/2013.
13. Ram Vinod Mahato et al. Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker. Biomedical Research 2011;22(3): 375-380.
14. Mahato RV, Gyawali P, Raut PP, Regmi P, Singh KP, Pandeya DR et al. Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker. Biomed Res. 2011;22(3):375-80.
15. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 1996;37:693-707.
16. Tavangar K, Murata Y, Pedersen ME, Goers JF, Hoffman AR, Kraemer FB. Regulation of lipoprotein lipase in the diabetic rat. J Clin Invest 1992;90: 1672-1678.
17. Khan, H.A. et al. Association betweenglycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predictsdyslipidaemia. Clin. Exp Med 2007;7:24-29.33.
18. Erciyas F et al. Glycemic control, oxidative stress and lipid profile in children with type 1 Diabetes Mellitus. Arch. Med. Res. 2004;35:134-140.

Review Article

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Role of Flow Cytometry in Cancer Detection

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Abstract

In this review, we will discuss the usage of flow cytometry as a diagnostic and prognostic tool for cancer. Flow cytometry analysis can help in selecting distinct therapies. Flow cytometry can be used to differentiate the typical immunophenotype of malignancies, recognition of tumor cell DNA aneuploidy and measurement of cell DNA content. Flow cytometry enables objective explanation of tumor cell heterogeneity with the help of probes that differentiate tumor and normal cells and evaluate all tumor cell properties. The cell fraction in the S phase (SPF) of cell cycle is a common parameter deduced from DNA histograms incurred by flow cytometry. Flow cytometry is a powerful tool for fast analysis of cells for marker expression, cell cycle position, apoptosis, proliferation and measurements of cell kinetics and the potential doubling time (Tpot). Tpot evaluation is helpful in speculating local control in head and neck squamous cell carcinomas. Analysis of cellular RNA content by Flow Cytometry is helpful for the objective differentiation of acute leukemia and of multiple myeloma. Various studies have been conducted to identify hematopoietic stem cells using flow cytometry. The cancer stem cell which may be responsible for the recurrence of a tumor are identified and effectively isolated by multiparametric flow cytometry. The number of Circulating tumor cells (CTCs) which have separated from the primary tumor and run into the blood or lymphatic circulation forming a secondary tumor could be a prognostic marker for cancer progression. Researchers have developed several assays to detect CTCs for cancer diagnosis.

Keywords: Cancer; Flow Cytometry; Neoplasms; DNA Content; Markers; DNA Analysis; Cancer Stem Cells; Circulating Tumor Cells.

Introduction

Cancers are among the major causes of death worldwide. There are more than 100 types of cancer, each with dissimilar causes, symptoms and treatments and can affect any part of the body. The survival of the patient is decided by the stage of the disease. With breakthroughs in research and treatment, cancer survival rates have doubled in the last four decades but still a large number of patients are either resistant to treatment or show disease reoccurrence. Many cancers can be cured if they are detected early and treated adequately. When early detection and treatment intervention is not done, patients are diagnosed at very late stages and then curative treatments have almost negligible chances of success. Flow cytometry analysis provides important diagnostic and prognostic information required to select distinct therapies. A review of studies reported over the past 6 years assessing flow cytometry, showed high sensitivity of flow cytometry compared with standard cytology for cancer detection. Numerous applications of flow cytometry

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are appearing now in the areas of pharmacology and cytoenzymology.

Overview of Flow Cytometry

Flow cytometry has evolved over the past three decades from a small lab technique to an important conventional tool for diagnosis and monitoring cancer and immune deficiencies. Flow cytometry is specifically important in situations when the investigator wishes to differentiate between multiple dissimilar cell types within a heterogeneous population and evaluate their frequencies or the expression of a particular molecule of interest. Measurement of DNA and surface antigens in hematology and immunology are the most common applications since the beginning of Flow Cytometry. However, applications of cell function measurements and measurements of cell components other than DNA are recently becoming more and more important.

Mainly a flow cytometer potentially allows for fast, sensitive and quantitative cytochemical measurements of any individual cell component that is specifically stained by distinct fluorochromes and monoclonal antibodies. Furthermore, a flow cytometer can also exploit properties of scattering, absorption and auto fluorescence, it can simultaneously acquire multiple parameters corresponding to different cell compartments of the same cells. Simultaneous measurement of nuclear DNA or RNA, and nuclear or cytoplasmic proteins, as well as membrane antigens can be achieved. The simultaneous acquisition of multiple parameters in flow cytometry is made out mainly to characterize heterogeneous cell populations. Cell sorting makes possible purification of homogenous cell, which then can be explored by other techniques.

Flow Cytometry in Cancer Detection

Flow cytometry permits high-speed analysis and sorting (1000 cells/sec) depending on morphological, biophysical, molecular, and functional cell features correlating them with each other in multi-parameter computer-aided instruments. All Flow cytometry examinations require quantitative cell staining, cell monodispersion, and high resolution analysis. For Flow cytometry to probe the heterogeneity of human cancer effectively the unequivocal discrimination of tumor from normal cells is a major requirement so that we can investigate the phenotypic diversity of tumor cells and the interaction with adjacent normal cells.

The pathways and the protein targets in cancer disease are greatly understood today, the application of biomarkers is resulting in more advanced knowledge about the disease process leading to progress in developing more effective drugs with minimum toxicity.

Measurement of DNA Content

Using probes which can distinguish tumor and normal cells and valuate differentiative as well as proliferative tumor cell properties, the objective elucidation of tumor cell heterogeneity and the tumor-host cell interaction can be achieved through quantitative cytology in the form of flow cytometry.

Abnormal nuclear DNA content is a definitive marker of malignancy. Benign and malignant human neoplasms sometimes contain clones with abnormal DNA content. Many models of DNA content aneuploidisation and evolution have been proposed but the exact mechanisms are not clearly understood. The extent of DNA aneuploidy relative to DNA diploid cells known as DNA index (DI) is a usual parameter derived from DNA histograms.

To analyze the existence of ploidy abnormality in human tumors we use DNA-specific fluorescent dyes. In Flow Cytometry cell proliferative activity does not affect the ploidy analysis unlike mitotic karyotyping but is limited only by cell preparative and staining protocols. In recent years a lot of progress has been made and the measurements of DNA content can now be achieved with high resolution and large reproducibility.

There is seen a noted variations in occurrence of DNA-defined aneuploidy. Flow cytometry studies have found that normal and reactive tissues and benign tumors show normal diploid DNA content. In some premalignant conditions like angioimmunoblastic lymphadenopathy and preleukemia and benign monoclonal gammopathy aneuploidy was detected. Diploid content is observed in chronic lymphocytic leukemia, Hodgkin's disease, and benign phase of chronic myelogeneous leukemia.

A 15 to 30% aneuploidy rate in acute leukemia and in indolent non-Hodgkin's lymphoma; a 50 to 80% aneuploidy rate in aggressive lymphoma and in multiple myeloma; and a 60 to 100% aneuploidy rate in solid tumors has been noticed (Bariogie et al., 1983).

Because of its low degree of dispersion, sensitive and selective association with neoplastia lesions and stable expression DNA content abnormality is generally used for tumor diagnosis and for detecting rare neoplastic cells in early stages of the disease.

Cellular RNA Content

Cellular or nuclear RNA content estimation gives useful information regarding transitions of cells between quiescent and proliferative states. It is known that the nucleoli are enlarged in actively growing cells and that they are distinguished in the cancer cells. Nucleolar RNA synthesis especially rRNA synthesis, is low in resting cells and increases in stimulated cells for proliferation. The role of the nucleolus in the regulation of cell proliferation and in neoplastic transformation is of particular interest in morphological investigations. Cellular RNA content is valuable for the objective differentiation of acute leukemia and of multiple myeloma. Andreeff et al introduced the method to distinguish acute lymphoblastic leukemia from acute myeloid leukemia. Following DNA digestion, propidium iodide staining confirms Frankfurt's notion that double stranded RNA content measurement in this way is a function of cellular proliferation and is very high in malignant cells than normal cells. RNA content is now being studied by using pyronine or acridine orange. The Sloan-Kettering group worked with acridine orange and has wholly published its experience.

RNA measurements have also been found to be useful for the distinction of low- and high-grade non-Hodgkin's lymphomas (Bariogie et al., 1983).

Cell Size Related Phenotypic Markers

Many cellular properties are related to cell size, like RNA and total protein content and forward-angle light scatter. Measurement of cell size is achieved by electronic Coulter volume analysis as well as by Forward-angle light scatter analysis. Two parameter analyses of DNA content and Coulter volume for the differentiation of low and high grade lymphomas was demonstrated by Shackney et al. Lymphomas which were constituted of cells with larger Coulter volumes exhibited higher proliferative activity and matched with the higher-grade histological subtypes. Forward-angle light scatter analysis is useful in distinguishing major marrow lineages and peripheral blood cells. Multi-parameter studies by Nicola et al considering lectin binding properties demonstrated enhancement of hemopoietic stem cells through these parameters.

Cytoplasmic Immunoglobulin

Cytoplasmic immunoglobulin is a characteristic feature of plasma cells and it can readily be determined by performing direct or indirect immunofluorescence, this is very useful for multiple myeloma specifically for the classification of nonsecretory myeloma. Unlike RNA content measurements, cytoplasmic immunoglobulin determination has the potential for more effective discrimination of DNA content-derived cell cycle distribution of normal and tumor marrow cells. This happens because cytoplasmic immunoglobulin is restricted to pre-B-cells and to plasma cells.

Plasma cells characterize the last stage of B lymphocyte differentiation and recognized by the occurrence of monotypic immunoglobulin in the cytoplasm. The plasma cells of multiple myeloma patients mostly have an abnormal DNA content and higher RNA content allowing flow cytometric quantitation of plasma cells.

Flow cytometric analysis of abnormal lymphocyte populations in chronic lymphocytic leukemia (CLL) has been widely reported to show weak expression of surface immunoglobulin [5]. It is seen that almost all cases of multiple myeloma are often preceded by asymptomatic monoclonal gammopathies.

Two-parametric flow cytometry of cytoplasmic light-chain immunoglobulin and DNA can be used for prediction of survival in a newly diagnosed Multiple Myeloma treatment. It provides efficient prognostic data for AMG and also helps in many ways in research and management of myeloma. It aids us in distinguishing patients with "low producing" and "low-secretary" myeloma. It brought into focus that development of plasma cell abnormality is accompanied by a persistent decrease in the production potential of immunoglobulin.

Cytokinetic Markers

Enhanced cell proliferation, an important hallmark of cancer can be easily identified using flow cytometry. DNA analysis through Flow cytometry allows rapid and accurate measurement of large number of cells for assessing the proliferation status. Evaluation of cell cycle kinetics, proliferation and apoptosis in human cancer permits differentiation of low and high grade malignant lymphomas. The S phase cell fraction from DNA histograms deduced by flow cytometry indicates that this fraction increases with rising DNA excess in many solid tumors. By studying the uptake of tritiated thymidine the magnitude of slowly or non cycling cells is computed and the cell cycle distribution can be evaluated by flow cytometry analysis of DNA content.

Cell Surface Membrane Antigens

With the application of hybridoma generated monoclonal antibodies, surface membrane antigens are now being largely studied in lymphoid and myeloid neoplasms as a phenotypic cell marker and have recently been found important in vivo and in vitro therapeutic application. Surface marker analysis by Flow Cytometry has brought forward the therapeutic use of monoclonal antibodies for the treatment of lymphomas and leukaemias.

An interesting new application of analysis has been introduced by Ault et al. introduced a new application in surface membrane immunoglobulin analysis, they demonstrated monoclonal light chain excess among peripheral blood lymphocytes extracted from malignant lymphoma patients. The reason behind this phenomenon is not known but similar studies of myeloma raises an interesting possibility circulating tumor cells express such monoclonal excess.

Circulating Tumor Cells

Circulating tumor cells (CTCs) were first spotted in 1869 by Thomas Ashworth and have since been of great significance in defining the metastatic spread of carcinomas. CTCs are cells which have disunited from the primary tumor and flow into the blood or lymphatic circulation producing a secondary tumor.

Current advancements in technology now reproducible detection of CTCs by a simple blood test. The number of CTCs can be used as a predictive marker for cancer progression. Researchers have created several assays to detect CTCs for cancer diagnosis. High CTC counts depict aggressive cancer, higher metastasis, and decreased relapse time. As blood collection is very easy, CTCs prove to be a useful marker for cancer progression and survival. CTCs can also help in therapeutic management, therapy effectiveness and give knowledge about drug resistance mechanisms.

The molecular characterization of CTCs offers a unique ability to assess genotypic and phenotypic features of a cancer without the need for invasive biopsy [16]. CTC isolation techniques rely on antibodies against epithelial cell-adhesion molecule (EpCAM), EpCAM is a protein that sticks out of the surface of CTCs not in healthy cells. Methods derived from immunocytochemistry and reverse transcriptase–polymerase chain reaction have also been useful in the detection and characterization of CTCs but neither technique leads to direct isolation of CTCs. These methods are generally deficient for any kind of functional characterization because they will need a cell-fixation step, which makes it unfeasible to preserve CTC viability.

The Cell Search system enables the reliable detection of CTCs in blood and is suitable for the routine assessment of metastatic breast cancer patients in the clinical laboratory. Blood samples should be shipped at room temperature and CTC counts are stable for at least 72 h [17]. Cell Search platform (Veridex LLC, Huntingdon Valley, PA, USA) and is currently the most widely practiced cytometric technology and the only one that is FDA approved for the counting of CTCs.

Cancer Stem Cell

Recently, a form of cancer cell called the cancer stem cell (CSC) has been observed and noted for tumors. CSCs might be accountable for the reoccurrence of a tumor after a successful therapy and are believed to stand a rich metastatic potential. For the progress of competent treatment strategies, formation of dependable methods for the recognition and proficient isolation of CSCs is really important. Equivalent to their stem cell counterparts in bone marrow or small intestine, several differentiation surface antigens have been defined, thus letting researchers to recognize them in the tumor mass and to find out their degree of differentiation. Moreover, functional properties and characteristic of CSCs can be examined.

Side population analysis is based on the stem cell-specific activity of certain ATP-binding cassette transporter proteins, which are able to transport fluorescent dyes out of the cells. Furthermore, the stem cell-specific presence of aldehyde dehydrogenase isoform 1 can be used for CSC labeling (Greve, Kelsch, Spaniol, Eich & Gotte, 2012). The method of choice for the examination of Cancer Stem Cells is Multiparametric flow cytometry. It enables the simultaneous analysis of varying cellular features with high performance and reliability. More than that, it enables the separation of living cells on the basis of marker expression or functional properties by fluorescence-activated cell sorting. The main plus point of this technique is its capability to isolate rare cells, which is a requirement for identifying small cell populations within the tumor bulk. Quantification is possible too and can be attained either by the addition of count check beads to the sample or by volume-based flow cytometry.

Sorting of CSCs

The mechanical sorting systems are completely isolated, thus cutting out the risk of aerosol

generation. FACS-Calibur, Becton Dickinson, NJ, operates with a catcher tube located in the upper part of the flow cuvette. If the analyzing unit finds out a cell as a sorting target, the mechanical unit enters the flow stream, collects the cell, and brings it into another separate tube.

Future Prospects of Flow Cytometry

Enumeration of circulating prostate micro particles (PMPs), a type of Extracellular vesicles discharged by prostate cancer in urine, blood and seminal fluid, may constitute a non-invasive method to prioritize and distinguish patients of prostate cancer with intermediary risk and high risk. Conventional flow cytometry isn't designed to analyze submicron events as the optics can detect light scatter from greater than 3 microns. New generation tools like nanoscale flow cytometry are able to examine cases 100-1000nm in diameter. Nanoscale flow cytometry of EVs in plasma, serum, or urine provides high-content information in a high-throughput manner.

Bead-based flow cytometric assays with high sensitivities could ease the detection of low abundant proteins, making better our knowledge of biological pathways and helps in disease diagnosis at early stages. Micro-bead based multiplexed protein immunoassays have rapidly grown in the past 10 years.

New creative concepts in radiation-induced signaling processes and the integration of radiation with new targeted agents are considerable areas for future flow cytometry-based research. Additionally high-throughput assays for radiation-associated population studies are very likely to be formed sooner.

Conclusion

The advancement and application of flow cytometry-based technologies is causing high impacts on the diagnosis, monitoring, and prognostication of patients with cancer and also classification of the disease. Flow Cytometry has had significant effects on patients receiving solid organ allografts and allogeneic hematopoietic stem cell transplants.

The capability of flow cytometry to bring forth huge amounts of multi dimensional, high-complexity data has certainly placed this technology as a major platform for use in clinical pharmacology for years to come. The instruments are getting smaller as well as less expensive and number of clinically useful antibodies is growing day by day, this is creating more chances for clinical laboratories to adapt flow cytometry in the diagnosis and prognosis of the disease.

The rising field of "cytomics" represents a structured, whole-cell–based elucidation of cellular physiology that encompasses aspects of genomics and proteomics and it attempts to design a clear comprehensive picture of individual cells. The flow cytometer can be seen as a rational platform for building the foundation of this emerging field.

Key Messages

"Flow cytometry analysis provides important diagnostic and prognostic information required to select distinct therapies. A review of studies reported over the past 6 years assessing flow cytometry, showed high sensitivity of flow cytometry compared with standard cytology for cancer detection"

"Flow cytometry is specifically important in situations when the investigator wishes to differentiate between multiple dissimilar cell types within a heterogeneous population and evaluate their frequencies or the expression of a particular molecule of interest."

"The pathways and the protein targets in cancer disease are greatly understood today, the application of biomarkers is resulting in more advanced knowledge about the disease process leading to progress in developing more effective drugs with minimum toxicity."

References

1. Barthel B, Martin N, Schumann J, Johnson T, Drewinko B, Swartzendruber D, Gohde W, Andreeff M, and Freireich E . Flow Cytometry in Clinical Cancer Research. Cancer Research 1983 Sep;43:3982-3997.
2. Verschoor C, Lelic A, Bramson J and Bowdish E. An Introduction to Automated Flow Cytometry Gating Tools and Their Implementation. Frontiers in Immunology 2015;6:380. doi:10.3389/fimmu. 2015.00380.
3. Jaye D , Bray R, Gebel H, Wayne, Harris A and Waller E. Translational Applications of Flow Cytometry in Clinical Practice. J Immunol 2012; 188:4715-4719; doi: 10.4049/jimmunol.1290017.
4. Giaretti W. Origins of ... Flow cytometry and applications in oncology. J Clin Pathol 1997;50:275-277. doi : 10.1136/jcp.50.4.275.
5. Lewis R, Cruse J, Pierce S, Lam J, Tadros Y. Surface and cytoplasmic immunoglobulin expression in B-

cell chronic lymphocytic leukemia. Experimental and Molecular Pathology. http://dx.doi.org/10.1016/j.yexmp.2005.04.009

6. Papasotiriou, I., Chatziioannou, M., Pessiou, K., Retsas, I,. Dafouli, G., Kyriazopoulou. Detection of Circulating Tumor Cells in Patients with Breast, Prostate, Pancreatic, Colon and Melanoma Cancer: A Blinded Comparative Study Using Healthy Donors. Journal of cancer therapy 2015 doi: 10.4236/jct.2015.67059.
7. Gupta V, Zhang Q, and Liu Y. Evaluation of Anticancer Agents Using Flow Cytometry Analysis of Cancer Stem Cells. Methods Mol Biol. 2011;716: 179–191. doi:10.1007/978-1-61779-012-6_11.
8. Lu Y, Liang H, Yu T, Xie J, Chen S, Dong H, Sinko P, Lian S, Xu J, Wang J, Yu S, Shao J, Yuan B, Wang L, and Jia L . Isolation and Characterization of Living Circulating Tumor Cells in Patients by Immuno-magnetic Negative Enrichment Coupled With Flow Cytometry. Cancer 2015, doi: 10.1002/cncr.29444
9. Greve B, Kelsch R, Spaniol K, Theodor H, Gotte M. Flow Cytometry in Cancer Stem Cell Analysis and Separation. Published online 6 February 2012 in Wiley Online Library (wileyonlinelibrary.com) doi: 10.1002/cyto.a.22022.
10. Siddiqui K, Pardhan S. Prostate extracellular vesicles in patient plasma as a liquid biopsy platform for prostate cancer using nanoscale flow cytometry. Oncotarget Jan 2016. doi: 10.18632/oncotarget.6983.
11. Mannello F. Understanding breast cancer stem cell heterogeneity: time to move on to a new research paradigm. BMC Medicine 2013;11:169. http://www.biomedcentral.com/1741-7015/11/169.
12. Ahluwalia, M., Wallace, P., & Peereboom, D. Flow Cytometry as a diagnostic tool in Lymphomatous or Leukemic Meningitis. Cancer, 2012;118(7):1747-1753. doi: 10.1002/cncr.26335.
13. Li, J., Ji, L., Chen J., Zhang, W., & Ye, Z. Wnt/5ØýÞ-Catenin Signaling pathway in Skin Carcinogenesis and Therapy. BioMed Research International Volume 2015, Article ID 964842, http://dx.doi.org/10.1155/2015/964842.
14. Zhang, B, Yang, J, Zou, Y, Chen, M, Hong, G, Dai, H. Plasmonic micro-beads for fluorescence enhanced, multiplexed protein detection with flow cytometry. Chemical science 2014 doi: 10.1039/C4SC01206B.
15. Miller, M.C., Doyle, G.V. and Terstappen, L.W. Significance of circulating tumor cells detected by the Cell Search system in patients with metastatic breast colorectal and prostate cancer. JJ Oncol. 2010;2010:617421. doi: 10.1155/2010/617421.
16. Krebs M, Hou J, Ward T, Blackhall F, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. Ther Adv Med Oncol 2010;2(6):351-365 doi: 10.1177/1758834010378414.
17. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, Janni W, Coith C, Beck K, Janicke F, Jackson S, Gornet T, Cristofanilli M and Pantel K. Detectionof Circulating Tumor Cells in Peripheral Blood of Patients with Metastatic Breast Cancer: A Validation Study of the Cell Search System. Clin Cancer Res 2007, doi:10.1158/1078-0432.CCR-06-1695.
18. Barlogie B, Alexanian R, Pershouse M, Smallwood L, and Smith L. Cytoplasmic Immunoglobulin Content in Multiple Myeloma. J. Clin. Invest (1985). 002 1-9738/85/08/0765/05.
19. Bhatt A N, Mathur R, Farooque A, Verma A and Dwarakanath B S. Cancer biomarkers - Current perspectives. Indian J Med Res 2010 Aug;132; 129-149.
20. Plaks V, Koopman C D and Werb Z. Circulating Tumor Cells. Science. 2013 Sept;13:341(6151): doi:10.1126/science.1235226.
21. Williams SCP, Circulating tumor cells. www.pnas.org/cgi/doi/10.1073/pnas.1304186110.
22. Papanikolaou X, Rosenthal A, Dhodapkar M, EpsteinJ, Khan R, Rhee1 F, Jethava Y, WaheedS, Zangari M, Hoering A, Crowley J, Alapat D, Davies F, Morgan G and Barlogie B. Blood Cancer Journal 2016;6:e410; doi:10.1038/bcj.2016.19.

Review Article

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Analysis of the Tests for New Life Extending Cancer Drugs

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Abstract

Since many decades basis of research for the detection of the cancer biomarkers is opted by many researcher to identify new drug for cancer. Specific biomarker assessment for the cancer will help in the diagnostic approach for the cancer diagnosis along with the assessment of the progress of the treatment. There are many cases where the association of the cancer with specific biomarker is possible. The example for reference may be the two types of colony stimulating factors, G-CSF and M-CSF. M-CSF promotes the production and activation of Monocytes, and G-CSF stimulates the production of granulocytes from stem cells in the bone marrow. G-CSF and GM-CSF, which are produced by recombinant DNA technology, have been approved by the FDA for use in patients with bone marrow depression, such as cancer patients and patients who receive bone marrow transplants. In these patients it is observed that the bone marrow depression is usually associated with intensive chemotherapy or irradiation, and the use of colony stimulating factors avoids the profound and prolonged neutropenia as seen in controls not receiving these compounds, with a corresponding decrease in morbidity and mortality. On the other hand, administration of G-CSF or GM-CSF is expensive and can be associated with severe side effects. The deployment of the new life extending cancer drugs is needed to be measured for its efficacy. The effect of drugs may be detected through the detection of the low amount of the secondary associated biomarkers of the cancer. The various oncologists have different opinion in use of the detection of the immunogenic biomarkers with cancers.

Keywords: B-Cell Signature; Biomarkers; Cancer; Immunogenic; T-Cell Signature.

Introduction

The human body consists of 1013 cells, and these are differentiated into many different types that form the different organs, and these differentiated into the many organs: skin, liver, kidney, blood cells and so on. It is the basis of research since long time for the detection of the cancer biomarkers. Specific biomarker assessment for the cancer can lead to the diagnostic approach for the cancer diagnosis as well as assessment of the progress of the treatment. There are many examples of association of the cancer with specific biomarker. The example for reference may be the two types of colony stimulating factors, G-CSF and M-CSF. M-CSF promotes the production and activation of Monocytes, and G-CSF stimulates the production of granulocytes from stem cells in the bone marrow. G-CSF and GM-CSF, which are produced by recombinant DNA technology, have been approved by the FDA for use in patients with bone marrow depression, such as cancer patients and patients who receive bone marrow transplants. In these patients it is observed that the bone marrow depression is usually associated with intensive chemotherapy or irradiation, and the use of colony stimulating factors avoids the profound and prolonged neutropenia as seen in controls not receiving these compounds, with a corresponding

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decrease in morbidity and mortality. On the other hand, administration of G-CSF or GM-CSF is expensive and can be associated with severe side effects.

Context

The various immunogenic signatures associated with cancerous cells includes the "Immune Score" in tumor microenvironment, the T-cells signatures, frequency of tumor-specific t cells in the circulation, apoptosis of CD8+ T Cells, differentiation Status of CD8+ T Cells, B-cell signature, suppressor cells in the tumor microenvironment, the neutrophils-to-lymphocyte ratio, cytokine expression and levels, tumor-derived exosomes, Programmed death-ligand 1 (PD-L1)

"Immune Score" in the Tumor Microenvironment

It is studied by the Whiteside in 1993 that nearly all the human solid tumors always infiltrated by immune cells (Whiteside, 1993). The composition and extent of these inflammatory infiltrates may vary among tumors. It was well established that the tumor microenvironment have a strong impact on immune cells, their identity, phenotype, localization, and density of immune cells present in the tumor has long been considered by immunologists to be critically important for tumor progression (von Kleist et al, 1987). Immune cells infiltrating human solid tumors have been extensively studied and found to exhibit unique phenotypic and functional characteristics (Pages et al., 2010; Fridman et al., 2012).

The T-Cell Signature

Naito et al, 1998 shown that the typing of tumor-infiltrating T lymphocyte (TIL) cells by immuno-histochemistry (IHC) and microscopic enumeration of these cells have been initially utilized to establish correlations between CD3+ CD8+ T-cell infiltrations and prognosis. The study of Sato et al, 2005 supports the potential significance of CD8+ T cells as predictors of risk. The human TIL were found to be functionally impaired relative to peripheral blood T cells of patients or of normal donors (Frey and Monu, 2008; Whiteside, 2010) and, in some instances, TIL were shown to contribute to tumor progression (Whiteside, 2006).

The Frequency of Tumor-Specific T Cells in the Circulation

In addition to scoring T cells at tumor sites, the frequency and functions of T cells circulating in the peripheral blood of cancer patients have been examined as potential biomarkers. The use of standardized single-cell assays able to detect tumor-antigen-specific T cells (ELISPOT, cytokine flow cytometry (CFC), and tetramer binding) has facilitated evaluation of epitope-specific T cells as potential biomarkers (Britten et al, 2011). These assays, especially ELISPOT, have been standardized for serial monitoring and can be reliably utilized to measure the frequency of epitope-specific T cells in blood or body fluids.

Apoptosis of CD8+ T Cells

Tumor-derived factors associated with the induction of death of immune cells at the tumor sites and in the peripheral circulation (Whiteside, 2010). The frequency of CD8+ T cells undergoing spontaneous apoptosis in the blood of patients with cancer was found to be significantly elevated relative to that in sex- or age-matched healthy controls (Hoffmann et al., 2002). CD8+ T cells were preferentially targeted for cell death compared to circulating CD4+ T cells (Tsukishiro et al., 2003).

The Differentiation Status of CD8+ T Cells

The functional potential of tumor epitope-specific T cells in situ or in the peripheral circulation of patients with cancer has been shown in some studies to correlate with outcome (Kirkwood et al., 2009) performing of functional immune assays is demanding and costly. A search for alternative biomarkers suggested that T-cell differentiation, as measured by the expression on CD8+ T cells of CCR7, a chemokine receptor for CCL19 and CCL21, discriminated cancer patients from normal controls (Czystowska et al., 2012).

The B-Cell Signature

Considerable evidence has existed for the presence of these cells in tumors, especially in breast cancer (Coronella et al., 2002). More recently, two independent reports have provided useful insights into the prognostic role of B cells in cancer.

Schmidt et al. (2008, 2012) have reported validation of the B-cell signature as the most robust prognostic factor in breast cancer and other human tumors. These investigators identified the immunoglobulin G kappa chain (IGKC) as an immunologic biomarker of prognosis and response to chemotherapy in hundreds of patients with breast cancer, non-small lung cancer, and CRC (Schmidt et al., 2012).

Suppressor Cells in the Tumor Microenvironment

Accumulations of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) in human tumors and their increased frequency in the circulation of cancer patients have been widely reported (Marigo et al., 2008; Whiteside, 2012). In ovarian carcinoma, melanoma, breast cancer, and glioblastoma, the frequency of Treg among TIL correlated with tumor grade and reduced patient survival (Lanca and Silva-Santos, 2012).

The Neutrophil-to-Lymphocyte Ratio

The chronic inflammation is closely associated with the development of specific human cancers. For example, inflammatory bowel disease predisposes to the development of CRC, and human papilloma-virus (HPV) infection is associated with oropharyngeal squamous cell carcinoma. Evidence has accumulated that the total white blood count and especially the high neutrophil-to-lymphocyte ratio (NLR) measured before oncological therapies predict adverse clinical outcome in patients with lung, breast, renal, ovarian, and HNC (Perisanidis et al., 2013).

Cytokine Expression and Levels

Cytokine gene or protein profiling, whether by multiplex immunoassays, microarrays, or proteomics technologies, is uniquely well suited to evaluations of the tumor microenvironment. Given the critical role it has in shaping local and systemic immune responses, events, and interactions between cells found in this milieu are of prime interest. Cytokines and chemokines mediate these interactions. Therefore, the potential for capturing polarization in the cytokine repertoire or differences in patterns of their production by immune or tumor cells and of relating them to a specific clinical response has a tremendous appeal.

Tumor-Derived Exosomes

Tumor-derived exosomes have recently come into the limelight as potential biomarkers in cancer. These membranous nano-vesicles (50–100nM in diameter) carry a large variety of cellular components, including proteins, RNA, microRNA, and DNA (Iero et al., 2008; Whiteside, 2013). TEX molecular content closely reflects that of tumor cells from which they originate, and thus TEX can serve as a sort of "liquid biopsy" in place of a conventional tissue biopsy. For this reason, molecular profiles of TEX are of great current interest.

Programmed Death-Ligand 1 (PD-L1)

Programmed death-ligand 1 (PD-L1) also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene (Chemnitz JM,2004).

Programmed death-ligand 1 (PD-L1) is a 40kDa type 1 transmembrane protein, and it is assumed that it played a significant role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. In usual case, the immune system reacts to foreign antigens where there is some accumulation in the lymph nodes or spleen which triggers a proliferation of antigen-specific CD8+ T cell and the formation of PD-1 receptor / PD-L1 or B7. The receptor /PD-L1 ligand complex transmits an inhibitory signal which reduces the proliferation of these CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen-specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2. (http://www.ncbi.nlm.nih.gov)

Test for deploying new life-extending cancer drugs is the new ray of hope for assessment of the cancer treatment progression. Before any diagnostic application of the same for the patients, intensive researches on the efficacy of the biomarker need to be done. This was the topic of debate over last many decades that whether and how the host immune system influences cancer development. The animal model studies on cancer reveal that cancer strongly supports the role of anti-tumor immunity in cancer development, progression, and therapy but the evidence from human clinical trials is not clear or straightforward. The underlying reason behind this may be in large part due to profoundly immunoinhibitory effects human tumors exert (Huang et al., 2008; Whiteside, 2008b). The study by Whiteside et al., 2012 revealed that cancer patients at best mount weak anti-tumor immune responses which are ineffective in controlling cancer progression. The tumor-induced immune suppression may promote tumor escape hence becomes a significant problem for cancer therapy (Whiteside et al., 2012).

Hence the measurement of (a) the degree of tumor-induced immune suppression by identifying a decrease in or absence of an anti-tumor immune response or (b) the degree of recovery from immune suppression after successful therapy (i.e., normalization of defective anti-tumor immune responses) will be beneficial. Both these approaches have been used in clinical trials with the hope that

intermediate biomarkers of immune suppression, as well as biomarkers of therapy-induced recovery, can be identified. Because of the complexity of host-tumor interactions and limited immune monitoring capabilities, both have been difficult to implement in practice. In a limited number of cases, such immune alterations have been shown to correlate with clinical outcome suggesting that upon validation, they might serve as future biomarkers of prognosis or response to therapy.

Discussion

Investigation for the progress of treatment through new anti-cancer drugs can be assessed by the assessment of the associated biomarker level. The new drug that boosts the immunity then it is possible to evaluate the biomarker related to the immune system as well as the development of cancer. In this context, the PD-L1 is the biomarker of choice. The understanding about its mode of actions needs to be more clear before deploying it as the test for implementing new life-extending drugs. It is well studied that the PD-L1 binds to its receptor PD-1 which is present on the activated T cells, B cells, and myeloid cells to modulate activation or inhibition. The affinity between PD-L1 and PD-1, as defined by the dissociation constant Kd is 770nM. Interestingly, PD-L1 also has an appreciable affinity for the co-stimulatory molecule CD80 (B7-1), but not CD86 (B7-2) (Butte MJ et al., 2008). CD80's affinity for PD-L1, 1.4μM, is in between its affinities for CD28 and CTLA-4 (4.0μM and 400nM, respectively). The related molecule PD-L2 has no such affinity for CD80 or CD86, but shares PD-1 as a receptor (with a stronger Kd of 140nM). Said et al. showed that PD-1, up-regulated on activated CD4 T-cells, can bind to PD-L1 expressed on monocytes and induced IL-10 production by the later (Elias A. Said et al. 2009).

Conclusion

The deployment of the new life-extending cancer drugs is needed to be measured for its efficacy. The effect of drugs may be detected through the detection of the low amount of the secondarily associated biomarkers of cancer. The up-regulation of PD-L1 may allow cancers to evade the host immune system. Thompson RH in 2004 found that on analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5-fold increased risk of death. The Ovarian cancer patients with higher expression of PD-L1 had a significantly poorer prognosis than those with lower expression. PD-L1 expression correlated inversely with intraepithelial CD8+ T-lymphocyte count suggesting that PD-L1 on tumor cells may suppress anti-tumor CD8+ T cells (Hamanishi J, et al., 2007). Following the FDA approval of some PD-1 inhibitors for cancer treatment, clinical trials have begun for PD-L1 inhibitors (http://www.medpagetoday.com, 2015). The effect might be tumor type dependent; a study on patients with non-small cell lung cancer showed that greater PD-L1 protein and mRNA expression is associated with increased local lymphocytic infiltrate and longer survival(Velcheti V, Jan 2014). The various oncologists have the different opinion in use of the detection of the immunogenic biomarkers with cancers.

References

1. Butte MJ, Peña-Cruz V, Kim MJ, Freeman GJ, Sharpe AH. "Interaction of human PD-L1 and B7-1". Mol Immunol. 2008 Aug;45(13):3567–72. DOI:10.1016/j.molimm.2008.05.014. PMID 18585785.
2. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. "SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of rogrammed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation". Journal of Immunology 2004 July;173(2):945–54. DOI:10.4049/jimmunol.173.2.945. PMID 15240681.
3. http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=29126#genomic-context, accessed on 03-10-2015.
4. Elias A. Said *et al*. PD-1 Induced IL10 Production by Monocytes Impairs T-cell Activation in a Reversible Fashion" Nature Medicine 2010;452-9.
5. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, Krejci KG, Lobo JR, Sengupta S, Chen L, Zincke H, Blute ML, Strome SE, Leibovich BC, Kwon ED. "Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target". Proc Natl Acad Sci USA 2004 Dec;101(49):17174–9. DOI:10.1073/pnas.0406351101. PMC 534606. PMID 15569934.
6. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, Honjo T, Fujii S. "Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer". Proc Natl Acad Sci USA 2007 Feb;104(9):3360–5. DOI:10.1073/pnas.0611533104. PMC 1805580. PMID 17360651

7. http://www.medpagetoday.com/MeetingCoverage/AACR/38388?utm_content=&utm_source=WC&xid=NL_DHE_2013-04- 11&eun= g431352d0r&mu_id=5529190,accessed on 03-10-2015.
8. Huang B., Zhao J., Unkeless J. C., Feng Z. H., Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. Oncogene 2008;27:218–224. DOI:10. 1038/onc.2008.208 [PubMed].
9. Velcheti V. "Programmed death ligand-1 expression in non-small cell lung cancer". Lab Invest. 2014 Jan;94(1):107–115. DOI:10.1038/labinvest.2013. 130. PMID 24217091.
10. Whiteside T.L. The tumor microenviron-ment and its role in promoting tumor growth. Oncogene 2008b;27:5904–5912. DOI:10.1038/onc.2008.271 [PMC free article] [PubMed].
11. Whiteside T.L. What are regulatory T cells (Treg) regulating in cancer and why? Semin. Cancer Biol. 2012;22:327–334.DOI:10.1016/j.semcancer.2012.03.004 [PMC free article] [PubMed].
12. Britten C. M., Janetzki S., Van Der Burg S. H., Huber C., Kalos M., Levitsky H. I., et al. Minimal information about T cell assays: the process of reaching the community of T cell immunologists in cancer and beyond. Cancer Immunol. Immunother. 2011;60:15–22. DOI:10.1007/s00262-010-0940-z [PMC free article] [PubMed].
13. Coronella J. A., Spier C., Welch M., Trevor K. T., Stopeck A. T., Villar H., et al. Antigen-driven oligoclonal expansion of tumor-infiltrating B cells in infiltrating ductal carcinoma of the breast. J. Immunol. 2002;169:1829–1836 [PubMed].
14. Czystowska M., Gooding W. E., Szczepanski M. J., Lopez-Abaitero A., Ferris R. L., Whiteside T. L al. The immune signature of CD8+CCR7+ T cells in the peripheral circulation associates with disease recurrence in patients with HNSCC. Clin. Cancer Res. 2012;19:889–899. DOI:10.1158/1078-0432.CCR-12-2191 [PMC free article] [PubMed] [Cross Ref].
15. Frey A. B., Monu N. Signaling defects in anti-tumor T cells. Immunol. Rev. 2008;222:192–205. DOI: 10.1111/j.1600-065X.2008.00606.x [PMC free article] [PubMed].
16. Fridman W. H., Pages F., Sautes-Fridman C., Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat. Rev. Cancer 2012;12:298–306. DOI:10.1038/nrc3245 [PubMed].
17. Iero M., Valenti R., Huber V., Filipazzi P., Parmiani G., Fais S., et al. Tumour-released exosomes and their implications in cancer immunity. Cell Death Differ. 2008;15: 80–88. DOI:10.1038/sj.cdd.4402237 [PubMed].
18. Kirkwood J. M., Lee S., Moschos S. J., Albertini M. R., Michalak J. C., Sander C., et al. Immunogenicity and antitumor effects of vaccination with peptide vaccine±granulocyte-monocyte colony-stimulating factor and/or IFN-alpha2b in advanced metastatic melanoma: eastern cooperative oncology group Phase II Trial E1696. Clin. Cancer Res. 2009;15:1443–1451. DOI:10.1158/1078-0432.CCR-08-1231 [PMC free article] [PubMed].
19. Naito Y., Saito K., Shiiba K., Ohuchi A., Saigenji K., Nagura H., et al. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res. 1998;58:3491–3494 [PubMed].
20. Pages F., Berger A., Camus M., Sanchez-Cabo F., Costes A., Molidor R., et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N. Engl. J. Med. 2005;353:2654–2666. DOI:10.1056/NEJMoa051424 [PubMed].
21. Perisanidis C., Kornek G., Poschl P. W., Holzinger D., Pirklbauer K., Schopper C., et al. High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. Med. Oncol. 2013;30:334.DOI:10.1007/s12032-012-0334-5 [PubMed].
22. Schmidt M., Bohm D., Von Torne C., Steiner E., Puhl A., Pilch H., et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res. 2008;68:5405–5413. DOI:10.1158/0008-5472.CAN-07-5206 [PubMed].
23. Schmidt M., Hellwig B., Hammad S., Othman A., Lohr M., Chen Z., et al. A comprehensive analysis of human gene expression profiles identifies stromal immunoglobulin kappa C as a compatible prognostic marker in human solid tumors. Clin. Cancer Res. 2012;18:2695–2703. DOI:10.1158/1078-0432.CCR-11-2210 [PubMed].
24. Tsukishiro T., Donnenberg A.D., Whiteside T.L. Rapid turnover of the CD8(+)CD28(-) T-cell subset of effector cells in the circulation of patients with head and neck cancer. Cancer Immunol. Immunother. 2003;52:599–607. DOI:10.1007/s00262-003-0395-6 [PubMed] [Cross Ref].
25. Von Kleist S., Berling J., Bohle W., Wittekind C. Immunohistological analysis of lymphocyte subpopulations infiltrating breast carcinomas and benign lesions. Int. J. Cancer 1987;40:18–23. DOI:10.1002/ijc.2910400105 [PubMed] [Cross Ref].
26. Whiteside T.L. Tumor Infiltrating Lymphocytes in Human Malignancies: Medical Intelligence Unit. Austin, TX: R. G. Landes, Co. 1993.
27. Whiteside T.L. The role of immune cells in the tumor microenvironment. Cancer Treat. Res. 2006;130:103–124.DOI:10.1007/0-387-26283-0_5 [PubMed].
28. Whiteside T.L. Immune responses to malignancies. J. Allergy Clin. Immunol. 2010;125:S272–S283. DOI:10.1016/j.jaci.2009.09.045 [PMC free article] [PubMed].
29. Whiteside T.L. Immune modulation of T cells and natural killer cells by tumor-derived exosomes. Biochem. Soc. Trans. 2013;41:245–251.DOI: 10.1042/BST20120265 [PMC free article] [PubMed].

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[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

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[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

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[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

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[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

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[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

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[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

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[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

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International Physiology	2	7500	7000	586	547
Journal of Animal Feed Science and Technology	2	78500	78000	6133	6094
Journal of Cardiovascular Medicine and Surgery	2	10000	9500	781	742
Journal of Forensic Chemistry and Toxicology	2	9500	9000	742	703
Journal of Geriatric Nursing	2	5500	5000	430	391
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Journal of Psychiatric Nursing	3	5500	5000	430	391
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New Indian Journal of Surgery	4	8000	7500	625	586
Ophthalmology and Allied Sciences	2	6000	5500	469	430
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