Correlation Between Clinical Stage of Solid Tumor and D Dimer as a Marker of Coagulation Activation

Ketut Suega, I Made Bakta

Department of Internal Medicine, Faculty of Medicine,Udayana University-Sanglah Hospital.
Jl. Kesehatan 1, Denpasar Bali, Indonesia

Correspondence mail to: *ksuega@yahoo.com*

ABSTRACT

Aim: *to examine the relationship between clinical stage of solid cancers and plasma D dimer value.*

Methods: *patients with solid cancer treated in Sanglah hospital who met study ctiteria were consecutively recruited and studied in order to examine the relationship between clinical stage of solid cancers and plasma D dimer value. Plasma D dimer was measured by ELISA (Nycocard) and TNM system to assign each patient into stage I,II,III and IV according to American Joint Committee on Cancer. Rank Spearman analysis was used to determine the relationship and one way Anova to compare the mean difference of D dimer between group of clinical stages.*

Results: *there were 79 patients included, mostly female (72,2%) and 57% was in age group of 40-59 years old. Level of D dimer > 500 ng/ml were found in 60 patients and 19 patients with D dimer < 500 ng/ml. The most frequent cancer was cervix (32.9%) then followed by nasopharyng cancer (16.5%). Clinical stage I,II,III, and IV were 6.3%, 16.5%, 53.2% and 24.1% respectively. Thrombocytosis (> 400.103/ uL) was found 50.6% as well as leukocytosis 62%. Although the differences of mean D dimer in each type of solid cancers were big enough but it was not statistically significant (p = 0.156). Plasma D dimer was positively correlated with clinical stage of solid cancers (r = 0.367; p = 0.001).*

Conclusion: *plasma D dimer level was positively correlated with clinical stage of solid cancers. High plasma D dimer could be a marker for advanced stage of a patient with solid cancer.*

Key words: *coagulation, D dimer, clinical stage, solid cancer.*

INTRODUCTION

Patients with cancer pose a major clinical challenge besides advance development of treatment modalities and more intensive care, which has led to prolonged survival during which complications may develop. Cancer is a prothrombotic state and thrombosis is one among frequent complications seen in cancer patients. Cancer patients account for as much as 20% of the total burden of venous thrombosis.[1] In a prospective register including 5451 patients with ultrasound-confirmed deep vein thrombosis (DVT), 32% of all patients suffered from cancer.[2] However, post mortem studies show a considerably higher incidence (30-50%) of venous thrombosis, the second most frequent cause of death in cancer patients.[3] Malignancies that commonly are associated with thrombosis are breast cancer, colon, and lung cancer. However, when adjusted for disease prevalence, the cancers most significantly associated with thrombotic disorders are derived from pancreas, ovary, and brain.[4] The underlying pathomechanism of thrombosis in cancer is multidimension and multicomplex rather than one unifying mechanism, and the etiology likely multifactorial. It is generally thought that both the tumor, through production of procoagulant factors, and the host, through its inflammatory response, take part in the processes. Much of the research in this area has focused on the intrinsic properties of tumor cells that lead to a prothrombotic state . The role of tissue factor (TF) has gathered the most attention.[1,4-6]

D dimer is a reactive marker of the haemostatic balance and an end product of the plasmin degradation process of the crosslinked fibrin clot. Systemic values of DD are an index of fibrin turnover in the circulation and are raised in a variety of clinical conditions. In addition to the diagnostic use of DD, it may also be of potential prognostic use in many conditions.[7] Elevated

D dimer levels have also been detected in patients with disseminated intravascular coagulation, vaso-occlusive crisis in sickle cell disease, thromboembolic events, and myocardial infarction. Accummulating evidence now suggests that critical oncogenic events may also trigger activation of the coagulation cascade, leading to a prothrombotic environment that not only manifest as venous thromboembolic disease but also promotes the growth and progression of the malignancy.[4,5]

Various solid tumor patients, including lung,prostate, cervical, and colorectal cancer patients are found with elevated D dimer level in the plasma. In patients with colorectal cancer, D dimer level has been shown to correlate with depth of tumor invasion at the time of surgical excision. Plasma D dimer level has also been shown to directly correlate with other tumor markers, including CA-125 and carcinoembryonic antigen. In one study D dimer was not associated with cancer-associated thrombosis, although other studies found solid tumor frequently followed thrombotic events.[8] Therefore, further study of D dimer as a risk factor for thrombosis among patients with active cancer may be useful. In this study we determine the relationship between coagulation activation indicators with clinical stage of solid cancers.

METHODS

A total of 79 solid tumor patients were enrolled in this cross sectional study. All of the solid tumors were treated in Sanglah hospital, consisted of several type of malignancies such as breast, lung, nasopharyng, cervix and others. Inclusion criteria were age >12 years of age, and consented to joint the study. Pregnancy, obesity, on-chemotherapy treatment, heart abnormalities, using oral contraception, and immobilization were all exluded. Sample size was determined using one sample formula for correlation coefficient.[9] Solid cancers were histologically proven. Clinical stage of cancer was based on TNM system according to The American Joint Committee on Cancer. Once the T, N and M are determined, they are combined and an overall stage of I,II,III,IV is assigned. Sometimes these stages are subdivided as well, using letters such as IIIA and IIIB. In this study we used stage I (T1N0M0), stage IIA (T0N1M0, T1N1M0, T2N0M0), stage IIB (T2N1M0, T3N0M0), stage IIIA (T0N2M0, T1N2M0, T3N1M0, T3N2M0), stage IIIB (T4N0M0, T4N1M0, T4N2M0), stage IIIC (anyTN3M0), stage IV (any T, any N, M1).[10-12] Demographic profile as well as other clinical variables were documented. Plasma D dimer assays were conducted using immunometric flowthrough sandwich ELISA (Nycocard Reader), with reference value <500 ng/ml. D dimer value was presented as continuum and dichotomous (less than 500 ng/ml and more than 500 ng/ml). Spearmen rank test was utilized to determine relationship between plasma D dimer and clinical stage of solid cancer. One way ANOVA to asses significant mean differences between clinical stage as well as between type of malignancies was also applied. Level of significance accepted at <0.5, and all statistical analyses were conducted using the SPSS, statistical package for Windows (SPSS16). This study was approved by ethical clearance committee of Sanglah Hospital/Udayana Medical School, and written informed consent was obtained.

Table 1. Characteristics of subjects

	N (%)	D Dimer	
		<500 ng/mL	>500 ng/mL
Sex			
- Male	22 (27.8)	5 (26.3)	17 (28.30
- Female	57 (72.2)	14 (73.7)	43 (71.7)
Age			
- 20 – 39	17 (21.5)	3 (15.8)	14 (23.3)
- 40 – 59	45 (57.0)	13 (68.4)	32 (53.3)
- > 60	16 (20.3)	3 (15.8)	13 (21.7)
Clinical stage			
- I	5 (6.3)	4 (21.1)	1 (1.7)
- II	13 (16.5)	6 (31.6)	7 (11.7)
- III	32 (53.2)	8 (42.1)	34 (56.7)
- IV	19 (24.1)	1 (5.3)	18 (30)
Type of malignancy			
- Nasopharyng	13 (16.5)	5 (26.3)	8 (13.3)
- Cervix	26 (32.9)	6 (31.6)	20 (33.3)
- Breast	9 (11.4)	2 (10.5)	7 (11.7)
- Lung	7 (8.9)	0	7 (11.7)
- Others	22 (27.8)	6 (31.6)	16 (26.2)
Level of hemoglobin			
- Anemia (< 10 qr/dl)	17 (21.5)	3 (15.8)	22 (36.7)
- No anemia (> 10 qr/dl)	60 (75.9)	16 (84.2)	36 (60.0)
Level of leukocyte			
- No leukocytosis (< 11.00/ µL)	49 (62.0)	15 (78.9)	24 (40.0)
- Leukocytosis (> 11.00/ µL)	28 (35.4)	4 (21.1)	35 (58.3)
Level of thrombocyte			
- No thrombocytosis (< 400.103/ µL)	40 (50.6)	15 (78.9)	35 (58.3)
- Thrombocytosis (> 400.103/ µL)	38 (48.1)	4 (21.1)	24 (40.0)

RESULTS

In this study 79 solid cancer patients were studied, consisting of nasopharyng, breast, lung, cervix and other cancers. The most frequent clinical stage of cancer was stage III in 53.2% and even higher (approximately 80%) in cervix cancer. As shown at **Table 1**, 57 (72.2%) out of 79 patients were female and 22 (27.2%) male with age group of 40-59 as seen in 57%. Plasma D dimer > 500 ng/ml was found more frequently as clinical stage increased as well as older ages. Nonetheless, the difference was not statistically significant.

Table 2 showed that no correlation was found between clinical variables and plasma D dimer, but thrombocyte was significantly correlated with sex.

Table 2. Correlation between clinical variables and coagulation activation markers

	D dimer (ng/ml)		Thrombocyte (10^3/uL)	
	Correlation(r)	p	Correlation(r)	p
Age	- 0.039	0.737	- 0.191	0.096
Sex	0.159	0.113	0.225	0.047
Leucocyte	0.168	0.140	0.346	0.002
Hemoglobin	- 0.209	0.068	- 0.172	0.134

As also can be seen in **Table 3** and **4** there were no significant correlation found between coagulation activation markers and type of malignancy (p= 0.156 and p= 0.691 with Spearman rank test).

Mean levels of D dimer of lung cancer were much higher than other type of malignancies, but this differences was not statistically significant.

Table 3. Correlation between coagulation markers and type of malignancy

	Type of malignancy	
	Correlation (r)	p
Thrombocyte	0.046	0.691
D dimer	0.163	0.156

Table 4. Mean difference within type of maligancy

Type of malignancy	N	Mean D dimer (ng/ml)	Mean platelet (10^3/uL)
Nasopharying	13	1012	375
Cervix	26	1184	339
Breast	9	959	349
Lung	7	2156	400
Others	22	1334	364
Total	77	1260	365

Analysis of variance test was to see the difference between group (Anova) p = 0.090 (D dimer) and p = 0.893 (thrombocyte).

Table 5. Correlation between coagulation markers and clinical stage of malignancy

	Clinical stage of malignancy	
	Correlation (r)[2]	p
Thrombocyte	- 0.052	0.64
D dimer	0.367	0.001

In this study significant coefficient correlation was found between plasma D dimer and clinical stage using Spearman rank test (r = 0.367, p = 0.001). This correlation was positively linear.

Analysis of variant test was to see the difference between group (ANOVA) p = 0,082 (D dimer) and p = 0,969 (thrombocyte).

Table 6. Mean difference within clinical stage and coagulation activation markers

Clinical stage	N	Mean D dimer (ng/ml)	Mean thrombocyte (10^3/uL)
Stage I	5	640	357
Stage II	13	851	377
Stage III	42	1376	355
Stage IV	19	1586	352

At **Table 6** showed that mean levels of plasma D dimer were much higher as cancer became more extensive. Although Anova showed no significant difference, post hoc analysis revealed significance difference between stage II and stage IV. On subgroup analysis in patient with nasopharyng cancer, strong correlation was seen between clinical stage and plasma D dimer with correlation coefficient r=0.81 and p=0.001.

DISCUSSION

The antigen fibrin D dimer is a unique marker of the primary enzymatic degradation product of cross linked fibrin by plasmin. Plasmin degrades the crosslinked fibrin to release fibrin degradation products and exposes the D dimer antigen. Systemic values of DD are an index of fibrin turnover in the circulation. Plasma D dimer levels are increased in many clinical conditions such as smoking, old age, pregnancy, trauma, infection, malignancies and others.[13,14] In addition to the diagnostic use of D dimer, it may also be of potential prognostic use in many conditions.[7] A study of Hughes et.al., (2005) reported that plasma D dimer value was significantly associated with old age (p= <0.001).[15] A quite similar study revealed that older age has much more higher level of plasma D dimer when compared with younger age.[16,17] Level of plasma D dimer rose linearly with increasing age, especially

when other comorbidities coexist. Diminishing kidney clearance and the presence of other occult disease might contribute for higher levels of D dimer in older age.[18] In the present study no significance difference was seen between age groups although older age has higher level of D dimer. Neither the difference between male and female was seen (p = 0.86). A cohort study on healthy traveller reported female population has strong association with D dimer value (p= 0.001), as also other study reported similar higher level of D dimer in female when compared with male patients.[15,19] There is no clear cut explanation as to what the underlying mechanism since some studies showed similar result of plasma D dimer level between male and female.[20-22]

The thrombocyte is an important cellular component of hemostasis processes and high counts of thrombocytes (thrombocytosis) is a risk factor for thrombosis to develop.[23] Thrombocytosis is associated with poor prognosis in solid tumors such as gastric carcinoma, esophageal, lung, colon and renal cancer.[24] A research on outpatient cancer population noted that leucocyte >11.000/uL and thrombocyte >400.000/uL were a new biomarker for thrombosis.[1] In this suty no significant correlation was found between thrombocyte and plasma D dimer.

Thrombus formation in cancer patients is a multifactorial and complex phenomenon. It is generally believed that inflammatory response of host together with cancer procoagulant interact to play a significant role. Tissue factor (TF) is a prominent cancer procoagulant initiated coagulation activation that lead to thrombus formation. Lung, colon, pancreas, ovary, brain cancer are known to have high insidence of thrombosis.[4,5] This study came up with lung cancer whose plasma D dimer was the highest (2156 ng/ml) compared to breast cancer which had the lowest D dimer (959 ng/ml). Also can be seen that in each type of malignancy higher level of D dimer (> 500 ng/ml) was found when compared to D dimer level < 500 ng/ml. Developments of thrombosis in cancer patients vary and largely depend on histologycal type of cancer cell, degree of differentiation and tumor burden.[5,22] Cancer cell derived from mucin-producing adenocarcinoma cell has the highest probability to have thrombosis. Mucin with other vascular proteoglycans, to some degree interact with anti thrombin to inhibit serine protease that lead to accelerating thombous formation.[3,20]

Clinical stages of cancer represent anatomical extent of the disease and prognosis of cancer influence primarily by the stage. Early and lower stage of cancer patient more likely to be cured. The worldwide application for reporting the extent of cancer is the TNM system, where the elements of the TNM consisted of T (tumor size), N (nodal involvement) and M (involvement of distance organ). Clinical stages of cancer are classified into stage I, II, III and IV after determination of the extent of the tumor combined with nodal involvement and presence or absence of metastasis process. The stage I comprised of the earliest and smallest element of TNM whereas stage IV is the latest and the most advanced of each element of TNM system.[23,24] In the future, the traditional anatomic staging will be closely linked with great number of nonanatomic prognostic markers that are currently in use or under study. All of these together will add up to a prognostic quilt that have a somewhat different from the traditional anatomic concepts.[12,13] Advance cancer stage with high tumor burden and high proliferation rate associated with high coagulation activation and finally fibrin formation providing suitable extracellular matrix for cancer cell to migrate and grows.[1,4,5] A cohort study of 821 cancer patients consisting of breast, lung, gaster, pancreas, renal, and prostate cancer reported that high D dimer value is a significant predictor for thrombosis.[25] Study in Italy with 343 cancer patients noted high plasma D dimer level together with other coagulation parameters related with poor prognosis.[26] Ko et al., (2009) stated in Japan that D dimer is a risk factor for thrombosis among ginecology malignancy.[27] Similar results was reported on prostate cancer.[28]

In this study we found a significant positive correlation between clinical stage of solid cancer with plasma D dimer value (r= 0.367; p= 0.001). This result was supported with study done by Kwon et.al., in South Korea. The study objective was to determine relationship between preoperative coagulation parameters and the extent of tumor involvement in gastric carinoma. Plasma D dimer was positively correlated with clinical stage with coefficient correlation r= 0.354 and p= 0.001.[29] Study in breast cancer revealed significant association between plasma D dimer and clinical stage with p= 0.002 using one way Anova.[8] Similar result was observed in a study of colorectal cancer. Significant higher D dimer level was found in malignant colorectal when compared to benign lesion. Higher D dimer level also related with deep invasion and advance stage. Study concluded preoperative plasma D dimer was important measurement to predict clinical stage preoperatively as well as postoperative survival. Hence, coagulation activation is associated with malignancy, though underlying involved mechanism is not clearly understood.[16] Duration and severity of coagulation activation strongly related with cancer prognosis. Almost 50% of cancer patients and 90% of metastased

cancer patients showed certain degree of coagulation abnormalities.[29] Fibrin formation and remodeling process (represented by high plasma D dimer value) provided suitable extra cellular matrix necessary for initial step of cancer cell to migrate, invade and finally grows and metastases. From this point of view, it is summarized that more advance the cancer more D dimer is produced as an indicator for coagulation activation. Nonetheless, succesfull treatment of solid tumors based on several recent reports demonstrated that not only traditional cancer staging but also nonanatomic biological prognostic markers status can also contribute to affect cancer mortality. Solid cancers have multiple and complex molecular transformation which occured during its progression and metastasis process within which several biological markers are developed, and it is proved to be significant prognostic factor in certain cancer.[12,30,31]

Plasma D dimer measurement has limited specificity because many conditions are associated with fibrin formation and D dimer assays are not directly comparable due to variation used of different antibodies. Correlation and degree of agreement between test is low.[7,18] Several factors related with variation of inter and intra assay are: antibody specificity, time dependence, assay format, purity of calibrator, matrix effect, interference by irrelevant analyses.[7]

CONCLUSION

From the study above we concluded that plasma D dimer level was positively correlated with clinical stage of solid cancers. High plasma D dimer could be a marker for advanced stage of a patients with solid cancer. The higher D dimer levels, more higher the clinical stage will be.

REFERENCES

1. Sousou T, Khorana AA. New insights into cancer-associated thrombosis. Arterioscler Thromb Vasc Biol. 2009;29:316-20.
2. Kroger K, Weiland D, Ose C, Neumann N, Weiss S, Hirsch C, et al. Risk factors for venous thromboembolic events in cancer patients. Ann Oncol. 2006;17:297-303.
3. Hillen HF.2000. Thrombosis in cancer patients. Ann Oncol;11 (S3):273-6.
4. Boccaccio C, Comoglio PM. A functional role for hemostasis in early cancer development. Cancer Res. 2005;65(19):8579-82.
5. Lee AYY. Thrombosis and cancer: the role of screening for occult cancer and recognizing the underlying biological mechanisms. Hematol. 2006;6:1-11.
6. Sallah S, Husain A, Sigounas V, Wan J, Turturro F, Sigounas G, et al. Plasma coagulation markers inpatients with solid tumors and venous thromboembolic disease receiving oral anticoagulation therapy. Clin Canc Res. 2004;10:7238-43.
7. Wakai A, Gleeson A, Winter D. Role of fibrin D-dimer testing in emergency medicine. Emerg Med J. 2003;20:319-25.
8. Blackwell K, Haroon Z, Broadwater G, Berry D, Harris L, Inglehart D. Plasma D-dimer levels in operable breast cancer patients correlate with clinical stage and axillary lymph node status. J Clin Oncol. 2000;18:600-8.
9. Madiyono B, Moeslichan SM, Satrosasmoro S, Budiman I, Purwanto SH. Perkiraan besar sampel. In: Sastrosasmoro S, Ismael S, eds. Dasar-dasar metodologi penelitian klinis. Jakarta: Sagung Seto; 2002. p. 259-87.
10. American Joint Committee on Cancer. What is cancer staging. Available from: www.cancerstage.org/stages/index.html. Access July 2011.
11. Edge SB, Compton CC. American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17:1471-4.
12. Greene FL, Sabine LH. The staging of cancer : a retrospective and prospective appraisal. CA Cancer J Clin. 2008;58:180-90.
13. Jong JB, Pohan HT, Zulkarmain I, Tambunan KL, Pangabean MM, Setiabudy RD, et al. Correlation of coagulation test and albumin with antithrombin III in dengue hemorragic fever. Acta Med Indones. 2004;36:61-5.
14. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. Blood. 2009;113:2878-87.
15. Hughes R, Thomson K, Hopkins R, Weatherall M, Wiltshire C, Wilsher M, et al. Determinants of plasma D-dimer levels in a traveling population. J Thromb Haemost. 2005;3:2445-8.
16. Oya M, Akiyama Y, Okuyama T, Ishikawa H. High preoperative plasma D-dimer level is associated with advanced tumor stage and short survival after curative resection in patients with colorectal cancer. Jpn J Clin Oncol. 2001;31(8):388-94.
17. Cushman M, Folsom AR, Wang L, Aleksic N, Rosamond WD, Tracy RP, et al. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003;101:1243-8.
18. Kelly J, Rudd A, Lewis RR, Hunt BJ. Plasma D-dimers in the diagnosis of venous thromboembolism. Arch Intern Med. 2002;162:747-56.
19. Rudnicka AR, Rumley A, Lowe GDO, Strachan DP. Diurnal, seasonal, and blood-processing patterns in levels of circulating fibrinogen, fibrin D-dimer, C-Reactive Protein, Tissue Plasminogen Activator, and von Willebrand Factor in a 45-year-old population. Circulation. 2007;115:996-1003.
20. Bick RL, Murano G. Phisiology of hemostasis. Disorders of thrombosis and hemostasis. 3rd ed. In: Bick RL, ed. Philadelphia: Lippincot Williams & Wilkins; 2002. p. 1-24.
21. Yamashita H, Kitayama J, Nagawa H. Hyperfibrinogenemia is a useful predictor for lymphatic metastasis in human gastric cancer. Jpn J Clin Oncol. 2005;35(10):595-600.
22. Robert F. The potential benefits of low-molecular-weight heparins in cancer patients. J Hematol and Oncol. 2010;3:3-15.
23. Greene FL, Sobin LH. The staging of cancer: a retrospective and prospective appraisal. CA Cancer J Clin. 2008;58:180-90.
24. Gospodarowicz MK, Miller D, Groome PA, Greene FL, Logan PA, Sobin LH. The process for continuous improvement of the TNM classification. Cancer. 2004;100:1-5.
25. Ay C, Vormittag R, Dunkler D, Simanek R, Chiriac A-L, Drach J, et al. D-dimer and prothrombin fragment 1+2 predict venous thromboembolism in patients with cancer: results from the Vienna cancer and thrombosis study. J Clin Oncol. 2009;27(25):4124-9.
26. Ferrigno D, Buccheri G, Ricca I. Prognostic significance of blood coagulation tests in lung cancer. Eur Respir J. 2001;17:667-73.

27. Ko J, Seki N, Masahiro S, Kusumoto T, Nakamura K, Hongo A, et al. D-dimer level as a risk factor for postoperative venous thromboembolism in Japanese women with gynecologic cancer. Ann Oncol. 2009;10:23-31.
28. Wong BY, Fink L, Symanowski J, Dincer A, Adcock D, Vogelzang N. Coagulation markers D-dimer and prothrombin fragment 1.2 compared to disease and treatment status in prostate cancer patients. J Clin Oncol. 2007;25(18S):15587.
29. Kwon H-C, Oh SY, Lee S, Kim S-H, Han JY, Koh RY, et al. Plasma levels of prothrombin fragment F1+2, D-dimer and prothrombin time correlate with clinical stage and lymph node metastasis in operable gastric cancer patients. Jpn J Clin Oncol. 2008;38(1):2-7.
30. Sudoyo AW, Hardi F. Cytogenetic in solid tumors: Lessons from Philadelphia chromosome. Acta Med Indones.2011;43:68-73.
31. Sudoyo AW. Biomolecular markers as determinants of patients selection for adjuvant chemotherapy of sporadic colorectal cancers. Acta Med Indones. 2010;42:45-50.