Saliva to Monitor Warfarin Therapy after Prosthetic Heart Valve Replacement

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Abstract: Patients with prosthetic heart valve are in need of anticoagulant therapy for protection against thromboembolic events. Warfarin therapy is monitored by Prothrombin time (PT) and International Normalized Ratio (INR). The clotting assays are invasive and may carry infection and prosthetic valve endocarditis. We try to use human saliva as noninvasive diagnostic fluid to access anticoagulant state. Our study was done in Cardiothoracic Department in Ain Shams University Hospital on 132 Patients had prosthetic valve (MVR, AVR or DVR) and under Warfarin therapy. We measure salivary TF activity by Quick one stage method; then calculate TF activity ratio and TF activity log to 10. The therapeutic value of salivary TF ratio ranges from (2.23 to 3.60) and salivary TF log to 10 ranges from (2.364 to 2.560). There is strong positive correlation between both salivary TF ratio and salivary TF log to 10 with INR (2.00 to 3.50). Salivary TF activity ratio has wider range than TF log to 10, and needs each laboratory to make its control under standardized conditions. Thus, the measurement of salivary TF activity ratio is a reliable test for follow up of patients on oral anticoagulant therapy.
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Key words: Prothrombin time (PT), INR, Saliva, Tissue factor (TF), Warfarin.

1. Introduction

Patients with prosthetic heart valve are in need of anticoagulant therapy. Warfarin is suitable for protection against thromboembolic events. Its activity has to be monitored by blood testing for Prothrombin time (PT) and International Normalized Ratio (INR). The target INR in patients with one or more mechanical heart valve ranges between (2.00- 3.50). Many medications and chemicals in certain foods may enhance or reduce Warfarin anticoagulant effect. Thus, close and repeated monitoring of the degree of anticoagulation is essential. In order to optimize the therapeutic effect without risking dangerous side-effect such as bleeding. (1, 2) The clotting assays have many drawbacks. First, they are invasive, requiring some blood from the patient. Secondly, to perform coagulation of blood in a vial, this requires delicate handling, accurate timing and citration of plasma. Thirdly, the assay depends on additional product (Thromboplastin) which differs from the reference substance of WHO. To ensure accuracy, we have to calculate complicated INR. Fourthly, multiple blood samples may carry infection and prosthetic valve endocarditis when antiseptic measurements not taken well. Finally, they indirectly reflect fibrino-peptides and fibrinogen degradation products. (3) We aim to use noninvasive diagnostic technique to measure the state of anticoagulant. The use of human saliva as a diagnostic fluid offers advantage of being collected non-invasively by individuals with modest training. (4, 5) Tissue factor (TF) is the key initiator of coagulation cascade. Tissue factor (TF) is a lipoprotein that with factor VII makes a complex that initiates blood coagulation by the extrinsic pathway. (6) TF antigen is elevated in hypertensive subjects as compared with normotensive subjects. (7)Tumor derived tissue factor is associated with venous thromboembolic events in malignancy.(8) The circulating pool of TF in blood is associated with increased blood thrombogenicity in patients during cardiopulmonary bypass.(9).TF pathway is considered as the primary physiological mechanism initiating blood coagulation. Induction of Warfarin anticoagulation reduces TF activity with vitamin K dependent clotting factors. The plasma clotting assays of PT measure vitamin K dependent clotting factors by adding Thromboplastin neglecting the effect of TF. (10) Saliva is a unique body fluid being free of factors V and VII and has TF activity. Measurement of salivary TF activities by Quick one stage method can give a direct image of the anticoagulant state of the blood. (11)

Aim of the study:

Use saliva as a biological fluid for monitoring TF activity and anticoagulant status.

2. Patients & Methods:

A total of 132 Patients in cardiothoracic department after open heart surgery for valve replacement using prosthetic valve (MVR, AVR or DVR) in Ain Shams University Hospital and under oral anticoagulant (warfarin) treatment after explaining the test for them and get their agreement

with written consent. Compared to 20 volunteers (control group); received no medication with normal liver function tests and normal PT (INR: 0.96-1.05) to compare their level of TF in saliva with those patients on oral anticoagulant.

Citrated blood samples are taken for measurement of their routine follow up PT & INR. Samples were usually collected between the hours of 8 to 11 A.M. Salivary flow was stimulated by chewing of sterile cotton swan (under tongue) for at least 2 minutes. Mixed oral saliva was collected into 17X100 mm plastic tube by hugging swab with the cover about 2 cm from the bottom of the tube and centrifugation at 500g for 15 min. at room temperature.

TF was assayed by mixing 0.05 ml of saliva with 0.05 ml of 0.02 M. $CaCl_2$ at 37◦ C for at least 5 min. then add 0.05 ml normal plasma control using coagulometer (DADE BEHRING BFTII). The results of all TF assays represent the average of duplicate determinations.

3. Results:

In the132 who are on oral anticoagulant we found that TF in second mean 294.6±98.45 and in the controlled group mean 107.3±11.93and there is a highly significant different between patient group and control group (p<0.001). (Table 1)

(Table 1): PT and TF in patient and control groups:

	Patient group			Control group
	PT sec.	INR	TF sec.	TF sec
Mean	**30.912**	**2.774**	**294.596**	**107.331**
± (SD)	**12.380**	**1.096**	**98.447**	**11.932**

We calculate TF ratio by this equation:

TF Ratio = TF activity of the patient / mean of TF activity of control group (in sec)

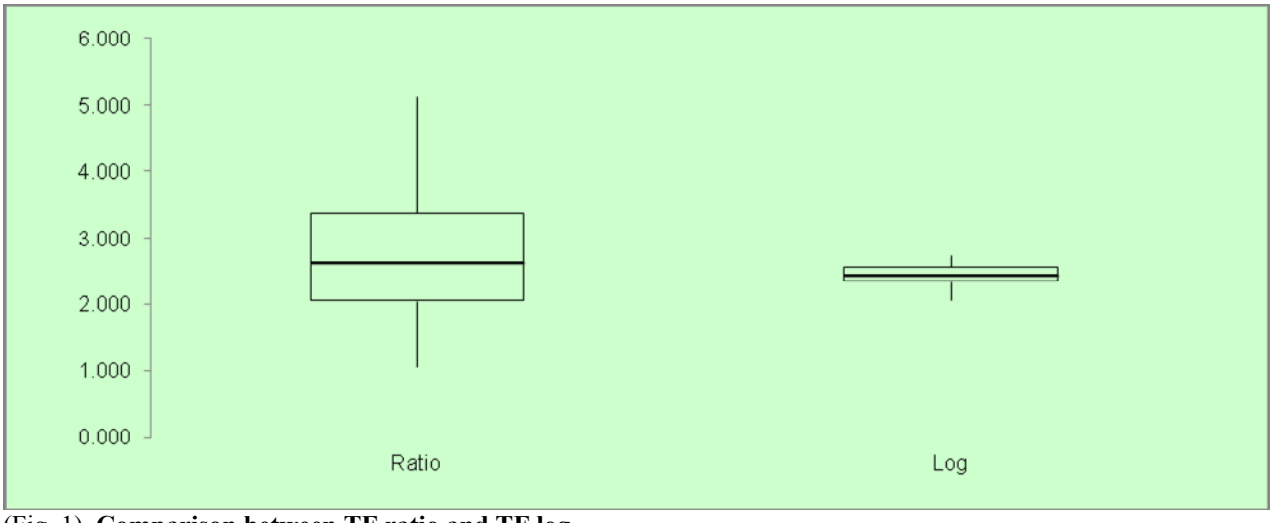
Then, calculate log to 10:

Log to 10 of TF activity of patient in sec

. (Table 2, Fig. 1)

(Table 2): Comparison between TF ratio and TF log:

	TF Ratio	TF log
Mean	**2.745**	**2.444**
± (SD)	**0.917**	**0.153**



(Fig. 1) **Comparison between TF ratio and TF log.**

There is positive significant correlation between INR and both TF ratio and TF log to 10. (Table 3)

(Table 3): r test between INR and both TF ratio and TF log to 10.

R test between INR & TF	INR r test	Significant (*p*)
Ratio	**0.796**	***p*<0.001(highly sig)**
Log to 10	**0.728**	***p*<0.001(highly sig)**

Considering of therapeutic target of INR in patients after prosthetic heart valve were in between 2.00 and 3.50 (INR), sensitivity, specificity and efficacy of both TF ratio and TF log to 10 were done by doing ROC curve, revealed very high sensitivity, specificity and efficacy of both TF ratio and TF log to 10. (Table 4)

(Table 4): Sensitivity & Specificity of Salivary Tissue Factor as control of oral anticoagulant therapy with target INR form 2.00 to 3.50

		Cut off value	Sensitivity	Specificity	Efficacy
TF ratio to control	**Low (INR ≥ 2.00)**	**2.229**	**86.21 %**	**83.33 %**	**84.11 %**
	High (INR ≤ 3.50)	**3.600**	**86.36 %**	**97.44 %**	**95.00 %**
TF log to 10	**Low (INR ≥ 2.00)**	**2.364**	**84.62 %**	**82.76 %**	**84.11 %**
	High (INR ≤ 3.50)	**2.560**	**95.45 %**	**89.74 %**	**91.00 %**

The cut off value of TF ratio (2.229-3.600) and TF log to 10(2.364-2.560) is used in monitoring the therapeutic dose of Warfarin. The cutoff value is indicator for the proper dose; the low cut off value is used in distinguishing under dose while the high cut off value is used for over dose. .

There is no significant difference between the sensitivity, specificity and efficacy of TF ratio and TF log to 10.

4. Discussion:

Patients after prosthetic heart valve replacement need to be on long life oral anticoagulant to avoid valve thrombosis. These have variable presentations; asymptomatic thrombosis, emboli, angina, hemodynamic compromise or shock from an obstructed valve. The number of prosthetic valves implanted is about figure of 60,000 per year. (12). Patients with mechanical valves are in need of long life oral anticoagulant. Patients with bio-prostheses or mitral repair may have other indications for long life anticoagulation, such as, atrial fibrillation (AF), heart failure, and impaired left ventricular function (ejection fraction 30%) (12) .The target INR is 2.00-3.00 for AVR, and 2.50-3.50 for MVR (13). The high variability of the INR is associated with reduced survival after valve replacement. (12-14)

So, accurate and reliable test as (PT, INR) is essential to evaluate these patients. It may be painful especially in children; also it may causes ecchymosis at the site of sample. Repeated tests may lead to loss of blood regularly especially if it was repeated at short interval. These drawbacks make patients reluctant to do test. If antiseptic measures are not probably taken during sampling, these may predispose endocarditis. Bettadapur *et al.* (13) found that 26% had poor compliance for treatment and follow up after open heart surgery for valve replacement. The risk of major bleeding begins to rise when the INR exceeds 4.5 and rises steeply above an INR of 6.0 (12, 13). So search for other reliable test with less side effects may be helpful for those patients to avoid over or under anticoagulation therapy .The analysis of saliva for TF activity may predict diagnostic application of saliva for monitoring warfarin therapy(5) . As a diagnostic fluid, saliva offers many advantages over plasma because it can be collected non-invasively. Furthermore, saliva may provide cost-effective approach for the screening of large population. Our study revealed that TF activity significantly reduced in patients on oral anticoagulant (294.6±98.45) compared to TF activity of control group (107.3± 11.93). T-test was highly significant, this result give us a hope for a promising reliable test to control oral anticoagulant therapy with the use of saliva (TF).

The therapeutic value of salivary TF ratio ranges from (2.23) to (3.60) and salivary TF log to 10 ranges from (2.364) to (2.56). There is strong positive correlation between salivary TF activity ratio and INR (2.00 to 3.50). Also, salivary TF log to 10 correlates significantly with INR.

So, measuring of tissue factor activity of saliva as a predictor test is highly specific (83.3%-97.4% for TF ratio) and (84.1%-95.0% for TF log to 10), with sensitivity (86.2%-86.4% for TF ratio) and (84.6%-95.5% for TF log to 10).The efficacy of salivary TF activity is (84.2%-95% for TF ratio) and (84.1%-91% for TF log to 10). We prefer to use TF activity ratio

because of its wider range than TF log to 10. Salivary TF ratio needs each laboratory to make its control under standardized conditions. Thus, the measurement of salivary TF activity ratio is a reliable test for follow up of patients on oral anticoagulant therapy. We hope to use the Nano technology in the future for making a strip to test salivary activity to be available for patients. This may be greatly helpful to save life for many patients die from valve thrombosis, thrombotic emboli or bleeding due to poor control of oral anticoagulant therapy.

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