Correlation between blood international normalised ratio (INR) value and saliva prothrombin concentration in patients under warfarin therapy: An observational analytics study

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ABSTRACT

Introduction: Oromaxillofacial surgery has a bleeding risk complication. Increasing bleeding risk can be caused by the use of blood clotting inhibitors, including warfarin. Warfarin is an anticoagulant that works by inhibiting coagulation factors in the cascade. International normalised ratio (INR) indicates warfarin monitoring and bleeding risk during surgical procedures. INR examination currently uses blood plasma. This research analyses the correlation between blood INR value and salivary prothrombin concentration. **Methods:** This research was an analytical observational with a correlational design on 19 samples examined using a human prothrombin reagent from Elabscience. The samples used were blood and saliva, and then ELISA tests were carried out on them. Data results were analysed with linear regression and correlation tests. **Results:** In this study, 19 samples were collected from 6 male and 13 female subjects. The average value of salivary prothrombin concentration in this study was 227.63 (ng/ml), and the blood INR value was 1.85. The normality test result showed that the data distribution was normal; therefore, the correlation and simple linear regression tests can be conducted. The correlation and regression tests showed a positive correlation between blood INR value and salivary prothrombin concentration with a correlation coefficient of r = 0.81 (p < 0.001). **Conclusion:** There is a positive relationship between blood INR value and salivary prothrombin concentration in oromaxillofacial patients under warfarin therapy.

Keywords

ELISA, prothrombin, international normalised ratio, saliva, warfarin

Hubungan antara nilai international normalised ratio (INR) darah dengan konsentrasi protrombin saliva pada pasien dengan terapi warfarin: penelitian observasional analitik

ABSTRAK

Pendahuluan: *Prosedur bedah mulut dalam kedokteran gigi memiliki risiko terjadinya komplikasi berupa perdarahan. Salah satu penyebab meningkatnya risiko perdarahan adalah penggunaan obat-obatan yang dapat menghambat pembekuan darah, termasuk warfarin. Warfarin merupakan obat antikoagulan yang dapat menghambat faktor koagulasi pada kaskade koagulasi. INR merupakan indikator pemantauan penggunaan Warfarin dan juga merupakan indikator risiko perdarahan selama prosedur operasi. Pemeriksaan INR saat ini menggunakan plasma darah. Penelitian ini bertujuan untuk menganalisis hubungan antara nilai INR dengan konsentrasi protrombin saliva.* ***Metode:*** *Penelitian ini merupakan penelitian observasional analitik dengan desain penelitian korelasional. Dalam penelitian ini, analisis laboratorium terhadap 19 sampel dilakukan dengan menggunakan reagen protrombin manusia dari Elabscience. Pengambilan sampel dilakukan dengan menggunakan sampel darah dan air liur, kemudian dilakukan uji ELISA pada sampel air liur dan INR pada sampel plasma darah. Selanjutnya dilakukan uji regresi linier dan korelasi.* ***Hasil:*** *Dalam penelitian ini 19 sampel dikumpulkan 6 laki-laki dan 13 perempuan. Nilai rata-rata konsentrasi protrombin ludah pada penelitian ini adalah 227,63 (ng/ml) dan nilai INR sebesar 1,85. Setelah dilakukan uji normalitas dinyatakan sebaran data normal, selanjutnya dilakukan uji korelasi dan uji regresi linier sederhana. Hasil uji korelasi dan regresi menunjukkan terdapat korelasi positif antara INR darah dengan konsentrasi protrombin ludah dengan koefisien korelasi r=0,81 (p<0,001).* ***Simpulan:*** *Terdapat hubungan positif dan signifikan antara INR darah dengan konsentrasi protrombin saliva.*

Kata kunci

ELISA, prothrombin, international normalised ratio, saliva, warfarin

INTRODUCTION

Oromaxillofacial surgery procedures are any surgery performed on the dental, gingival, periodontal, and facial structures.[1] These procedures, including tooth extraction, have a risk of complications, including soreness, nerve injury, swelling, infection, and bleeding. Tooth extraction is one of the most common procedures in oromaxillofacial surgery in routine dental practice. Post-extraction bleeding is a frequent complication found in dental practice, which is defined as bleeding that continues more than 8-12 hours after tooth extraction. This type of bleeding can be easily controlled and almost completely stops within 8 hours post-extraction. However, in a few cases, it can cause prolonged bleeding up to systemic conditions that require blood thinner therapy to prevent a life-threatening situation.[1,2,3] A systematic review database from Cochrane Library in 2018 found that the incidence of post-extraction bleeding varies from 0 to 26%.[2,3] If post-extraction bleeding is left untreated, complications can range from tissue hematoma to fatal blood loss.

The causes of post-extraction bleeding are classified as local and systemic. Local causes of bleeding include bleeding from soft tissue and bone, while systemic causes include platelet problems, coagulation disorders, excessive fibrinolysis, and heredity. Bleeding control is an essential step during oromaxillofacial surgery procedures because excessive bleeding can increase the risk of morbidity. According to a study conducted by Eichhorn *et al.*, 7.4% of patients experienced bleeding after oromaxillofacial surgery, while 2.4% required hospitalised treatment.[4,5,6]

Excessive bleeding that requires blood thinner therapy affects the blood clotting system. Warfarin is a drug that is often used in patients with myocardial infarction, deep vein thrombosis, pulmonary embolism, and arterial fibrillation. An FDA-approved oral anticoagulant drug since the 1950s, warfarin remains the first-line treatment for prophylaxis and treatment of venous thromboembolism, thromboembolic complications, myocardial infarction, and stroke.[7,8] Although direct-acting oral anticoagulants (DOAC), including dabigatran and rivaroxaban, are available in Indonesia, the use of warfarin remains high in many health facilities in Indonesia. Warfarin has advantages over DOAC. It is relatively cheaper, easy to obtain, and also safer because it has an antidote in case of an overdose. The disadvantages of warfarin are that it requires monitoring of the INR value and has a higher risk of bleeding compared to other anticoagulants.[7,8]

Patients who take warfarin require a coagulation status examination prior to surgery. The purpose of the prothrombin time measure is to check the coagulation status. The International Sensitivity Index (ISI) is used to convert the PT Reagen. The International Normalised Ratio (INR) value of 1.1 or lower is considered normal. The INR value of 2.0 to 3.0 is generally the optimal range for surgical procedures. In this range, patients have a low risk of thrombosis or bleeding. The blood is clotting more slowly when the INR is higher than the recommended range. In the therapeutic range, the patient is considered safe for surgery. Oromaxillofacial surgery in daily practice is an action with a low to moderate risk of bleeding. Thus, knowing the patient's coagulation status is essential, especially in patients taking blood thinners.[8,9,10]

The INR examination was conducted by taking the blood plasma. According to the European consensus recommendations by the SDCEP (Scottish Dental Clinical Effectiveness Program) in 2015, an INR examination considered valid is at most 24 hours. It takes at least 24 hours to report the results of a patient's INR examination from the first time the sample is received. Completion time is defined as the number of days from the date of specimen collection until the time the results are obtained.[11]

The INR examination using blood plasma, while effective, is quite expensive and limited. However, a promising alternative has emerged, which is saliva. Saliva, secreted by the salivary glands, is composed of the majority of water (99%), protein (0.3%), and inorganic substances (0.2%). The oral cavity houses major and minor salivary glands, including three major salivary glands and several minor salivary glands in the oral and throat areas.[11]

The major salivary glands are the parotid, submandibular, and sublingual glands and contribute 90% of the total salivary secretion. Among the minor salivary glands, the labial, buccal, lingual, and palatal glands contribute 10% of the total salivary secretion.[12] Each salivary gland has high permeability and is covered by capillaries. The porous capillary structure allows the transfer of blood molecular components to saliva. Researchers concluded that blood components can enter saliva employing transcellular (passive and active transport) or paracellular (extracellular ultrafiltration).[13]

While many researchers believe in the potential of saliva as a molecular diagnostic tool, its use as a sample is still uncommon. This is due to the substantial differences in the levels of blood components detected in saliva. For instance, in healthy adults, blood serum levels of IgG (5 to 30 mg/ml versus 5 to 30 g/ml) and IgM (0.5 to 1 mg/ml versus 5 to 10 g/ml) are higher than those found in saliva. However, this unique correlation between salivary and blood components, although separate, suggests a potential at the molecular level. Therefore, it is crucial for us to further explore saliva as a potential alternative for blood and tissue-based diagnostics.[13,14]

Prothrombin has been the subject of many previous studies as it can be measured in saliva, revealing a significant difference in salivary prothrombin concentrations in warfarin users. However, the correlation between the two variables still needs to be clarified. Studies on salivary prothrombin concentrations have been conducted by De Nora *et al.*[15] in the United States, however, this investigation has yet to be carried out in Indonesia. Therefore, it is crucial to research the correlation between blood INR value and salivary prothrombin concentrations in Indonesian subjects. This study analyses the correlation between blood International Normalised Ratio (INR) value and salivary prothrombin concentration in oral surgery patients on warfarin therapy.[15]

METHODS

This research was observational analytics, with the population of patients under warfarin therapy at Hasan Sadikin Hospital of Bandung who required oral surgery treatment. This research was conducted from September to December 2022 at Hasan Sadikin Hospital, Bandung. Consecutive sampling takes subjects from a portion of the population that meets the inclusion criteria until a minimum sample size is met. The inclusion criteria were adult patients over 18 years of age and under warfarin therapy. Exclusion criteria were patients who received a combination of drugs with antiplatelets and patients with hepatic and thyroid disorders.

This research was conducted by taking patients' blood plasma and salivary samples under warfarin therapy. Based on the Lemeshow sample calculation formula, a total sample of 19 people was obtained.[17] Prior to the sample taking, the patient was instructed to rinse his mouth with 0.9% NaCl for 1 minute, not to eat food 1 hour before, and not to take drugs for the previous 12 hours.

The 0.5 cc saliva sample was taken using a passive drooling technique. After collection, an ELISA examination was performed in the laboratory to determine the prothrombin concentration using spectrophotometry. The 2 cc blood plasma samples were taken from the veins in the elbow area. After collection, a prothrombin time examination was performed, and then the PT value was standardised to obtain the blood INR value. The confounding variables in this study were the body temperature, environment temperature, and exposure temperature during the period of sample collection until being stored in the laboratory storage refrigerator.

Using the Shapiro-Wilk test, a data normality test was performed on salivary prothrombin concentration data and blood plasma INR values. A correlation and simple linear regression tests were performed to determine the correlation between blood plasma INR values and salivary prothrombin concentrations in oromaxillofacial surgery patients under warfarin therapy.

RESULTS

Study subjects were grouped based on sex, blood INR results, and salivary prothrombin concentration (Table 1), which showed more female patients (13; 68.4%) than male (6; 31.6%). Table 2 presented that the average value of salivary prothrombin concentration was 227.63 ng/mL, with the highest of 370 ng/mL and the lowest of 40 ng/mL. Meanwhile, the average value of blood INR was 1.85, with the highest blood INR value of 3.01, and the lowest was 1.16.

Table 1. Characteristic of research subjects

No	Characteristics	Value	Description
	Sex		
1.	Male	6	68.4%
2.	Female	13	32.6%
	Salivary prothrombin concentration	X	
1	Median	200 ng/mL	Median value of prothrombin concentration in salivary samples
2	Mean	227.63 ng/mL	Average value of prothrombin concentration in salivary samples
	INR value	Y	
1	Median	1.78	Median value of blood INR samples
2	Mean	1.85	Average value of blood INR samples

Table 2. Blood INR and salivary prothrombin concentration

No sample	Salivary prothrombin concentration (ng/mL)	Blood INR
	X	Y
1	370.00	3.01
2	375.00	2.93
3	395.00	2.67
4	320.00	2.49
5	337.00	2.33
6	395.00	2.22
7	120.00	2.14
8	230.00	2.03
9	275.00	1.86
10	380.00	1.78
11	200.00	1.61
12	146.00	1.59
13	200.00	1.41
14	132.00	1.35
15	55.00	1.27
16	185.00	1.20
17	40.00	1.16
18	150.00	1.11
19	20.00	1.03

Hypothesis analysis determined the correlation between blood INR value and salivary prothrombin concentrations in oromaxillofacial surgery patients under warfarin therapy. The Shapiro-Wilk test was initially performed to determine the data distribution, and the data was normally distributed. Therefore, the hypothesis analysis used the Pearson product-moment correlation test.

Table 3. Normality test of blood INR value and saliva prothrombin concentration

Normality test			
Variable	**Shapiro-Wilk**		
	Statistic	**Df**	**p-value**
Salivary prothrombin concentration	0.923	19	0.128
Blood INR value	0.936	19	0.226

The hypothesis was analysed using a linear regression correlation test to estimate the magnitude of the independent variable's influence on the dependent variable and whether the effect was positive or negative. The result was derived from the value of r. If the value of r approaches 1, it indicates a strong influence. However, it's important to note that body temperature, environment temperature, and exposure temperature during sample collection and storage in the laboratory refrigerator can act as confounding variables in this study.

Table 4 Correlation test of blood INR value and salivary prothrombin concentration

Variable	Correlation coefficient	p-value
Salivary prothrombin concentration <--> Blood INR	0.814	<0.001**

** p < 0.001; CI 95%, α 5%

DISCUSSION

Age and sex can affect the use of warfarin. Age affects the warfarin dose, as also discovered by Shendre[6], that middle age requires a 10% dose reduction, and older adults require an additional 10% dose reduction. Wong[6] also stated that warfarin users were also more dominated by women, and one of the factors that caused it was the factor of pregnancy, which can increase the risk of atrial fibrillation, eventually increasing the need for warfarin.[16,17]

Warfarin is one of the medications of choice in cases of atrial fibrillation. Data from the Women's Health Study demonstrated a linear increase in the risk of atrial fibrillation with increasing parity, ranging from a hazard ratio of 1.15 for one pregnancy to 1.46 for more than six pregnancies. This condition may reflect repeated exposure to pregnancy's physiological, inflammatory, and hormonal stresses, which affect the heart, particularly the left atrium. During pregnancy, hypertension, gestational diabetes, and preeclampsia are independently associated with the long-term development of cardiovascular disease.

Laboratory examinations in this research were carried out using the ELISA technique, and concentration values were obtained from the results of optical density calculations on the spectrophotometer. ELISA is effective in detecting antibodies and was also used by other research of Ozgocer[18] to detect salivary cortisol accurately. The optical density value was proportional to the value of salivary prothrombin concentration.[18-20]

ELISA is a plate containing 96 wells, a place where the binding process of antibodies to proteins occurs. The ELISA procedure is easy to perform and includes easy application and rinsing material to be used several times, making the ELISA kit very specific. The ELISA examination procedure begins with the antigen and antibody coating process on the surface of the well above the plate. After, the blocking stage of antigen and antibody binding to nonspecific sites is carried out with the blocking agent.

After incubation and rinsing, the plates are incubated with enzyme-bound antibodies. Then, the plate is rinsed and continued with the addition of substrate so that a colour change is produced, and the OD (optical density) value can be read by an ELISA reader. The washing step is a crucial step to remove antibodies that are not bound to antigens. Washing liquid must be ensured that it is not left on the plate to prevent it from affecting the next examination stage. Although the ELISA technique is very sensitive and specific, ELISA usually measures a single biomarker, so the sample volume will increase excessively if more than one biomarker is measured.[18-20]

The current research showed that the salivary prothrombin concentration was much lower than the prothrombin from other samples. In the research on salivary cortisol, Sarah *et al.*[21] also obtained lower concentration values in salivary samples compared to the serum. This condition can be influenced by many factors affecting salivary biomarkers, such as smoking, daytime sampling, age, food consumption, periodontal disease, saliva collection methods, tooth brushing, and sample storage methods.[19,20] This study also illustrates the importance of maintaining oral health to ensure the salivary examination can be conducted optimally, thus minimising the influence of the oral environment on salivary components.[20,21]

Saliva can be collected through unstimulated or stimulated methods (unstimulated or stimulated saliva). Unstimulated saliva represents basic saliva, which is present in the oral cavity for most of the 24 hours. They often showed systemic clinical conditions more accurately than stimulated saliva because agents used to stimulate salivary flow can change the composition of the saliva. Unstimulated saliva is generally elicited by having the subject sit quietly with the head tilted forward and allowing the saliva to drip passively from the mouth into the reservoir or by asking the subject to gently spit the saliva into the reservoir for a specified time. This collection method is considered the "gold standard" for obtaining many salivary components of saliva.[21-22]

Salivary collecting methods play an essential role in the quality of saliva samples. A good saliva sample must follow a protocol to prevent saliva from accumulating with other contaminating substances that could interfere with the immunoassay interpretation results. Based on the salimetrics from the Saliva Collection Handbook23, things that need to be followed before saliva sample collection include: avoiding high sugar or acid food or beverages consumption and high caffeine because it can lower the salivary pH and increase bacterial growth; avoiding alcohol, caffeine, nicotine, or drugs consumption within 12 hours; avoiding eat for 60 minutes before sample collection; gargling with NaCl to remove debris from the oral cavity and let stand 10 minutes before the sample collection is carried out.[23]

Our research findings demonstrate the significant impact of oral hygiene, saliva sample collection methods, and oral cavity conditions on the components of the samples. Chen-Zi-Zhang *et al.* has previously suggested that the oral cavity condition and pre-sampling food consumption could influence sample components, thereby highlighting the sensitivity of salivary examination.[19-24] Interestingly, we found that stimulation of saliva by chewing gum during collection increased salivary flow and the rate of total salivary protein secretion. Nitrite secretion and total antioxidant capacity also increased with stimulation. Moreover, the accumulation of saliva for 30 seconds prior to unstimulated collection increased salivary alpha-amylase concentrations, potentially related to sympathetic excitability. Salivary flow and total protein secretion levels from samples taken after toothbrushing were lower than those collected without toothbrushing.

This study compared blood INR with salivary prothrombin concentration values and discovered a positive and strong relationship and correlation (Table 4). This finding suggested that if the INR value increases, salivary prothrombin concentration will also increase. No previous research has examined this specific correlation, making this discovery particularly intriguing. Conversely, if the INR value decreases, the salivary prothrombin concentration will also decrease.

The examination of salivary prothrombin concentration holds great promise as an alternative method for measuring INR values when a blood examination cannot be carried out. As previously explained, the path of blood components into saliva can be through 3 ways: passive intracellular diffusion, active transport, and extracellular ultrafiltration in the salivary glands, thus supporting the correlation between salivary prothrombin levels and blood INR.[23,24] Diffusion is the most common route for protein components to migrate from blood to saliva. The capillaries surrounding the salivary glands are porous enough to allow the passage of many small molecules. By diffusion, serum molecules that reach saliva must pass through 5 barriers: capillary walls, interstitial space, basement cell membrane, acinar

cytoplasm or duct cells, and the luminal cell membrane.[25-27] The ability of a molecule to passively diffuse through the cell membrane depends partly on its size and partly on the electrical charge it carries. If a molecule is polar or dissociates into charged ions while in solution, it will have difficulty crossing the ductal cell membrane composed of phospholipids. For example, steroid hormones are relatively small in size and consist mainly of fatty acids, so they tend to escape quickly by diffusion.[24]

Other molecules bound to large carrier proteins, such as albumin serum, are too large to enter by this route. Active transport is a second pathway for the entry of molecules into saliva through glandular secretory cells, which is the route used by IgA secretory cells. The molecule then binds to IgA receptors found on the acinus cells and is released into saliva. Ultrafiltration (extracellular mechanism) is the third means of transporting molecules from the bloodstream to the saliva. Ultrafiltration is possible, but the molecules must be relatively small. Components that cannot pass through the cell membrane layers due to their electrical charge are believed to enter via this route.[24-28]

In addition, molecular ultrafiltration can also occur through gap junctions between secretory unit cells (intercellular nexus). Only molecules with 1900 Da (such as water, ions, catecholamines, and steroids) are transferred by the ultrafiltration mechanism, and their concentration in saliva is 300 to 3000 times lower than in the blood plasma. Protein components, including prothrombin, are more likely to exit into the saliva by diffusion and active transport.[24-28]

Only a few studies have been conducted on blood coagulation factors in saliva. However, our study aligned with other studies by De Nora et al.[15], which concluded that blood coagulation factor fibrinopeptides could be measured in saliva samples. These tests are non-invasive, fast, and easy compared to INR blood tests. This study did not exclude systemic diseases or other diseases that could affect the quality of saliva.

Future studies can compare the ratio scale of prothrombin concentrations in blood and saliva. They can also compare salivary prothrombin concentrations with the incidence of bleeding complications after oromaxillofacial surgery in patients taking warfarin without stopping the therapy.[15]

It's important to note that this research, while significant, still has limitations. There are confounding variables, namely body temperature, environment temperature, and exposure temperature of the sample after collection until it reaches the laboratory storage refrigerator. These variables could potentially impact the results of this study. As previously known, body temperature can also influence the rate of saliva flow, which could introduce potential bias in the results. These limitations should be considered in future research to ensure the validity and reliability of the findings.

CONCLUSION

There is a positive relationship between blood INR value and salivary prothrombin concentration in oromaxillofacial patients under warfarin therapy. This research implies that the relationship between blood INR and salivary prothrombin concentration makes it possible to obtain INR values through saliva sampling.

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Institutional Review Board Statement: This study and its protocols were approved by the Universitas Padjadjaran Research Ethics Committee. Universitas Padjadjaran Research Ethics Committee was formed in order

to protect the rights and welfare of the research subject and to guarantee that the research using survey questionnaire/registry/surveillance/epidemiology/humanities/social-cultural/archived biological materials/stem cell/other non-clinical materials, that carried out according to ethical, legal, social implications and other applicable regulations. Nineteen samples were taken from the research participants using the human prothrombin reagent from Elabscience. Samples taken were blood and saliva samples. ELISA tests were carried out on saliva samples and INR on blood plasma samples. The protocols were approved by the ethics committee and hospital laboratory.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper if applicable.

Data Availability Statement: Data availability can be obtained through the author's correspondence email.

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