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Ethnic differences in platelet aggregation in healthy non-kava drinking and kava drinking, Fijians and Indofijians

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Abstract
Platelet aggregation was measured in healthy Fijian and Indo-Fijian participants predominantly residing in Suva, Fiji islands. The Fijians were divided into three groups: non-kava drinkers (n=58), occasional kava drinkers (n=60) and regular kava drinkers (n=56). Similarly the Indo-Fijians were also divided into three groups: non-kava drinkers (n=58), occasional kava drinkers (n=58) and regular kava drinkers (n=52). A comparison of platelet aggregation was done between the Fijian and Indo-Fijian groups. Blood Samples were collected from randomly selected healthy volunteers and platelet aggregation was measured using the whole blood platelet aggregometer (Chronolog corporation). The aggregating agent used was collagen. Analysis of results was done using the t tests.
Platelet aggregation was within the normal range (15-27Ω) in both the ethnic groups, Fijians and Indo-Fijians. No Significant differences in platelet aggregation were seen between the groups ($p>0.05$).
Based on the results of this study, platelet aggregation between the two groups was found to be similar and does not seem to have been affected by difference in ethnicity or genetic and dietary factors.

Keywords: ethnicity, fijians and indo-fijians, kava drinkers, non-kava drinkers, platelet aggregation

Introduction
Various studies have highlighted the existence of racially determined differences in platelet aggregation. The haemostatic characteristics of populations, the possible association between the ethnic differences in the levels of the variables including platelet aggregation and the incidence of thromboembolic disease has been researched in various populations. The populations previously studied were mainly Europeans Africans, Asians and Arabs. So far none of these studies have been done on the Fijian and Indo-Fijian population.
Fiji is a Pacific island country, located in the southwest Pacific. It has two main ethnic groups-the original Melanesian/Polynesian inhabitants of the islands, and the descendants of the Indian labourers who were brought in the late 19th century to work on the sugar plantation. Fiji is a country where rapid changes have occurred in the social and economic aspects since its independence in 1970. There is an acute awareness of race, and especially of the entitlements, advantages and disadvantages of the two main groups Fijian and Indo-Fijian (Indians). Each group has strong stereotypes about the other. Most of the research studies done in Fiji present extensive tables under the racial headings Fijian, Indian, and Others, even though these terms are not clearly defined in the medical literature (*Sorokin M, et al. 1973*) [21].
Cardiovascular diseases have emerged as the major public health problem in Fiji (*J. Toumilehto et al., 1984*). According to the latest WHO data published in May 2014, deaths due to coronary heart disease has reached 24.23% of total deaths.
Epidemiological studies show that heart disease of all types are extremely common in Fiji and the incidence of chronic non-communicable disease such as IHD (Ischaemic heart disease) has lead to the highest mortality rate in Fiji amongst the Pacific Island nations (Menzies center for population research, 1999). It has been seen that hospital admissions and mortality due to cardiovascular disease has been increasing steadily over the past 20 years. They were seen more frequently in the Indian population but now an increase is the prevalence rate has been noticed among the Melanesian population. IHD has been known to be the main cause of mortality and morbidity among the Indian population.
In Fiji, relatively high prevalence of NCD increases the risk of CVD episodes and death (*Gyaneshwar R, et al. 2016)* [1].

Platelets
Platelets play a very critical role in response to injury that involves the process of haemostasis, thrombus formation, vascular and connective tissue healing. Platelet reactions contribute to thrombus formation and these can be inhibited by various platelet inhibiting agents. Therefore, pharmacological inhibition of platelets is considered to be the cornerstone in acute and prophylactic treatment of ischaemic heart disease, peripheral vascular disease and stroke. (*Poulsen TS 2005*) In vitro platelet aggregation can be induced by a number of agents including ADP, adrenaline, collagen and thrombin as well as other non-physiological compounds such as ristocetin.

Kava kava and platelet function
Kava (*Piper methysticum,* also called the pepper plant*)* is an ancient crop of the western Pacific. It is found in Polynesia, Melanesia, and Micronesia. Kava is the term used for both the plant and the beverage made from its roots. Kava is consumed

by both the Fijians and the Indo-Fijians. In most cases it forms a part of their daily lives and is consumed regularly by both the ethnic groups.

One *in vitro* study showed that the kava-lactone, kawain, appears to decrease thromboxane-2 production and inhibit cyclo-oxygenase, indicating that kava may have significant inhibitory effect on platelet aggregation (Gleitz *et al.*, 1997). All of the above kava compounds tested demonstrated better or similar COX-1 inhibition activities as compared to ibuprofen, aspirin and naproxen (Wu *et al.*, 2002). Therefore this study included both the non-kava drinkers and kava drinkers in both ethnic groups.

To explore this warranted issue of the interaction between kava and platelet aggregation, a series of experiments were conducted to determine and compare platelet aggregation in response to collagen (as an agonist) between healthy Fijians (F) and Indo-Fijians (IF), kava drinking (KD) and non-kava drinking (NKD) adult volunteers which might partly be able to explain the differences in the incidence of IHD (ischaemic heart diseases) between the two ethnic groups.

Methodology

This clinical study was carried out in Suva city, Fiji Islands. The analytical part of the research work was conducted at the Fiji School of Medicine (FSM), Pacifica Campus Research Laboratory located on Extension Street, Suva. The lab is well equipped both for teaching and research. The platelet aggregometer was installed in this research lab. The volunteers for this research work were selected randomly.

A request for participation in this study was sent out to the academic and support staff at the Fiji School of Medicine, The University of the South Pacific and Fiji Institute of Technology via e-mail and information about this study was attached. For this study apparently healthy adult volunteers were selected and whosoever agreed to participate in the study was selected as a volunteer. To maintain consistency and to prevent bias both males and females were included as volunteers in the study. The age group of the participants was between 21 - 50yrs and comprised of Indo-Fijian and Fijian non-kava drinking and kava drinking volunteers.

In this study the kava drinkers have been classified as regular drinkers and occasional drinkers. The non-kava drinking (NKD) volunteers were those who abstained from drinking kava completely. The occasional kava drinking (OKD) volunteers were those who drank kava occasionally i.e only once a week, whereas the regular kava drinkers included individual who drank kava regularly i.e everyday and more than or equal to 20 bowls per day. Both the occasional and the regular kava drinkers have been drinking kava for more than 2 years.

Informed consent was obtained from the participant. To participate in the study the participants were requested not to take any drugs that included NSAID (such as aspirin), or any herbal medication which could potentially impair platelet function) for a period of 2 weeks before the initial sampling. Women taking oral contraceptives or estrogen based therapy or other hormonal based medication, were requested to discontinue with the medications for a period of one cycle. Social and medical history of the participants was recorded to confirm that they are free from disease or medication.

10ml of blood was collected with minimal stasis in blue top tubes containing 3.2% sodium citrate. The samples were immediately analysed for platelet aggregation. The chronolog model 591whole blood aggregometer was used for platelet function testing of whole blood specimens using impedance aggregometry. Collagen was used as the aggregating agent.

Ethical consideration and approval

Required human ethics approval was sought from the Fiji National Research Ethics Review Committee (FNRERC) and from the National Health Research Committee (NHRC) before the start of the experiment. The approval was granted, FNRERC Reference Number: 2008-001 dated 14TH July 2008.

Results

A Comparison of platelet aggregation was done between the Fijian and the Indo-Fijian non-kava drinking, occasional kava drinking and regular kava drinking participants.

An Independent t test was used to compare the platelet aggregation between the groups.

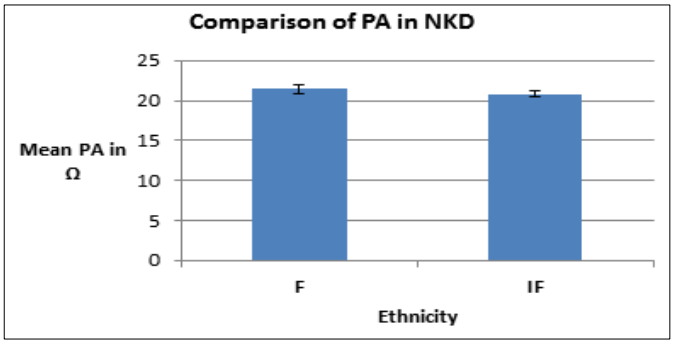
Table 1

Drinkers	ethnicity	N	Mean	Std. Deviation
non kava drinkers	fijians	58	21.43	3.858
	Indo- Fijians	58	20.86	3.192
Occassional kava drinkers	fijians	60	20.30	3.504
	Indo- Fijians	58	20.88	3.112
Regular kava drinkers	fijians	56	20.61	3.855
	Indo-Fijians	52	20.87	3.625

PA was compared between the Fijian and the Indofijian, NKD, OKD and RKD, to see if there is any difference in PA between these two major ethnic groups. No significant difference in PA was seen between the Fijians and Indo-Fijians.

NKD (Non kava drinkers)

An independent t-test was used to compare the average platelet aggregation in Fijian NKD with the Indo-Fijians NKD No difference in PA was found between the two groups. The t-test was non significant, t (114) = 0.865, p=0.38, two tailed.



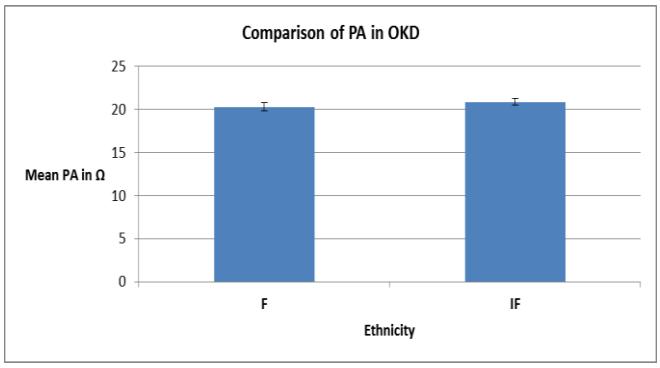
NKD (mean±sd)		
Fijians n=58	Indo-Fijians n=58	p=0.38
21.4 ±3.9	20.9 ±3.2	

Fig 1: Comparison of platelet aggregation PA **(Ω**, mean±se) between the Fijian and Indo-Fijian NKD before aspirin

OKD (Occasional kava drinkers)

An independent t-test was use to compare the average platelet aggregation between Fijian OKD and the Indo-Fijian OKD**.** It

was found to be statistically non significant, t (116) = -0.948, *p*=0.345, two tailed. Both the Fijian and the Indo-Fijian OKD groups had similar platelet aggregation.

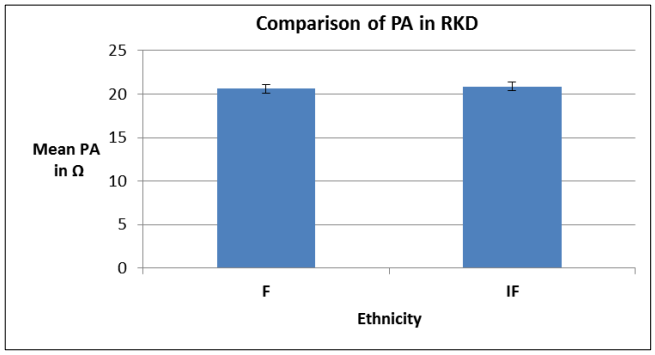


OKD (mean±sd)		
Fijians n=60	Indofijians n=58	*p*=0.345
20.3 ±3.5	20.9 ±3.1	

Fig. 2: Comparison of PA (Ω, mean±se) between the Fijian and Indo-Fijian OKD

RKD (Regular kava drinkers)

An independent t-test was use to compare the average platelet aggregation between the Fijian RKD and the Indo-Fijian RKD. It was found to be statistically non-significant, t =0.358, df = 0.106, *p*=0.721, two tailed. Both the ethnic groups had similar PA.



RKD (mean±sd)		
Fijians n=56	Indofijians n=52	*p*=0.721
20.6 ±3.9	20.9 ±3.6	

Fig 3: Comparison of PA (Ω, mean±se) between the Fijian and Indo-Fijian RKD

Discussion

This study assessed the ethnic differences in platelet aggregation taking into consideration the Fijian and Indofijian population of Suva Fiji Islands. No significant difference in platelet aggregation was seen between two major ethnic groups of Fiji (Fijians and the Indo-Fijian).

Platelet aggregation in the NKD Fijians and Indo-Fijians was compared. Similarly PA in the Fijian OKD was compared with the PA in the Indofijian OKD and Fijian RKD was compared with Indo-Fijian RKD. Unpaired t-test was used to compare these groups and the results were found to be non significant. So far no study is available in literature to compare our results, where we studied kava- aspirin effect on platelet aggregation in two major ethnic populations in Fiji.

According to some studies, ethnic differences seem to have an impact on platelet aggregability in response to various aggregating agents used like ADP, AA, EPI and COL (Meade *et al.*, 1985) [6]. Various explanations have been provided for the differences in platelet aggregation between the ethnic groups. Genetic variations between the ethnic groups may affect the platelet function and the hemostatic platelet response may be influenced by the genetic profile of the platelet enzymes and its membrane glycoprotein. While the ethnic differences in glycoprotein IIIa gene polymorphism remain to be established (Park, 2005) [3], the cyclooxygenase polymorphisms have been confirmed in the African-American population (Urlich *et al.*, 2002 and Urlich *et al.*, 2005) [2, 5]. Thromboxane synthase (TXS) polymorphism (TBXAS1) has particular importance, since TXS, a cytochrome P450 enzyme, converts prostaglandin H2 into thromboxane A2, a potent inducer of platelet aggregation (Urlich *et al.*, 2005) [5]. Polymorphism could probably indirectly explain the reduced platelet aggregability in response to AA in 40% of Hispanics and 50% of African- Americans.

Results of studies by Otahbachi *et al.* (2010) [19] demonstrate that, ethnicity might also influence platelet response to agonists. Caucasians had more aggregation induced by ADP, AA and EPI as compared to the Hispanics and the effect of COL was similar in the tested ethnic groups. Gader *et al.* (1991) [7] observed significant differences in the aggregation responses between the ethnic groups. While Saudi Arabs and Westerners (Europeans/Americans) had greater aggregation responses to ADP than Asians and Africans, aggregability in response to COL was more pronounced in Saudis and Africans than in Westerners and Asians. A population study in London by Meade *et al.* (1985) [6] to confirm the higher incidence of IHD in whites as compared to blacks, reported greater aggregability in white men when compared with black men. ADP was used as an aggregating agent. The study was done using a slightly different procedure of ADP dose-response aggregometry. Much greater aggregability in Caucasian than African men was consistent with the higher incidence of ischemic heart disease in whites, as reported by Meade *et al.* (1985) [6]. The study conducted by Gader confirms the observation by Mead when comparing Africans to Westerners. Platelet aggregation was estimated using a slightly different procedure of ADP dose-response aggregometry. However, when comparing Saudi Arabs and Westerners, no obvious differences between the ADP response of Saudi Arabs and Westerners were evident. On the other hand, the responses to ADP in South East Asians, like Africans, were significantly less than both Arabs and Westerners. Ina study of disparity in cardiovascular disease rates between Asian Indians and Caucasians. Patel *et al.* (2007) [8] indicated that there were no differences in platelet aggregation in response to ADP and AA. There is limited information on the differences in platelet aggregation between Hispanic and other ethnic groups. The reported differences in platelet aggregability between ethnic groups are often linked with genetic and dietary factors. (Salo *et al.*, 1985 and Renaud *et al.*, 1981) [13, 14].

Racial differences in ristocetin-induced platelet aggregation (RIPA) has been reported in at least other studies as well in

which blacks have exhibited markedly inhibited aggregation responses to ristocetin (Buchanan *et al.,* 1981) [9] when compared to whites. The diminished aggregability to ristocetin was also noted, but lesser in magnitude, in other non-Caucasians groups. In a study by Gader *et al.* (1991) [7], the diminished RIPA (ristocetin induced Platelet aggregation) in blacks was explained on the basis of a plasma inhibitor against RIPA. However, the authors recently observed and have reported a significant inhibition of RIPA in children with sickle-cell disease (SCD) when compared to normal children (Babikar *et al.,* 1987). Furthermore, West African students in King Saud University in Riyadh, who come from an area where sickle cell haemoglobin is prevalent, demonstrated, inhibited RIPA which was very similar to Saudi patients with Sickle Cell Disease. Therefore, sickle cell haemoglobin may be the cause for the abnormal RIPA seen in blacks. These inhibited aggregation responses to ristocetin may explain the low frequency of thromboembolic disease in Africans (Dupuy *et al.,* 1978) [11] and perhaps non Caucasians at large, since it indicates a diminished tendency of von Willibrand factor-mediated platelets stickiness to the endothelium. Aggregation using arachdonic acid, is a test of the integrity of the prostaglandin pathway involved in platelet activation. The higher prevalence of inhibited AA aggregation in Westerners compared to Arabs or Asians remains unexplained. The remarkably higher prevalence **(**51%) of abnormal aggregation responses to adrenaline in Asians compared to other ethnic groups was also an unexpected finding in the study by Gader *et al.* (1991) [7].

Most Asian subjects with markedly inhibited aggregation responses to adrenaline, showed normal response to AA and ADP. ADP and AA aggregations in Asians were similar to Arabs. The anomalous adrenaline aggregation seems to be unique to Asians. According to Gader, this could be due to different dietary habits; for example construction workers from South East Asia (mainly Filipinos) living in Riyadh, were shown to consume a considerable amount of fish (different types of fish being served in the three meals every day (Al-Mufarraj *et al.,* 1990) [12]. A similar phenomenon. i.e., dietary-related anomalous aggregation responses have been reported recently in a Finnish study where platelets collected from farmers were found to be less responsive to adrenaline but have the same sensitivities to other agonists (ADP and thrombin) when compared to platelet responses in semi-urban populations (Salo *et al.,* 1985) [13]. These differences were related to the different types of fats consumed by rural versus semi-urban subjects an observation which was confirmed in three farming communities in Britain (Renaud *et al.*, 1981) [14]. Similar selective diminution of platelet aggregation responses to adrenaline has also been related to consumption of alcoho1 (Fenn *et al.*, 1984 and Mikhailidis *et al.*, 1986) [15] but the study by Gader *et al.* (1991) [7] could not find any evidence that alcohol consumption is the causative factor in the Asian subjects. Their study established ethnic differences in platelet aggregation responses to ADP, adrenaline, arachidonic acid and ristocetin, with Asians and Africans showing the greatest deviations from other ethnic populations.

Dietary factors such as fish, onions and garlic are known to inhibit platelet function (Ogstun, 1983) [18]. These ingredients in addition to a mixture of different spices are used in cooking the ordinary Saudi diet. The study by Bertrand *et al.* (1987) [17] comparing the platelet aggregation in 50 Ivorian and 50 European showed a lower platelet aggregability in the Ivorian people as compared to the Europeans. The authors have attributed environmental factors in the genesis of such differences. Hypocholesteraemia, lower smoking and drinking levels and high fish consumption in the Ivorian population has been put forth as the basis for reduced platelet aggregability.

The dietary habits of the Fijians and Indofijians differ in a way that the Indofijian food is rich in spices and contains a lot garlic and onion as compared to the Fijian diet. Despite these differences no difference in PA between the Fijian and Indofijians was seen in our study.

Conclusion

The results of this study revealed that there was no difference in platelet aggregation in the Fijian and Indo-Fijian non-kava drinking and kava drinking population. There seem to be wide variation in results regarding the platelet aggregation in different ethnic groups based on the method and aggregating agent used for aggregation. Important factors like, rural versus semi urban, smoking status has been taken into consideration. In this study collagen has been used as the aggregating agent compared to these other studies where different aggregating agents like ADP, adrenaline, ristocetin, have been used. Diet, smoking status, and alcohol consumption of the volunteers has been taking into consideration, yet, no difference in platelet aggregation was seen among the two ethnic groups.

Study limitations the results of this study have certain limitations. One is the lack of genotyping to directly determine genetic variation and associated platelet phenotypes in studied subjects. The strict inclusion and exclusion criteria used made the study groups relatively uniform, largely preventing extraneous variables from affecting platelet aggregability. Only one methodology has been employed and only one aggregating agent (collagen) used in the determination of platelet aggregation.

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