BLOOD FLOW INDEX IN LOWER EXTREMITIES OF HEALTHY VOLUNTEERS BY IMPEDANCE PLETHYSMOGRAPHY (IPG)

Yogesh Kacha*, Anup Vegad, Maulik Varu**, Hemant Mehta***, C J Shah******

* Assistant Professor, Pacific Medical College, Udaipur, Rajasthan, ** Assistant Professor, ***Professor and Head, **** Associate Professor, Department of Physiology, Government Medical College, Bhavnagar, Gujarat

Abstracts: **Background & Objectives:** Impedance plethysmography (IPG) is the recording of instantaneous volume by measurement of electrical impedance. It is an indirect assessment of blood volume changes in any part of the body segment from changes in the electrical impedance of that segment. Our study was aimed to measure the blood flow index (BFI) of lower extremities in healthy Volunteers. **Methods:** The study was carried out in different segments of lower extremities (knee, calf and ankle) of healthy volunteers of different age groups of either sex by Nivomon series computerized software of L&T Company at cardiovascular laboratory in department of Physiology, Government Medical college, Bhavnagar, Gujarat. **Results**: Arterial parameters measured were Basal impedance (Z_0) & Blood flow index (BFI) in knee, calf ankle regions and analyzed statistically. **Interpretation & Conclusion:** Z_0 increases as the age increases and BFI remains more or less same throughout the age groups with higher BFI in proximal than in distal part of lower extremities. It is suggested that IPG may become an important clinical tool to study the central as well as peripheral blood flow. This simple, harmless, inexpensive, objective and non-invasive hemodynamic test may replace other invasive hemodynamic test for screening peripheral vascular diseases (PVDs) at any age provided that one has predetermined normative data.

Key Words: Blood flow index, Impedance plethysmogrphy (IPG), Basal impedance (Z_0)

Author for correspondence: Dr. Yogesh Kacha, Assistant Professor, Department of Physiology, Pacific Medical College & Hospital, Udaipur, Rajasthan, 313001.E- mail ID: dr.yogesh.kacha@gmail.com

Introduction:

Peripheral vascular disease (PVD), commonly referred to as peripheral artery occlusive disease (PAOD), refers to the obstruction of large and medium sized arteries not within the coronary, aortic arch vasculature, or brain [1]. PVD can result from atherosclerosis, inflammatory processes leading to stenoses, an embolism, or thrombus formation. Risk factors contributing to PAD are smoking, diabetes mellitus, dyslipidemia, hypertension, obesity etc. Angiography is a routinely used invasive procedure to investigate PVD [1]. While in case of non-invasive methods, Doppler Ultrasound [2], Strain gauge plethysmography [3], Pulse volume recorder [4], Electrical impedance plethysmography [5-9] has been made in developing to investigate PVD. Ankle brachial pressure index.[10] has been made in developing new non-invasive methods to investigate PVD. The literal meaning of Impedance Plethysmography is “Recording of instantaneous volume (of an object) by measurement of electrical impedance.” It has, however become a synonym for “indirect assessment of blood volume changes in any part of the body from changes in the electrical impedance of the body segment”, introduced by Nyboer [11]. In this technique, the electrical impedance of any part of body is measured by either constant current method or bridge method and variations in the impedance are recorded as a function of time. Since blood is a good conductor of electricity, the amount of blood in a given body segment is reflected inversely in the electrical impedance of the body segment. Pulsatile blood volume increase in the body segment caused by systemic blood circulation therefore, causes proportional decrease in the electrical impedance. Variation in the electrical impedance thus yields adequate information about the blood circulation [12].

Any flow limiting stenoses found in the x-ray can be identified which give information about the anatomical status of the arterial and venous tree but not about the hemodynamic of the circulation in the limb. The site of block is very important in patients who are likely to undergo surgery. In these patients, arteriography is essential. In rest of the patients undergoing conservative therapy, arteriography need not be performed. Impedance plethysmography thus offers a good screening procedure for the patients with PAD.[13]

Doppler ultrasound, [1] a popularly used method for diagnosing of PVD, can detect and measure blood flow by measurement of high-frequency sound waves that are reflected off of tissues. It has the advantage of locating the block anatomically but is insensitive to the deeper blood vessels. Threefold cost of the Doppler system and requirement of a skilled operator gives an edge to impedance technique over the Doppler.[14] The ankle-brachial index (ABI) is the most documented of these methods, but in diabetic patients, calcification of artery walls (medial sclerosis) frequently causes falsely elevated ankle pressure values.[15, 16] A complementary methods might be necessary to exclude medial sclerosis.

Impedance Plethysmography is superior to other plethysmographic methods (volume displacement plethysmograph, strain gauge plethysmograph and photo plethysmograph), as it is directly related to the electrical property of the blood [3].The sensitivity of IPG technique is 97.5% and the specificity of IPG technique is 98.1 % in the diagnosis of PAOD.[17]

Work carried out in our laboratory using this technique has been documented in several publications.[18] In our country, few institutes [13,19-22] have added IPG studies to their routine procedures as these techniques are simple, non invasive, low cost, objective and informative. In future, Impedance Plethysmography (IPG) may become a useful non-invasive tool in determining the hemodynamic of the circulation in limbs or in diagnosis of peripheral vascular diseases. Impedance Plethysmography (IPG) may become an useful non-invasive tool in future to know the following things in peripheral arterial occlusive diseases (PVDs) [19]: (1) To determine the extent of the arterial obstruction. (2) To decide the status of the collateral circulation. (3) To determine the flow in the artery proximal and distal to the block (arterial run off). (4) To study the effect of various drugs on the peripheral arterial circulation. (5) To select patients for arteriographic study who need surgery. (6) To provide aid in the selection of modality of therapy. (7) To study the benefits derived from surgical procedures such as sympathectomy, vascular bypass operations etc.

So far in India, very few studies are carried out to detect vasculopathy with the use of IPG hence the aim of this study was to set up a normative baseline data for future study of BFI (Blood flow index) of lower limb segment for cardiovascular laboratory in Department of Physiology, Government Medical College, Bhavnagar, Gujarat.

Material and Methods:

The present study of arterial parameters using Impedance Plethysmography (IPG) in 100 healthy subjects of either sex of different age group of Bhavnagar district was conducted in the Department of Physiology, Government Medical College, Bhavnagar after obtaining prior approval of IRB (Institutional Review Board) & Ethical committe of Government Medical College, Bhavnagar and taking informed consent from the participants. The study was carried out in the period extending from May 2012 to August 2013.

The study population was consisted of healthy medical staff of Government Medical College and Sir Takhtsinhji Hospital, Bhavnagar. There was also included other volunteers attending Sir Takhtsinhji Hospital and UHTC Bhavnagar, after proper screening of inclusion and exclusion criteria. **Inclusion Criteria*:*** a) 100 healthy subjects. b) Age : > 30 year in healthy c) Sex: either sex. d) Those who can give written informed consent. **Exclusion Criteria*:*** a) Age group ≤ 30 years. b) H/O significant illness of other system especially cardiovascular system. c) Factors affecting blood flow like smoking, obesity, diabetes. d) Hypertension, other Peripheral vascular disease like burger disease etc. e) those who not give informed consent.

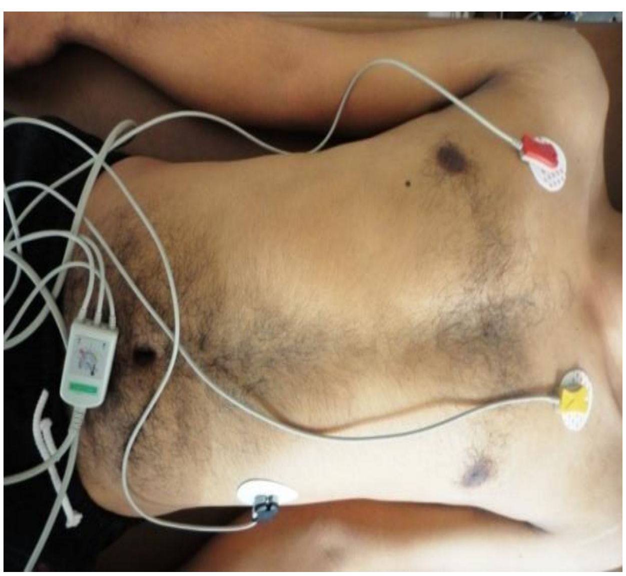
From 100 healthy subjects ranging from age group 31-90 years 45 were females and 55 were males. We devided them into different age groups: A. 31-40 (Males-13 , Females-12), B. 41-50 (Males-16 , Females-9), C. 51-60 (Males-12 , Females-13), D. > 60 years (Males-14 , Females-11).

Materials requirement*:* IPG cable, Accessory band electrodes, E.C.G. cable and E.C.G. electrodes were used to capture the IPG (impedance plethysmography) waveform and to get the value of arterial parameters like Zo (basal impedance), BFI (blood flow index), from each subject participated in the study.

Subject preparation:

The age, sex, height and weight of the subjects were recorded. Each subject was given a thorough Clinical Examination as a preliminary measure as detailed out in proforma to exclude any pathology affecting vascular function. No subject had a history of relevant cardiovascular disease, diabetes, and hypertension or of drug use which affect vascular functions. After giving brief information about the procedure to alleviate any apprehension and to assure full relaxation during the test & obtaining written consent from each volunteer participated in the study, the IPG recording was done in supine position at room temperature on Nivomon Series Product computerized software by L & T company in the cardiovascular laboratory, Department of physiology, Govt. Medical College, Bhavnagar.
Surface E.C.G. electrodes were used on the chest of the subject in 3 lead configurations as shown in figure 1.

Figure 1: E.C.G. electrodes placement in 3 lead configuration on chest



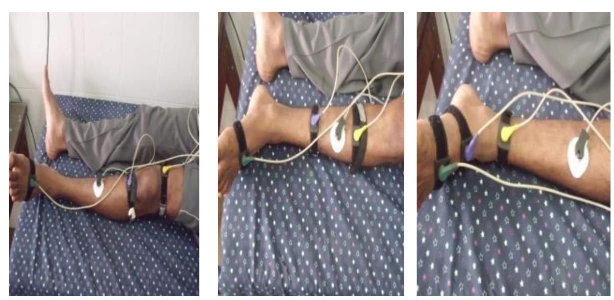
The accessory band electrodes (I_1, I_2 - current electrodes and V_1, V_2 – sensing electrodes) were strapped to the body segment of interested area of the subject by choosing appropriately sized bands at the desired location to capture the IPG waveform in following table 1.

Table 1: **Placement of accessory band electrodes on lower limb segment**

No.	Segment	Placement of Accessory Band Electrode			
		I_1	I_2	V1	V_2
1.	Knee	Forehead	Feet	Above knee	Below knee
2.	Calf	Forehead	Feet	Below knee	Above ankle
3.	Ankle	Forehead	Feet	Above ankle	Below ankle

In present study, Inter-electrode distance between V_1 and V_2 was tried to maintain less than 10 cm for each site as mentioned above. I_1, V_1, V_2 and I_2 of IPG cable were connected to the band electrodes at the extremities of the measurement area to capture the IPG waveform.

Figure 2: Placement of accessory band electrodes on lower limb segment



Knee Calf Ankle

The IPG waveform was recorded at least for 30 seconds but to reduce the noise interference in the averaged waveform, long recording to a maximum of 150 seconds was taken. As soon as waveform acquisition was stopped, all results of arterial parameters (Z_0, and BFI) displayed on the screen. After recording was over from all the desired sites, surface electrodes and band electrodes from the body of subject were carefully removed with minimal discomfort to subject.

The statistical analysis of the data obtained in the above recordings was performed by computer programs using Microsoft excel and statistical software named GraphPad InStat 3 Demo version. The data were presented if form of mean ±/-SD and analyzed by applying anova test. Kruskal - Wallis test with post test - Dunn's multiple comparison tests was used to compare each arterial parameter among the 4 age groups.

Result:

The present study was comprised of IPG recordings from 100 normal subjects in the age range of > 30 years. The physical parameters of the subjects are shown in Table 2. The mean ± SD of the age of the subjects in years, height in cm, Body mass index (BMI) in kg/m^2, heart rate in beats/min and systolic blood pressure in mm Hg are depicted for males and females.

TABLE 2: Physical parameters of males and females (Mean ± SD)

Physical parameters	MALE (n=55)	FEMALE (n=45)
Age (years)	50.02± 15.57	59.79± 10.20
Height(cm)	168.02± 6.20	150.93± 3.40
Weight(kg)	66.70± 11.22	64.52± 13.84
BMI (kg/m2)	23.55± 3.26	28.25± 5.70
Systolic BP (mm Hg)	122.35± 7.29	124.42± 11.54
Diastolic BP (mm Hg)	81.25± 6.67	80.90± 5.91
Heart Rate (beats/min)	79.09± 4.52	78.55± 4.89

The Mean ± SD of the arterial parameters like BFI and Z_0 that were recorded in Males 100 normal subjects (45 females; 55males) of different age groups (A, B, C, D). Tables no. 3, 4, 5, 6, 7, and 8 shows recording of IPG parameters in region of Knee, Ankle, and Calf.

Table 3: Zo and BFI in knee of males (Mean ± SD)

SITES	KNEE			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	30.21± 8.70	0.76± 0.13	32.13± 7.11	0.78± 0.14
B	34.93± 14.85	0.75± 0.09	35.28± 11.35	0.73± 0.20
C	34.86± 8.03	0.72± 0.06	34.71± 7.94	0.72± 0.07
D	34.56± 8.35	0.74± 0.15	37.92± 7.85	0.73± 0.15

Table 4: Zo and BFI in calf of males (Mean ± SD)

SITES	Calf			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	35.14± 13.16	0.75± 0.13	30.75± 14.96	0.79± 0.14
B	36.09± 13.45	0.74± 0.12	40.69± 15.10	0.78± 0.14
C	47.47± 12.20	0.72± 0.11	41.94± 16.99	0.76± 0.14
D	48.87± 14.32	0.73± 0.14	40.60± 13.62	0.76± 0.18

Table 5: Zo and BFI in ankle of males (Mean ± SD)

SITES	Ankle			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	61.17± 11.39	0.81± 0.19	63.70± 11.90	0.82± 0.22
B	58.21± 15.41	0.80± 0.18	61.23± 15.66	0.79± 0.11
C	59.8±3 7.31	0.76± 0.11	63.00± 8.90	0.69± 0.13
D	63.31± 16.31	0.77± 0.13	65.59± 8.02	0.64± 0.12

Table 6: Zo and BFI in knee of females (Mean ±SD)

SITES	Knee			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	26.15± 8.65	0.72± 0.15	29.13± 12.58	0.72± 0.33
B	27.61± 8.29	0.75± 0.17	28.09± 6.99	0.74± 0.14
C	46.36± 20.48	0.70± 0.04	43.55± 16.46	0.71± 0.12
D	43.43±	0.71±	34.58±	0.75±

	9.68	0.12	9.56	0.22

Table 7: Zo and BFI in calf of females (Mean ± SD)

SITES	Calf			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	32.52± 18.32	0.72± 0.14	33.26± 14.52	0.70± 0.45
B	33.17± 13.86	0.77± 0.18	39.39± 13.64	0.78± 0.16
C	33.78± 10.49	0.71± 0.07	36.76± 13.37	0.74± 0.11
D	35.50± 11.61	0.72± 0.11	37.22± 15.49	0.73± 0.11

Table 8: Zo and BFI in ankle of females(Mean ±SD)

SITES	Ankle			
GROUPS	RIGHT		LEFT	
PARAMETERS	Zo (Ω)	BFI	Zo (Ω)	BFI
A	58.55± 13.25	0.78± 0.13	58.23± 10.23	0.77± 0.12
B	61.44± 11.03	0.78± 0.17	60.42± 11.12	0.68± 0.13
C	60.06± 22.68	0.77± 0.10	62.31± 19.18	0.77± 0.15
D	61.15± 12.12	0.77± 0.12	64.15± 12.36	0.66± 0.17

TABLE 9: Comparison of Zo and BFI in both sides of males subjects among the 4 groups

SITES	KNEE JOINT (p value)		CALF (p value)		ANKLE JOINT (p value)	
	Zo	BFI	Zo	BFI	Zo	BFI
GROUPS	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. B	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. C	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
B vs. C	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
B vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
C vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Note: p value < 0.05 indicates significant.

In male subjects intergroup variations in arterial parameters of both sides in all three regions were found statistically not significant by applying ANOVA (p values for Zo: knee- 0.2519, calf-0.4279, ankle-0.6556, p values for BFI: knee- 0.4005, calf-0.6723, ankle-0.3907).

TABLE 10: Comparison of Zo and BFI in both sides of females subjects among the 4 groups

SITES	KNEE JOINT (p value)		CALF (p value)		ANKLE JOINT (p value)	
	Zo	BFI	Zo	BFI	Zo	BFI
GROUPS	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. B	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. C	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
A vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
B vs. C	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
B vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
C vs. D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Note: p value < 0.05 indicates significant.

In female subjects intergroup variations in arterial parameters of both sides in all three regions were found statistically not significant by applying ANOVA (p values for Zo: knee- 0.2430, calf-0.6145, ankle-0.5124, p values for BFI: knee- 0.6351, calf-0.3536, ankle-0.9873).

Discussion:

Blood flow index (BFI) and Z_0 that were recorded in males and females of healthy volunteers as shown in Table no. 3 - 8. Although statistically not significant but we can say visually from our findings that with aging, Z_0 increases in all 3 sites – knee, calf and ankle and there may be decrease in BFI with increasing age. Probably it may be because of different vessels sizes (length and diameter) and organization (series or parallel circuits).[23] Which affect the Z_0 and BFI according to Poiseuille's equation. These may be due to various factors affecting the blood flow through the tissue besides age like circumference of limb, temperature, occupation, hematocrit, vessels size and organization etc.[24] Basal impedance Z_0 of various biological tissues like plasma, blood, skeletal muscle, cardiac muscle, bone, fat etc. show markedly variation and out of it, fat shows greater resistance.[14] Z_0 also depends on vessels size (diameter, length), vessels arrangements (series or parallel) and viscosity of blood.

This may be due to age related changes occurring in blood vessels. Blood vessels show gradual decrease in number of elastic fibres and increase in number of collagen fibres and also there occurs deposition of calcium salts in elastic and muscular type of arteries in later ages. These all lead to decrease in the distensibility and windkessel effect of the blood vessels and also cause arteriosclerosis.[25] Thus, People with diabetes are more prone to PAD than nondiabetics. Regular follow up assessment allows early identification of a reduced arterial blood flow to the lower extremities which, in turn, allows the implementation of management strategies earlier to prevent PAD. An accurate vascular assessment can prove invaluable in clinical practice as a screening tool to predict ulcer risk, identify patients in whom vascular conditions require further investigation and inform a prognosis for wound healing in patients with active ulceration. However, vascular assessment of the diabetic foot can be complicated by a number of factors: calcification of the arterial walls is common in diabetes, with approximately one third of diabetic patients developing calcified arteries (medial arterial calcification) [26] this causes the vessels to lose elasticity and become rigid. The presence of medial arterial calcification and/or neuropathy can also render some assessment techniques unreliable.[27] it is imperative that practitioners have an awareness of the potential limitations of commonly utilised assessments to ensure accuracy when assessing a patient's vascular status. So IPG may become a valuable non-invasive test in making diagnosis, prognosis and therapeutic decision in diabetic mallitus & other peripheral vascular diseases because of their so many advantages compared to other methods.

Conclusion:

Z_0 increases as the age increases and BFI remains more or less same throughout the age groups with higher BFI in proximal than in distal part of lower extremities. It is suggested that IPG may become an important clinical tool to study the central as well as peripheral blood flow and for diagnosis, prognosis and therapeutic intervention in peripheral vascular diseases.

Acknowledgment:

We are thankful to our Head of department Dr. H. B. Mehta Sir & entire physiology department for their kind support. We are also very thankful to our healthy volunteers for taking part in our study.

References:

1. Peripheral Arterial Disease at Merck Manual of Diagnosis and Therapy Professional Edition, Retrieved on August 9, 2010. Available from:http://en.wikipedia.org/wiki/Peripheral_vascular_disease.

2. Franklin, D L., Schlegal, W. A. and Rushmer, R. F.: Blood flow measured by Doppler frequency shift of back scattered ultrasound. Science, 1961, 134: 564-565.
3. Sigdell, J. E.: A critical review of the theory of the mercury strain-guage plethysmograph. Med. Biol. Eng., 1969, 7: 365-371.
4. Raines, J. K., 1972, "Diagnosis and Analysis of Arteriosclerosis in the Lower Limbs from the Arterial Pressure Pulse." Ph.D. Thesis, Massachusetts Institute of Technology.
5. Jindal GD: Impedance plethysmography for screening vascular disorders. J Postgrad Med, 1986. 32, 1, 1-3.
6. Jaffrin, M. Y. and Vanhoutte, C.: Quantitative interpretation of arterial impedance plethysmographic signals. Med. & Biol. Eng. Compute., 1979, 17: 2-10.
7. Kubicek, W. G., Kottke, F. J., Patterson, P., Witsoe, D. A., LaBree, J. W., Ransole, W., Layman, T. E., Schoening, H. and Smith, D.: The Minnesota impedance cardiograph theory and applications. Bio, Med. Eng., 1974, 9: 410-416.
8. Van De Water, J. M., Cough, N. P., Dmochowski, J. and Dove, C. B.: Development and evaluation of the impedance flow meter in arterial surgery. Surgery, 1971, 70: 954-961.
9. Van De Water, J. M., Dove, C. B., Mount, B. E. and Linton: Application of bioelectric impedance to the measurement of arterial flow. J. Surg. Res., 1973, 15: 22-29.
10. Al-Qaisi, M; Nott, DM, King, DH, Kaddoura, S (2009). "Ankle brachial pressure index (ABPI): An update for practitioners.". Vascular health and risk management 5: 833–41.
11. Nyboor, J.: Regional pulse volume and perfusion flow measurements; Electrical impedance plethysmography. Arch. Intern. Med., 1960, 105: 264-276.
12. Nyboer J, Bango S, Barnett A, Halsey RH. Radio-cardiograms. J Clinical Investigation, 1940.
13. MD Kelkar, Padmashree R Bharadwaj, GD Jindal and GB Parulkar : Impedance Plethysmography in Arterial Occlusive Disease : Correlation with Arteriography. Indian Heart Journal 1983; 35; 2 : 94-97.
14. Baker LE. Principles of the impedance technique. IEEE Engg Med Biol Magazine, 8:11-15, 1989.
15. Lepa¨ntalo M, Kangas T, Pietila¨ J, Scheinin T, Scheinin TM: Non-invasive characterization of angiopathy in the diabetic foot. Eur J Vasc Surg 2:41–45, 1988
16. Emanuele MA, Buchanan BJ, Abraira C: Elevated leg systolic pressures and arterial calcification in diabetic occlusive vascular disease. Diabetes Care 4:289–292, 1981
17. Jindal GD, Nerurkar SN, Pedhnekar SA, Babu JP, Kelkar MD, Deshpande AK, Parulkar GB. Diagnosis of peripheral arterial occlusive diseases using impedance plethysmography. J Postgrad Med [serial online] 1990 [cited 2012 Feb 22];36:147.
18. Divyesh L Prajapati*.Chintan V Parmar, Pradnya A Gokhale, Hemant B Mehta, Chinmay J Shah..Non-Invasive Assessment Of Blood Flow Index In Healthy Volunteers Using Impedance Plethysmography. Int J Med Health Sci. January 2013,Vol-2;Issue-1.
19. Parulkar GB, Padmashree RB, Bapat RD, Rege RV, Bhagtani KC, Jindal GD : A new electrical impedence plethysmogram : observations in peripheral arterial occlusive diseases: J Postgrad Med, 27:66-72, 1981.
20. Jindal GD, Nerurkar SN, Pednekar SA, Babu JP, Kelkar MD, Deshpande AK and Parulkar GB : Diagnosis of peripheral arterial occlusive diseases using impedance plethysmography. J Postgrad Med 1990;36:147.
21. Jindal GD, Pednekar SA, Nerurkar SN, Masand KL, Gupta DK, Deshmukh HL, Bapu JP and Parulkar GB : Dignosis of venous disorders using impedance plethysmography. J Postgrad Med 1990;36:158.
22. Jindal GD, Kelkar MD, Bhardwaj PR, Dewoolkar SD, Suraokar SB, Babu JP, Parulkar GB, et al.: Non-invasive diagnosis of aortic and arterial occlusive diseases using on-line impedance. Clinic (Italy): 1984; 2:3-14.
23. Richard E. Klabunde. Cardiovascular Physiology Concepts. Vascular function, 2nd ed. Lippincott Williams & Wilkins publisher, 2011, pp. 108.
24. Text book of Medical physiology by Guyton & Hall: 12th Edition, 164-170.
25. Suzuki E, Yoshimura T, Omura Y, Sakaguchi M, Nishio Y, Maegawa H, Hisatomi A, Fujimoto K, Takeda J, Kashiwagi A. Higher arterial stiffness, greater peripheral vascular resistance and

lower blood flow in lower-leg arteries are associated with long-term hyperglycaemia in type 2 diabetic patients with normal ankle-brachial index. Diabetes Metab Res Rev. 2009 May;25(4):363-9. doi: 10.1002/dmrr.955.
26. Jagruti C, Chaugule N, Ananthakrishnan TS,Prakash Babu J and Jindal GD (2000) : Normalized DZ/DT waveform for easyassessment of peripheral blood flow, Proc. SBME-NM 2000, 67.
27. Falanga V (2005) Wound healing and its impairment in the diabetic foot. Lancet 366: 1736–43.

Disclosure: No conflicts of interest, financial, or otherwise are declared by authors