ABSTRACT

Aim: To evaluate the applicability of Ultrasound Doppler Flowmetry (2D, Duplex and colour flow imaging) in recording the gingival blood flow and to assess the blood flow changes prior to and following periodontal flap surgery. Methodology: Nine patients with Generalized chronic periodontitis with a probing pocket depth of ≥5mm were included in this study. After completion of the basic periodontal therapy, Ultrasound Doppler measurements of Peak Systolic Velocity were performed on the day of surgery (0 day-baseline) prior to local anesthesia in the upper anterior segment. The study area was treated with an access flap. Ultrasound Doppler measurements of Peak Systolic Velocity were recorded on 7th, 15th, 30th, 60th days following surgery. Results: At baseline, each patient presented with variations in the gingival blood flow. The gingival blood flow presented an overall increase in comparison to baseline values until 7th, 15th, 30th post-operative days. At 60th day, the gingival blood flow values were similar to baseline values. Probing pocket depth reduced significantly from baseline to 60th post operative day. Conclusion: The results of the present study, suggests that Ultrasound Doppler Flowmetry with colour imaging facility presents clinical applicability in recording and assessing the blood flow changes following surgery.

Keywords: Gingival blood flow, color doppler, flap surgery, healing

INTRODUCTION

The objective of periodontal surgery is to preserve the periodontium by facilitating plaque removal and plaque control. Healthy gingiva is characterized by subepithelial vascular plexus consisting of a capillary network with loops arching towards the epithelium. Gingival inflammation results in increased vascularity with more capillary loops, larger blood vessel size and slowed blood flow, and restriction of the afferent blood vessels. Wound healing following periodontal surgery is influenced by the revascularization rate, and by the preservation and reconstruction of the microvasculature of the gingival tissues. Repair of connective tissue also depends on the development of a new vascular system, which can supply blood and nutrients to the wound area. Several methods have been used to measure gingival blood flow (GBF) which includes vital microscopy of gingival margin, implantation of microspheres into the...
internal carotid artery in animals, infused radio isotopes and radio labeled microspheres and high speed cinematography. Most of these are invasive and not suitable for clinical application on patients. Laser Doppler Flowmetry (LDF), is non-invasive and a real time method, currently used for perfusion measurements but it has certain shortcomings. Only a localized small area of gingival tissue beneath the area of placement of the laser probe over any one selected tooth could be studied at a given time. Also, comparison of blood flow changes between different sites in the same patient and different patients is not possible. To overcome this, we have measured the perfusion status of the gingiva using Ultrasound Color Doppler. This study attempts to evaluate the applicability of Ultrasound Doppler (2D and Duplex Imaging) in recording gingival blood flow and to assess its changes before and after periodontal flap surgery.

**MATERIALS AND METHODS**

Nine patients with generalized chronic periodontitis from the patient pool of the Department of Periodontology, Meenakshi Ammal Dental College & Hospitals, Chennai were recruited for the study. The study sample consisted of 4 males and 5 females between the age group of 33 to 52 years. The Ultrasound Doppler recordings were performed in the Department of Radiology and Imaging, Meenakshi General Hospital, Chennai.

**Inclusion Criteria**

1. Patients with generalized chronic periodontitis with probing pocket depth of ≥5mm.

**Exclusion Criteria**

1. Smokers.
2. Patients with systemic diseases and complications.
3. Patients who have undergone periodontal treatment in the past six months.
4. Patients who have used systemic antibiotics/NSAIDs for at least 6 months prior to the commencement of the study.

**Presurgical Protocol**

The surgical procedure (access flap surgery) was planned in the upper anterior segment for all patients. Treatment protocol was explained to the patient and informed consent was obtained. All patients underwent initial phase of scaling and root planing. They were all educated and motivated on proper oral hygiene maintenance and were recalled after 1 month for periodontal flap surgery. The Ultrasound Doppler recordings of Peak Systolic Velocity (PSV) were recorded on 0 day (baseline) prior to surgery.

**Ultrasound Methodology**

Ultrasoundography studies were performed in the Department of Radiology and Imaging, Meenakshi General Hospital, utilizing PHILIPS HDI 1500- ATL® UNIT with 2D, 3D and Colour Doppler facilities (Figure 1). For routine soft tissue USG (Ultrasoundography), high definition linear transducer probe with 12–5MHz capacity was used for all patients. For intra oral and gingival study, our choice was intracavitory convex transducer with 9–5MHz capacity, since this probe facilitated adequate and satisfactory intra-oral accessibility. The ultrasound waves produced by the 9–5MHz transducer possess adequate penetrating capacity up to a depth of 3cm to 7.5cm. For the study of anterior segment gingiva, a cheek retractor was used and a water filled glove finger was placed over the gingiva. The transducer was applied over the glove
finger in a coronal plane with interspersed ultrasonic gel for proper contact. The water filled glove finger served as a water path for the sound waves and provided better clarity and detail (Figure II).

Sound waves produced at higher MHz have less penetrating capacity but provide better soft tissue characterization and detail. USG settings were adjusted for 4.5cm depth which would be adequate for covering water path and the gingiva. Initially two dimensional (2-D) images of the gingiva were obtained to provide tissue detail of varying echoarchitecture (Figure IIIa).

Gingiva consists of keratinized epithelium with connective tissue containing collagen fibers and vascular structures each with varying echogenicity. Fluid containing areas (blood vessels, cyst or abscess) will be less echogenic (hypoechoic) than fibrous tissue which are more echogenic (hyperechoic). High definition 2-D soft tissue images of gingiva depict excellent soft tissue morphology with mixed echogenic architecture. These images are obtained in cine mode from which suitable images are selected and recorded.

In Doppler settings, sound waves pass through blood vessels and get scattered (reflected) by moving red blood cells. For color Doppler study, the color box is adjusted over the region of interest (R.O.I.) and color gain must be adjusted to avoid any spillover of color outside the R.O.I. Color Doppler settings delineate the color coded blood vessels as rounded or oval dots or linear or cylindrical structures. The number of color coded structures will indicate the number of blood vessels in the region under study. Thus, color Doppler gives information about the amount of vascularity in the region of interest. In color Doppler images, whenever the blood flow is towards the transducer, it is color coded as red. When the blood flow is away from the transducer, it is color coded as blue (Figure III b).

For spectral Doppler studies, Pulse wave Doppler cursor is focused over the blood vessels which are depicted as anechoic areas in 2-D or as color coded blood vessels in color Doppler settings and the update key is pressed. This enables delineation of flow pattern in venous channel graphically. Recording the flow pattern in the form of graph (Spectral Doppler) allows measurement of velocity of blood flow in Peak Systolic Velocity (PSV) phase. These values are displayed on the monitor once the tracing over the graph is marked by auto or manual mode. This helps to measure the velocity of flow. Arterial channel depicts a pulsatile curve. Venous channel shows a continuous flow pattern. Spectral Doppler curve is projected above the base line when the blood flow is towards the transducer and the velocity values are depicted as +. The curve is projected below the base when the flow is away from the transducer and the velocity values are depicted as – (Figure III c).

**BASELINE (0 DAY)**

The Ultrasound Doppler recordings of Peak Systolic Velocity (PSV) were recorded on 0 day (baseline) prior to surgery.

**SURGICAL PROCEDURE**

The surgical area was anesthetized with 2% lignocaine hydrochloride containing 1:2,00,000 epinephrine. Sulcular incisions were given and a full thickness mucoperiosteal flap was reflected labially and lingually to expose the diseased root surface, which were carefully debrided. Saline irrigation was done. Flaps were replaced to their original position and secured with interrupted black silk (4-0).
sutures. The surgical site was covered with Coe-Pak®.

All patients were placed on antibiotics (Amoxicillin 500mg tid for 5 days), analgesics (Ibuprofen 400mg tid for 3 days) and 0.2% Chlorhexidine digluconate mouth rinse twice daily for one week. Patients were given handouts of postoperative instructions. Patients were recalled on 7th postoperative day for suture removal. Ultrasound Doppler measurements were recorded prior to suture removal (Figure IV).

Follow up visits
Follow up visits were scheduled on 15th day, 30th day & 60th day, on which Peak Systolic Velocity (PSV) was recorded (Figure V, VI, and VII).

Statistical Analyses
Mean and standard deviation were estimated from the sample for each variable at different time points. Mean values were compared between different time points using Students Paired t-test. The Ultrasound Doppler recordings were performed for all the patients for PSV at different time points. The data was statistically analyzed to find the Mean, Standard Deviation, test of significance of PSV at different time points.

RESULTS
The mean Peak Systolic Value (PSV) on day 0 was 2.33±0.25cm/s, which increased to a mean value of 3.32±0.34cm/s on day 7 and 3.00±0.23cm/s on day 15, 2.75±0.19cm/s on day 30 and 2.34±0.15cm/s on day 60 (Graph I). The mean increase in PSV from day 0 to all time points was statistically significant except for the time period between day 0 to day 60 (p<0.0001) (Table-I) (Graph II).

The mean decrease in PSV from day 7 to day 15, 30 and 60 was 0.32±0.23 (p<0.003), 0.57±0.30 (p<0.001), and 0.98±0.39 (p<0.0001) respectively. The mean decrease in PSV was significant for all the time periods (Table II) (Graph III). The mean decrease in PSV from day 15 to day 30 and 60 was 0.25±0.20, 0.66±0.28 respectively. The mean decrease in PSV was significant for both the time periods (Table III) (Graph IV).

The mean decrease in PSV from day 30 to day 60 was 0.41±0.19cm/s, which was statistically significant (p<0.0001) (Table IV) (Graph V). When the percentage change in PSV was evaluated (Table V), there was an increase in PSV from day 0 to day 7 by 42.49%, from 0 day to 15th day by 28.76%, 0 day to 30th day by 18.03%, and 0 day to 60th day by 0.43%. There was a decrease in PSV from day 7 to day 15 by -9.64% from day 7 to day 30 by -17.17% and from day 7 to day 60 by -29.52%. There was a decrease in PSV from day 15 to day 30 by -8.33% and from day 15 to 60 by -22%. There was a decrease in PSV from day 30 to day 60 by -14.91%.

DISCUSSION
Although the bacterial etiology of periodontal diseases is universally accepted, the exact mechanism of disease progression is unclear. Investigations of the pathogenesis of periodontitis focus on the initiation and progression of the disease (Gleissner et al 2006). One of the earliest signs of any inflammatory process is change in the vasculature architecture and microvasculature. The free gingiva receives its blood supply from the gingivo-periosteal plexus and the periodontal ligament plexus, which are connected with the Volkman canals passing through the alveolar bone (Lindhe et al 2003).
Periodontal flaps are commonly used in surgical treatment in order to gain access for professional scaling and root planing (Wennstrom et al. 2003). It is well known that, during the early-healing period, the blood reperfusion of the flap is a critical determinant in achieving optimal wound healing and avoiding partial flap necrosis (Retzepi et al. 2007). The present study has demonstrated the clinical applicability of soft tissue Ultrasonography (USG) with colour Doppler facility in recording the changes in gingival blood flow following periodontal flap surgery. One of the first reports on USG in Periodontology was by Spranger (1971) who tried to determine the height of the alveolar crest in patients with periodontitis. He concluded that, if carefully applied, this technique could add some information to X-ray diagnosis. In the present study we have performed USG with 9–5MHz capacity convex intracavitory transducer with color Doppler facility to determine the gingival vascularity.

The results of the 2D picture of the present study reveal whether the area is anechoic, hypoechoic or hyperechoic. Normal gingiva appears as hypoechoic, fluid filled regions (abscess) appear as dark anechoic, fibrotic and edematous regions as white hyperechoic. Color Doppler studies depict vascular areas by color coding the hypovascular and hypervascular areas depicted on 2D images. Marginal hypervascularity indicative of inflammation associated with infection is well depicted. Also, the flow pattern by Spectral Doppler clearly indicates the velocity of venous blood flow to the region. At baseline, (mean PSV 2.33cm/s) each patient presented with variations in the gingival blood flow measurements which are influenced by the thickness of the connective tissue and local distribution of different blood vessels.

In this study, Doppler measurements were performed prior to local anesthesia as the studies done by Hinrichs et al (1995), Ketabi and Hirsch (1997), Ahn J (1998)11, Donos N et al (2005)2 and Retzepi M et al (2007)7 have shown a significant decrease in the gingival blood flow following local anesthesia with vasoconstrictor, irrespective of the flap area.

Full thickness mucoperiosteal flap surgery was done (day 0) in order to increase the accessibility to root deposits and to reduce the pocket depth. At 7th day following flap surgery increased gingival blood flow was observed (from PSV 2.33cm/s to 3.32cm/s) which was statistically significant. This is in accordance with the recent Laser Doppler Flowmetry studies (LDF) done by Ambrosini et al (2002)3 where they have evaluated the modification and changes occurring in human gingival blood flow following periosteal stimulation, Donos N et al (2005)2 and Retzepi M et al (2007)7 where they investigated the pattern of gingival blood flow changes following periodontal access flap surgery.

It is well established that the vascular network within the mucoperiosteal flap presents hyperemic response after the 1st day following surgery, which continues until the 3rd day with simultaneous proliferation of the blood clot in areas of close flap adaptation (Caffesse et al 1981). This prompt hyperemic response of the microcirculation could be attributed to the action of vasoregulatory factors, inducing vasodilatation as the predominant microvascular response to a wound (Rendell et al 1998). By 4th day there is vascular proliferation in the organizing blood clot, with anastomotic channels connecting the cancellous bone circulation.
with the flap and mucosa. By 7th day the gingival vessels show continuity with those of periodontal membrane (Donos N et al 2005).2

By 15th day (PSV 3.00cm/s) and 30th day (PSV 2.75cm/s) following surgery, the gingival blood flow was increased when compared to the 0 day (baseline PSV 2.33cm/s) which was statistically significant, and the findings of the present study is supported by the studies done by Donos N et al (2005)2 where they have shown increased blood flow changes at buccal papillae and Retzepli M et al (2007)7 also have shown increased blood flow changes at palatal papillae on 15th and 30th days following surgery. In the present study, the increased blood flow changes that were still observed at 15th day and 30th days following surgery can be explained by the fact that the elevation of a mucoperiosteal flap initiates a significant vascular trauma. It has also been observed that the elevation of a mucoperiosteal flap serves as a trigger for angiogenesis of the periodontal ligament vascular plexus (Nobuto et al 2003).14

By 60th day (mean PSV 2.34cm/s) following surgery, the gingival blood flow was similar to baseline values (mean PSV 2.33cm/s) which were not statistically significant. The findings of the present study is in line with the study done by Donos N et al (2005)2 where they have shown the gingival blood flow changes in the alveolar mucosa on 60th day were very similar to baseline values. Study done by Retzepli M et al (2007)7 also have shown the gingival blood flow changes in the palatal papillae on 60th day were very similar to baseline values. Therefore, it would be reasonable to assume that the prolonged increased gingival blood flow would represent a combination of an increased vascular trauma of the areas and possible blood flow contamination from the deeper tissue layers such as the periodontal ligament vascular plexus. (Vongsavan et al 1993).15

USG is a non-invasive, radiation free, patient friendly procedure and is safe in children and pregnant women too. It may be performed as an outpatient procedure and is often repeatable with no contraindications or complications. This procedure provides direct visualization of quantum of vascularity and blood vessels. This procedure can be used for:

- Pre-treatment evaluation of gingival soft tissue and treatment planning prior to implant placement.
- Assessment of donor site vascularity for grafts and mucoperiosteal access flap area.
- Periodical post-operative monitoring of vascular status of various flaps and grafts.
- Evaluation of healing after periodontal surgery (Regenerative, muco-gingival or implant surgery).

The limitations of this study were the small sample size. More studies with larger sample sizes are required to better understand and detect the changes in the gingival blood flow at different time points and to establish their association with the critical wound healing process.

REFERENCES


2. Donos N, D’Aiuto F, Retzepli M, Tonetti M. Evaluation of gingival blood flow by the use of Laser Doppler Flowmetry following periodontal
Table – I: Comparison of Mean Values between Day 0 and Days 7, 15, 30, 60 for Peak Systolic Velocity (PSV) in cm/s.

<table>
<thead>
<tr>
<th>Time points compared</th>
<th>Mean ± S.D (PSV in cm/s)</th>
<th>Change Mean ± S.D (PSV in cm/s)</th>
<th>p-Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day – 0</td>
<td>2.33 ± 0.25</td>
<td>0.99 ± 0.43</td>
<td>&lt; 0.0001 (sig)</td>
</tr>
<tr>
<td>Day – 7</td>
<td>3.32 ± 0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 0</td>
<td>2.33 ± 0.25</td>
<td>0.67 ± 0.33</td>
<td>&lt; 0.0001 (sig)</td>
</tr>
<tr>
<td>Day – 15</td>
<td>3.00 ± 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 0</td>
<td>2.33 ± 0.25</td>
<td>0.42 ± 0.25</td>
<td>0.001 (sig)</td>
</tr>
<tr>
<td>Day – 30</td>
<td>2.75 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 0</td>
<td>2.33 ± 0.25</td>
<td>0.01 ± 0.18</td>
<td>0.87 (Ns)</td>
</tr>
<tr>
<td>Day – 60</td>
<td>2.34 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Student’s paired t-test was used to calculate the p-value.

Table – II: Comparison of Mean Values between Day 7 and Days 15, 30, 60 for Peak Systolic Velocity (PSV) in cm/s.

<table>
<thead>
<tr>
<th>Time points compared</th>
<th>Mean ± S.D (PSV in cm/s)</th>
<th>Change Mean ± S.D (PSV in cm/s)</th>
<th>p-Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day – 7</td>
<td>3.32 ± 0.34</td>
<td>0.32 ± 0.23</td>
<td>0.003 (sig)</td>
</tr>
<tr>
<td>Day – 15</td>
<td>3.00 ± 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 7</td>
<td>3.32 ± 0.34</td>
<td>0.57 ± 0.30</td>
<td>0.001 (sig)</td>
</tr>
<tr>
<td>Day – 30</td>
<td>2.75 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 7</td>
<td>3.32 ± 0.34</td>
<td>0.98 ± 0.39</td>
<td>&lt; 0.0001 (sig)</td>
</tr>
<tr>
<td>Day – 60</td>
<td>2.34 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Student’s paired t-test was used to calculate the p-value.

Table – III: Comparison of Mean Values between Day 15 and Days 30 & 60 for Peak Systolic Velocity (PSV) in cm/s.

<table>
<thead>
<tr>
<th>Time points compared</th>
<th>Mean ± S.D (PSV in cm/s)</th>
<th>Change Mean ± S.D (PSV in cm/s)</th>
<th>p-Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day – 15</td>
<td>3.00 ± 0.23</td>
<td>0.25 ± 0.20</td>
<td>0.006 (sig)</td>
</tr>
<tr>
<td>Day – 30</td>
<td>2.75 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day – 15</td>
<td>3.00 ± 0.23</td>
<td>0.66 ± 0.28</td>
<td>&lt; 0.0001 (sig)</td>
</tr>
<tr>
<td>Day – 60</td>
<td>2.34 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Student’s paired t-test was used to calculate the p-value.
Table – IV: Comparison of Mean Values between Day 30 and Day 60 for Peak Systolic Velocity (PSV) in cm/s.

<table>
<thead>
<tr>
<th>Time points compared</th>
<th>Mean ± S.D (PSV in cm/s)</th>
<th>Change Mean ± S.D (PSV in cm/s)</th>
<th>p-Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day – 30</td>
<td>2.75 ± 0.19</td>
<td>0.41 ± 0.19</td>
<td>&lt; 0.0001 (sig)</td>
</tr>
<tr>
<td>Day – 60</td>
<td>2.34 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Student’s paired t-test was used to calculate the p-value.

Table – V: Percentage change of Peak systolic velocity between Day 0 and Days 7, 15, 30, 60

<table>
<thead>
<tr>
<th>Time points compared</th>
<th>Mean % Change of PSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 – Day 7</td>
<td>42.49 %</td>
</tr>
<tr>
<td>Day 0 – Day 15</td>
<td>28.76 %</td>
</tr>
<tr>
<td>Day 0 – Day 30</td>
<td>18.03 %</td>
</tr>
<tr>
<td>Day 0 – Day 60</td>
<td>0.43 %</td>
</tr>
<tr>
<td>Day 7 – Day 15</td>
<td>-9.64 %</td>
</tr>
<tr>
<td>Day 7 – Day 30</td>
<td>-17.17 %</td>
</tr>
<tr>
<td>Day 7 – Day 60</td>
<td>-29.52 %</td>
</tr>
<tr>
<td>Day 15 – Day 30</td>
<td>-8.33 %</td>
</tr>
<tr>
<td>Day 15 – Day 60</td>
<td>-22.0 %</td>
</tr>
<tr>
<td>Day 30 – Day 60</td>
<td>-14.91 %</td>
</tr>
</tbody>
</table>

Graph 1 Represents the line diagram for Peak Systolic Velocity (PSV) at different time points.
Graph II  Comparison of Mean values between day 0 and days 7,15,30,60 for Peak Systolic Velocity (PSV).

Graph III  Comparison of Mean values between day 7 and days 15, 30, 60 for Peak Systolic Velocity (PSV).
Graph IV  Comparison of Mean values between day 15 and days 30, 60 for Peak Systolic Velocity (PSV).

![Graph IV](image1)

Graph V  Comparison of Mean values between day 30 and 60 for Peak Systolic Velocity (PSV).

![Graph V](image2)
**Figure VIb** Color Doppler

**Figure VIc** Spectral Doppler

**Figure VIIa** 2D Image

**Figure VIIb** Color Doppler

**Figure VIIc** Spectral Doppler

**60th POST-OPERATIVE DAY**

**Figure VIIa** 2D Image
Legends

Figure I - PHILIPS HDI 1500 ATL unit with 3D and Color Doppler facilities.
Figure II- 9 – 5MHz Intracavitary Transducer probe and water filled glove finger as water path.
Figure III a - 2D image of upper anterior segment shows mixed echogenic architecture.
Figure III b- Color Doppler study demonstrates marginal hypervascularity due to infection (color coded areas) and reduced vascularity in rest of the areas due to edema (not color coded).
Figure III c- Venous Spectral Doppler shows venous type of flow with Peak Systolic Velocity (PSV) of -2.48cm/s.
Figure IV a- 2D image revealing normal echoarchitecture
Figure IV b- Color Doppler delineates reduction in marginal hypervascularity with increased vascularity in rest of the areas.
Figure IV c- Venous Spectral Doppler shows normal flow pattern with Peak Systolic Velocity (PSV) of -2.98cm/s.
Figure V a- 2D image reveals uniform mottled echoarchitecture.
Figure V b- Color Doppler shows normal uniform vascularity.
Figure V c- Spectral Doppler shows both pulsatile arterial flow pattern with Peak Systolic Velocity (PSV) of 13cm/s and continuous venous flow pattern with Peak Systolic Velocity PSV of-2.92cm/s.
Figure VI a and VII a- 2D image shows normal echoarchitecture.
Figure VI b and VII b- Color Doppler shows normal vascularity.
Figure VI c- Venous Spectral Doppler shows normal flow pattern with Peak Systolic Velocity (PSV) of -2.66cm/s and arterial Peak Systolic Velocity (PSV) of 7cm/s.
Figure VII c- Venous Spectral Doppler shows normal flow pattern with Peak Systolic Velocity (PSV) of -2.30cm/s.