Comparative Study between Angiosome Directed Therapy and non-Angiosome Therapy in Treatment of lower limb ischemia

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Abstract
Revascularization of the source artery to the angiosome result in better wound healing and limb salvage rates in comparison to failure of its revascularization as in our study showed that 90% better healing in directed Angiosome concept and 20% in the which Angiosome concept not applied direct revascularization of arteries supplying the target angiosome (wound area) resulting in less complication and no further debridement is needed as in our study only 10% need post operative debridement in comparison to 80% in the other group where Angiosome therapy couldn’t be applied.

Keywords: angioplasty, angiosome, foot ulcers.

1. Introduction
Peripheral arterial disease is a manifestation of atherosclerosis that leads to arterial stenosis and occlusions in the major vessels supplying the lower limbs. The most common symptom of Peripheral arterial disease is intermittent claudication, characterized by reproducible muscular leg pain on exercise that is relieved by short rest. In more severe cases there may be chronic ischemic rest pain, which usually indicates critical limb ischemia (1).

Peripheral arterial disease is 2-4 times more common in patients with diabetes than in those without (2) and in diabetic patients with foot ulcers the prevalence of Peripheral arterial disease can reach up to 50% (3). The most feared complication of Peripheral arterial disease is amputation and diabetic foot complications remain the main cause of non-traumatic amputation in most western countries (4).

Neuropathy and ischemia are usually the initiating factors, bearing a different weight in
different patients. Infection is rarely the direct cause of an ulcer, but is strongly related to the probability of amputation, especially in combination with Peripheral arterial disease (5).

The main goal when treating critical limb ischemia is preservation of the limb. Ischemia is primarily responsible for 90% of amputations in these patients (6). When left untreated, the poor arterial perfusion in critical limb ischemia may lead to the need for amputation (7) and potentially fatal complications from the progression of gangrene and the development of sepsis (8).

The vast majority of ischemic foot complications resulting in amputation and it is usually begin with the formation of skin wounds. Early detection and appropriate treatment of these wounds may prevent up to 85 percent of amputations, Optimal wound healing requires adequate tissue perfusion. Thus, arterial insufficiency should be suspected if wounds fail to heal (9).

Patients with chronic limb ischemia typically have disease involving multiple levels (i.e., aorto-iliac, femoro-popliteal, and infra-popliteal), but fewer than 10% have hemodynamically significant disease in all three levels. Approximately 33% of patients with infra-inguinal disease (popliteal, and infra-popliteal) present with predominantly isolated infrapopliteal disease, and the other 67% present with both femoropopliteal and infra-popliteal disease (10).

Isolated and infra-popliteal disease is mainly seen in the elderly (age> 80 years) patients with diabetes, or dialysis-dependent patients. These patients are at higher risk for amputation. Multilevel arterial occlusion requires either single-level revascularization to provide indirect flow to the ischemic foot or multilevel revascularization to provide direct flow to the ischemic foot, direct flow revascularization is straightforward (11).

The angiosome concept was derived from plastic surgery for the purpose of healing of skin flaps. An angiosome is an anatomic unit of tissue (consisting of skin, subcutaneous tissue, fascia, muscle, and bone) fed by a source artery and drained by specific veins. The entire body can be divided into 40 angiosomes, and the foot itself consists of six. The posterior tibial artery feeds three angiosomes, the anterior tibial feeds one, and the peroneal artery feeds two. The posterior tibial artery gives rise to a calcaneal branch, which supplies the medial ankle and lateral plantar heel, a medial branch that feeds the medial plantar instep, and a lateral branch that supplies the lateral forefoot, plantar midfoot, and entire plantar forefoot. The anterior tibial artery continues on to the dorsum of the foot as the dorsalis pedis. The peroneal artery supplies the lateral ankle and plantar heel via the calcaneal branch and the anterior upper ankle via an anterior branch (11).

From that point of view, it can be presumed that revascularization of the source artery to the
angiosome might result in better wound healing and limb salvage rates and respective vessels leading to these areas are treated in a distinctive way\textsuperscript{(13)}.

For peripheral arterial occlusive disease such reversible ischemia areas might be open wounds at the foot level. A proof for this concept might be the fact that ischemic heel ulcerations perfused by the dorsalis pedis are able to heal in approximately 86.5\% of cases. Therefore, direct revascularization of arteries supplying the target angiosome (wound area) might be more successful for complete wound healing than indirect revascularization\textsuperscript{(14)}.

2. Patients and Methods

This is a prospective study on twenty patients in Beni-Suef University Hospital. Ten patients were treated by angiosome direct therapy in the revascularization process (Group A) and ten patients angiosome concept was not applied (Group B), at the vascular surgery unit on the department of General Surgery, Beni-Suef University Hospital, after approval from the local ethical committee (Starting from January 2018).

**Inclusion criteria**

1. Age more than 35 years.
2. Any gender.
3. Ischemic patient with tissue loss or ulcer or gangrene.
4. The angiosome concept of revascularization will be applied when target vessel is suitable for angioplasty that serving angiosome concept for wound healing (group A).
5. The angiosome concept of revascularization will not be applied when target vessel is not suitable for angioplasty that serving angiosome concept for wound healing (group B).
6. All patients will apply consent to be in the study and for follow up to six months with orientation of all possible complications.

**Exclusion criteria**

1. Patient who has history of hypersensitivity to dye.
2. Patient with neuropathic ulcer.
3. Patient with impaired renal function (elevated serum creatinine level more than normal value).
4. Ischemic patient without tissue loss or gangrene.
5. Acute on top of chronic ischemia
6. Neglected embolism or traumatic occlusions
7. Connective tissue disorders or immunological disease and Entrapment syndromes.

**Pre-intervention preparation**

These included initial prophylactic antibiotics, the wounds were subsequently reassessed for possibility of limb salvage and assigned according to the territory of the involved angiosome. Cardiac, pulmonary, renal, and glycemic status were optimized preoperatively with the assistance of the concerned specialist physicians.
All patients underwent preoperative
1- duplex
2- Computerized axial tomography angiography to determine the extent of the disease and to identify the crural vessel crossing the ankle to perfuse the foot.
3- Peri-procedural medications used were clopidogrel loading dose (300 mg) and oral acetylcysteine 600 mg once with good hydration at a rate of 0.5 ml/kg/hr normal saline for 6 hrs before and after the procedure.

**Intra-procedural** 5000 - 10000 units of unfractionated heparin were administered. Revascularization followed the standard techniques of below the knee angioplasty, using an antegrade ipsilateral femoral access or contralateral through cross over sheath. Crossing and dilatation were performed using mainly low-profile equipment (0.018", 0.035" or 0.014"). The choice of the type of endovascular intervention was left to the discretion of the operating surgeon and was also based on the patient’s individual clinical status.

**After the procedure:** All patients were prescribed low molecular weight heparin (LMWH) anticoagulation for 48 hrs. Then aspirin (150 mg/day), clopidogrel (75 mg/day) and statins (20 mg/day) were given for 90 days. The patients followed an appropriate risk factor modification, in addition to postoperative debridement and minor amputations (toe/s or trans metatarsal) for patients presenting with wet gangrene/ necrotic tissue or sloughs in the wound bed.

All dressings were performed Depending on the wound status. Dressings and wound evaluation were performed daily, initially after the arterial intervention and later at alternate day/every third day, once adequate granulation tissue was noted to cover the wound and wound epithelialization had started. Intermittent debridement was performed as dictated by the wound status either as an outpatient procedure or in the operating room. Once complete epithelialization was achieved, the patients were evaluated for podiatric assessment. Foot counseling and appropriate offloading footwear were advised to all patients. This treatment protocol was similar in all groups.

The wounds were photographed preoperatively and immediately post-debridement and were followed up with photographs at 1, 3, 6 months.

Limb salvage was considered if the ulcer/gangrenous segment had healed completely or if at the end of 6 months the ulcer persisted but with a significant reduction in size of more than 50 %. Major amputation was defined as amputation performed either above or below knee. All patients were followed up to the endpoints of limb salvage at 6 months, major amputation, or death.
Contractions. A positive test was considered when there were four or more contractions per hour before the 30th week of gestation and from 30 weeks onward, 6 or more contractions per hour.

3. In case of positive test or threatened preterm labor in progesterone group we doubled the prophylactic dose of vaginal progesterone, but in (CCBs) group the prophylactic dose was repeated every 15-20 minutes up to a maximal total dose of 40 mg during the first hour of treatment, then 20 mg orally every 6-8 hours for 2-3 days.

4. The net result was stopping of contractions and other symptoms of threatened preterm labor, so returning to prophylactic doses again.

Statistical methodology

✓ The collected data were tabulated, coded and analyzed using SPSS for Windows, version 23.
✓ Continuous variables were presented as mean values ± standard deviation (SD).
✓ Categorical data were presented as frequency and percentage.
✓ Cross tabulation and Chi Square test: For comparison between categorical variables and percentage values.
✓ Non parametric statistical tests if significant were used in comparing the means (Mann–Whitney U test).
✓ Graphs were used to illustrate simple information.
✓ P-value <0.05 was considered statistically significant.

3. Results

This study included twenty patients suffering from chronic Limb Ischemia (CLI) underwent revascularization through two different methods. The studied patients were divided into two groups according to the surgical procedure:

Group (A): ten patients treated by angiosome direct therapy in the revascularization process

Group (B): ten patient were angiosome concept not be applied.

The mean age was 57.35 years with a SD of 12.7 years with no statistically significant difference between both studied groups; (p-value= 0.328). The studied cases were 13 males and 7 females with a male: female ratio near to 2:1 and with no statistically significant difference between both studied groups regarding sex; (p-value= 0.350).
Age and Sex distribution of the studied Cases; (N=20):

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A N= 10</th>
<th>Group B N= 10</th>
<th>TOTAL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age; (years)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean ±SD</td>
<td>54.50 ±14.3</td>
<td>60.20 ±10.8</td>
<td>57.35 ±12.7</td>
<td>0.328</td>
</tr>
<tr>
<td>Minimum</td>
<td>38</td>
<td>40</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>80</td>
<td>75</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><strong>Sex; N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2 (20.0)</td>
<td>5 (50.0)</td>
<td>7 (35.0)</td>
<td>0.350</td>
</tr>
<tr>
<td>Male</td>
<td>8 (80.0)</td>
<td>5 (50.0)</td>
<td>13 (65.0)</td>
<td></td>
</tr>
</tbody>
</table>

Screening for co-morbidities as a risk factor; more than two thirds of the studied cases had comorbidities in the form of DM and HTN (65%) and only 7 cases (35%) had no comorbidities. The distribution of comorbidities between the both studied groups was nearly similar with no statistically significant difference (p-value= 0.5).

Associated Comorbidities among the studied population; (N=20):

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A N= 10</th>
<th>Group B N= 10</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No comorbidities</strong></td>
<td>4 (40.0)</td>
<td>3 (30.0)</td>
<td>7 (35.0)</td>
<td>0.500</td>
</tr>
<tr>
<td><strong>DM &amp; HTN</strong></td>
<td>6 (60.0)</td>
<td>7 (70.0)</td>
<td>13 (65.0)</td>
<td></td>
</tr>
</tbody>
</table>

Associated Comorbidities among the studied population.
Regarding the ischemic lesion among the studied patients; sixteen cases (80%) had ischemic lesion at infra-popliteal while four cases (20%) had SFA and infra-popliteal. The ischemic lesion distribution was the same between the both studied groups; (p-value =0.999).

Table (): Ischemic lesion Distribution among the studied patients in each group ; (N= 20):

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A (N= 10)</th>
<th>Group B (N= 10)</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infra-popliteal</td>
<td>8 (80.0)</td>
<td>8 (80.0)</td>
<td>16 (80.0)</td>
<td>0.999</td>
</tr>
<tr>
<td>SFA, infra-poplite</td>
<td>2 (20.0)</td>
<td>2 (20.0)</td>
<td>4 (20.0)</td>
<td></td>
</tr>
</tbody>
</table>

Ischemic lesion Distribution in each group

The site of lesion among the studied cases were distributed as; eleven cases (55%) at the Toes and dorsum of foot, four cases (20%) at the Planter aspect of foot and medial side of heel and five cases (25%) at the Lateral side of heel. Site of lesion had nearly similar distribution in the both studied groups with no statistically significant difference (p-value =0.236).

Site of lesion among the studied patients; (N= 20):

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A (N= 10)</th>
<th>Group B (N= 10)</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toes and dorsum of foot</td>
<td>6 (60.0)</td>
<td>5 (50.0)</td>
<td>11 (55.0)</td>
<td>0.236</td>
</tr>
<tr>
<td>Planter aspect of foot and medial side of heel</td>
<td>3 (30.0)</td>
<td>1 (10.0)</td>
<td>4 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Lateral side of heel</td>
<td>1 (10.0)</td>
<td>4 (40.0)</td>
<td>5 (25.0)</td>
<td></td>
</tr>
</tbody>
</table>
Intra-operative dilated arteries were ATA at seven (35%) cases, PTA at seven (35%), peroneal at 2 cases (10%) and 4 cases had ATA and PTA dilatation (20%) with no statistically significant difference between both studied groups regarding the dilated arteries intra-operative; (p-value = 0.099).

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A N= 10</th>
<th>Group B N= 10</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATA</td>
<td>3 (30.0)</td>
<td>4 (40.0)</td>
<td>7 (35.0)</td>
<td>0.099</td>
</tr>
<tr>
<td>PTA</td>
<td>3 (30.0)</td>
<td>4 (40.0)</td>
<td>7 (35.0)</td>
<td></td>
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<tr>
<td>Peroneal</td>
<td>0 (0.00)</td>
<td>2 (20.0)</td>
<td>2 (10.0)</td>
<td></td>
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<tr>
<td>ATA &amp; PTA</td>
<td>4 (40.0)</td>
<td>0 (0.00)</td>
<td>4 (20.0)</td>
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</table>
Intra operative details (dilated arteries) among the studied patients in group A

Intra operative details (dilated arteries) among the studied patients in group B

Intra operative details (dilated arteries) among the studied patients in both group A and B.

Healing rate within 6 months post-operative was significantly better among the patients group with Angiosome Directed Therapy (group A) surgical procedure where 9 cases showed good healing rate in opposite to 2 cases in the Non Angiosome (group B). Therapy group with a statistical p-value (0.003).
Table (14): Healing rate and prognosis assessment within 6 months post-operative among the studied patients; (N= 20):

<table>
<thead>
<tr>
<th>Surgical Application</th>
<th>Group A N= 10</th>
<th>Group B N= 10</th>
<th>Total</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Bad prognosis</td>
<td>1 (10.0)</td>
<td>8 (80.0)</td>
<td>9 (45.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Good prognosis</td>
<td>9 (90.0)</td>
<td>2 (20.0)</td>
<td>11</td>
<td>(55.0)</td>
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</tbody>
</table>

Healing rate and prognosis assessment within 6 months post-operative among the studied patients

Post-operative complications as stump infection, major amputation below or above knee and further debridement, were significantly higher among Non Angiosome Therapy group were Eight cases (80%) showed complication in opposite to only one case among the Angiosome Directed Therapy group with a statistically significant p-value (0.020).

Table (16): Post-operative Complications among the studied patients; (N= 20):

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<tr>
<th>Surgical Application</th>
<th>Group A N= 10</th>
<th>Group B N= 10</th>
<th>Total</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>9 (90.0)</td>
<td>2 (20.0)</td>
<td>12 (60.0)</td>
<td>0.020</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (10.0)</td>
<td>8 (80.0)</td>
<td>8 (40.0)</td>
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</table>
Post-operative Complications among the studied patients.

Case 1: male patient 50 year with toe gangrene in 2nd and 3rd toe, we determine the site of the gangrene belong to which area according to the angiosomal concept to choice which infrapopliteal vessel is our target in angioplasty

Before angioplasty a

After the angiography of this patient showed occlusion of both ATA, PTA and peroneal arteries after application of direct Angiosome concept and balloon dilation of the target vessel balloon angioplasty of ATA is done. amputation of 2nd and 3rd toe is done and following up to assess the healing process with repeated dressing daily and following up the healing of the stump after one and three and six months
The healing process of the stump after application of directed Angiosome concept are excellent after 6 months in comparison to the other group treated by indirect concept which need further debridement and end by amputation

Case 2: Female patient of directed Angiosome concept balloon dilation of PTA, ulcer at planter aspect of the foot
**Case 3:** Male patient of direct Angiosome concept balloon angioplasty of ATA & PTA gangrene of little toe

![Before and After Images]

**Case 4** male patient with infection and gangrene of dorsum of foot (indirect Angiosome concept group B) balloon angioplasty of peroneal artery.

![Before and After Images]
Case 5: Male patient with infection and gangrene of toes and dorsum of the foot, balloon dilation of ATA, PTA (non Angiosome concept group B).

Before

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Case 6: Female patient with infection and gangrene at the planter aspect of the foot, balloon dilation of ATA (non Angiosome concept group B).

Before

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After
4. Discussion

A few dedicated studies available seem to favor this strategy as encouraging ulcer healing and limb preservation are reported in connection with both bypass and endovascular techniques based on these principles. The theory on the angiosome model of revascularization may help the clinician to better refine vessel selection, vascular access, and specific strategies in the revascularization of critically ischemic legs with tissue lesions (15). If the critical limb ischemia is not revascularized, up to 40% of extremities with ischemic non healing ulcers, gangrenous digits, or rest pain may require a major amputation within 6 months of onset (16).

The main purpose of this study is to evaluate the concept of angiosomal distribution of the vessels of the lower limb as well as the completeness of foot arch and to assess their role in choosing the rescue vessel of revascularization especially in patients with tissue loss. All risk factors were similar in both groups making no groups associated with more or less morbidities. All risk factors were similar in the two groups making no groups associated with more or less morbidities. Our study include 20 patient with infrapopliteal disease divided into two groups ten of them are treated with Angiosome concept (group A) and the others are treated with Non angiosome concept (group B).

In Iida O, et al., 2015 Limbs were classified into direct (n=200) and indirect (n=169) groups by whether feeding artery flow to the site of ulceration or gangrene was successfully achieved, based on the angiosome concept (17).

In Kabra, et al., 2013 Direct revascularization (DR) of the ischemic angiosome was performed in 61% (n=39), indirect revascularization (IR) in 39% (n=25) (18). In Söderström et al., 2013 direct flow to the angiosome feeding the ulcer area was achieved in 121 legs (48%) compared with indirect revascularization in 129 legs (19).

In our study the mean age was 57.35 years with a SD of 12.7 years with no statistically significant difference between both studied groups; (p-value= 0.328). The studied cases were 13 males and 7 females with a male: female ratio near to 2:1 and with no statistically significant difference between both studied groups regarding sex.

All risk factors were similar in all groups making no groups associated with more or less morbidities. The screening for co-morbidities as a risk factor; more than two thirds of the studied cases had comorbidities in the form of DM and HTN (65%) and only 7 cases (35%) had no comorbidities. The distribution of comorbidities between the both studied groups was nearly similar with no statistically significant difference.

In our study Intra-operative dilated arteries were ATA at seven (35%) cases, PTA at seven (35%), peroneal at 2 cases (10%) and 4 cases had ATA and PTA dilatation (20%) with no
statistically significant difference between both studied groups regarding the dilated arteries intra-operative; (p-value = 0.099). In group A, dilation of ATA in 3 cases, dilation of PTA in 3 cases in 3 cases, dilation of both vessels in 4 cases. In group B, dilation of ATA in 4 cases, dilation of PTA in 4 cases, dilation of peroneal artery in 2 cases.

In Kabra et al. (2013). The runoff involved the anterior tibial artery in 42.2%, posterior tibial artery in 34.4%, and the peroneal artery in 23.4 % (18). 65% of the patients have other comorbidites as DM or HTN and other patient has no comorbidites

In our study the site of lesion among the studied cases were distributed as; eleven cases (55%) at the Toes and dorsum of foot, four cases (20%) at the Planter aspect of foot and medial side of heel and five cases (25%) at the Lateral side of heel. Site of lesion had nearly similar distribution in the both studied groups with no statistically significant difference. In group A the site of lesion among the studied cases were distributed as; six cases (60%) at the Toes and dorsum of foot, three cases (30%) at the Planter aspect of foot and medial side of heel and one case (10%) at the Lateral side of heel. In group B the site of lesion among the studied cases were distributed as; five cases (50%) at the Toes and dorsum of foot, one cases (10%) at the Planter aspect of foot and medial side of heel and four cases (40%) at the Lateral side of heel.

In another study, in Kabra et al., 2013, 22.6% of patients had single toe ischemic ulceration, 16 % had ischemic multiple toes up to forefoot ischemic ulceration, 24% had foot sole ischemic ulceration, 17.3% had foot dorsum ischemic ulceration and 21.3% had foot heal ischemic ulceration (18). 81.2% of patients had forefoot ischemia, 17.2% had ischemic heel, whereas 1.6% had mid foot non healing ischemic ulceration.

We choose which vessel to be dilated according to the site of the ulcer or the gangrene direct and trying application of the Angiosome therapy and following up the healing process after one ,three and six months. Intra-operative dilated arteries were ATA at seven (35%) cases, PTA at seven (35%), peronial at 2 cases (10%) and 4 cases had ATA and PTA dilatation (20%) with no statistically significant difference between both studied groups regarding the dilated arteries intra-operative; (p-value = 0.099).

Healing rate within 6 months post-operatively was significantly better among the patients group with Angiosome Directed Therapy (group A surgical procedure where 9 cases showed good healing rate in opposite to 2 cases were Angiosome(group B) concept not applied . Therapy group with a statistical p-value (0.003). In group A the healing rate of the
ulcers is 90% which is treated by direct Angiosome concept (direct revascularization) which was good prognosis while in group B which is treated by indirect Angiosome concept (indirect revascularization) the healing rate was 20% which is bad prognosis.

Other studies comparing wound healing were mostly supporting the direct angiosomal revascularization. Kabra, et al. showed ulcer healing after 6 months for direct revascularization of 96.4% versus 83.3% following indirect revascularization (18). Söderström, et al. reported ulcer healing rate of 72% at 12 months for the direct group compared with 45% for the indirect group (P <0.001) (19).

Post operative complication were significantly higher among Non Angiosome Therapy (group B) were Eight cases (80%) showed complication in opposite to only one case among the Angiosome Directed Therapy (group A) with a statistically significant p-value (0.020).

In Kabra et al., 2013 the limb salvage in the DR group (84%) and IR group (75%) was not statistically significant (p=0.06). The mortality was 10.2% for DR and 20% for IR at 6 months (18). In a review by McCallum and Lane some studies showed better healing with direct revascularization while others did not. (20).

Our study had the advantage of being prospective not retrospective as most previous studies, important information that had a significant impact on the outcome.

5. Conclusion and Recommendations

- The study denotes that the site of the lesion is the key factor for success of the angiosomal concept in management of critical lower limb ischemia.
- Revascularization of the source artery to the angiosome result in better wound healing and limb salvage rates in comparison to failure of its revascularization as in our study showed that 90% better healing in directed Angiosome concept and 20% in the which Angiosome concept not applied.
- Direct revascularization of arteries supplying the target angiosome (wound area) resulting in less complication and no further debridement is needed as in our study only 10% need post operative debridement in comparison to 80% in the other group where Angiosome therapy couldn’t be applied (20).

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