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Avascular Necrosis

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Abstract: Avascular Necrosis (AVN), also known as osteonecrosis, is a degenerative bone disorder characterized by the death of bone tissue due to insufficient blood supply, commonly affecting weightbearing joints like the hip. This study provides a comprehensive overview of AVN, including its types, causes, symptoms, diagnostic methods, risk factors, and treatment approaches. It focuses particularly on a comparative analysis between allopathic and Ayurvedic treatment methods. The experimental work involved six case studies, with five patients undergoing Ayurvedic treatments and one receiving surgical intervention through allopathy. Results indicated significant improvements in pain management, joint mobility, and overall quality of life among Ayurvedically treated patients, with no reported side effects. Ayurvedic therapies incorporated herbal formulations, Panchakarma procedures, and lifestyle modifications. The study concludes that Ayurvedic treatment offers a holistic, cost-effective, and non-invasive alternative in managing AVN, especially beneficial in early stages, potentially delaying or eliminating the need for surgery.

Keywords: Avascular Necrosis

I. INTRODUCTION

Avascular necrosis (AVN), also known as osteonecrosis, is a debilitating condition characterized by the death of bone tissue due to a lack of blood supply. This phenomenon predominantly affects weight-bearing joints, such as the hip and knee, and can lead to significant pain, joint dysfunction, and, if left untreated, irreversible damage. [1]

TYPES:

Avascular necrosis (AVN) can be categorized into two main types: traumatic and non- traumatic:

- Traumatic: Occurs after an injury, such as a bone fracture or dislocation.
- Non-traumatic: Occurs when there is no history of injury, and can be caused by a medical condition or illness that prevents blood from flowing to the bone tissue.[2]

SIGN AND SYMPTOMS:

Some people have no symptoms in the early stages of avascular necrosis. But as the condition worsens the following symptoms may be observed :

- Mild or severe pain
- Pain in the areas:
- hip
- buttocks
- groin
- knee
- Thigh[3]
- Periodic discomfort that intensifies and subsides in response to applying and removing pressure to your bone.
- Increasing pain and stiff joints
- Limited range of motion.
- Limping if it is AVN of knee or hip.

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• Difficulty in climbing, walking and standing. [2]

CAUSES:

Avascular necrosis may be the result of the following:

Injury:

Injury, particularly fractures or dislocations, can cause avascular necrosis (AVN) by damaging nearby blood vessels and disrupting the blood supply to the bone, leading to bone tissue death.

Fracture :

Fractures can cause avascular necrosis (osteonecrosis) by damaging blood vessels and disrupting the blood supply to the bone, leading to tissue death and potentially joint collapse.

Damage to blood vessels:

Avascular necrosis, or bone death, occurs when the blood supply to a bone is interrupted or reduced, leading to the death of bone tissue due to lack of oxygen and nutrients.

Damage to blood vessels, whether from trauma, disease, or other causes, can disrupt this blood flow and cause avascular necrosis.

Long-term use of medicines, such as corticosteroids:

Corticosteroids reduce the inflammatory cell function in the body. The other effect directly linked with the avascular necrosis is lipogenesis or an increase in lipid/fat content of the blood. The increased lipid content causes blockage of the blood flow of the small vessels.

Excessive, long-term use of alcohol:

Excessive, long-term alcohol use can lead to avascular necrosis (osteonecrosis) by disrupting lipid metabolism, increasing triglyceride and cholesterol levels, and

causing fat deposition in bone marrow, ultimately leading to a lack of blood flow and bone cell death.

Specific chronic medical conditions:[4]

Hypertension can contribute to avascular necrosis (AVN) by damaging blood vessels, leading to reduced blood flow and oxygen supply to bone tissue, ultimately causing bone death.

Diabetes can increase the risk of avascular necrosis (AVN) or osteonecrosis, a condition where bone tissue dies due to a lack of blood supply, potentially through mechanisms like impaired bone formation, accelerated bone cell death, and reduced new blood vessel formation.

DIAGNOSIS:

Avascular necrosis (AVN) is diagnosed by a doctor who reviews your medical history, performs a physical exam, and orders imaging tests:

1. Medical history:

Your symptoms, their onset date, and whether they have gotten worse overtime will all be questions the doctor will ask you. In order to identify risk

factors, such as a history of hip injuries or corticosteroid use, they will also examine your medical history.

2. Physical exam:

The doctor will examine your hip and move it around to see if certain positions cause pain.[5]

3. Imaging tests:

These can include:

- X-rays: Can show bone changes in the later stages of AVN.
- Magnetic resonance imaging (MRI): A sensitive and specific imaging procedure that can detect AVN in the earliest stages.
- Computed tomography (CT) scan: Uses X-rays and a computer to create detailed images of the body.
- Radionuclide bone scan: Injects a small amount of radioactive material into the blood to show blood flow and cell activity in the bone.[5,6]

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4. Biopsy:

A procedure that removes tissue samples from the body for examination under a microscope.[5,7]

RISK FACTORS:

- Serious injury: Serious injuries, particularly those involving fractures or dislocations, can be a risk factor for avascular necrosis (AVN) because they can disrupt or damage the blood vessels supplying the bone, leading to reduced blood flow and potentially bone cell death.
- Excessive alcohol consumption: Excessive alcohol consumption is a significant risk factor for avascular necrosis (AVN) because it can lead to abnormal lipid metabolism, increasing the risk of fat deposition in bone marrow and disrupting blood flow, ultimately causing bone cell death.
- High dose steroids:
- systemic lupus erythematosus(SLE):

Some less common risk factors may include:

- HIV
- Blood disorders
- Radiation therapy
- Organ transplant
- Bisphosphonates
- Decompression disease

TREATMENT:

Treatment for avascular necrosis includes non-surgical and surgical interventions. The best treatment option for people may vary according to :

- the person's age
- stage of disease
- what bone is affected
- the extent of the damage to the bone

Nonsurgical treatment:

If avascular necrosis is in its early stages or only affects a small area, nonsurgical treatment may be helpful. On the other hand, when the disease affects the hip or knee, or in individuals with advanced versions of the disorder, it is ineffective.

It is best to start treatment early before the bone collapses. Some treatment options include:

- Physical therapy and rehabilitation
- Anti-inflammatory medications
- Using crutches or canes

Surgical treatment:

Most people eventually need surgery, which involves removing some dead cores of the bone. Options include:

- Core decompression
- Osteotomy
- Bone graft
- Total joint replacement[8]

PREVENTION:

The main methods of preventing avascular necrosis involve addressing the risk factors that are possible to change. This may include:

• consuming alcohol in moderation.

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- quitting smoking , if applicable.
- discussing alternatives to corticosteroids with a doctor, if a person takes these medications.

SURVEY RESULTS:

Decade	No. of estimated patients
1970s	500-1000(cases per year)
1980s	1000-2000(cases per year)
1990s	2000-5000(cases per year)
2000s	5000-10000(cases per year)
2010s	10000-15000(cases per year)
2020s	20000+(cases per year)

PLAN OF WORK :

1. Preparation Phase:

Conduct a literature review on AVN to establish the research context. Design and validate a standardized questionnaire for patient interviews. Obtain necessary ethical approvals and informed consent from participants.

2. Patient Recruitment:

Collaborate with orthopedic clinics and hospitals to identify AVN patients. Include patients at various stages of AVN to ensure comprehensive data collection.

3. Data Collection:

Conduct detailed patient interviews to gather medical history, lifestyle information, and treatment history.

Review medical records and imaging studies to confirm diagnosis and staging.

4. Data Analysis:

Categorize patients based on age, gender, stage of AVN, and treatment received. Use statistical tools to analyze trends, correlations, and outcomes.

5. Comparative Analysis:

Compare findings with existing national and international studies. Highlight similarities, differences, and potential gaps in current knowledge.

6. Report Writing:

Prepare sections for the research paper, including an abstract, introduction, methods, results, discussion, and conclusion.

Include tables, graphs, and charts to present data effectively.

7. Publication and Dissemination:

Submit the research paper to a reputable medical journal. Present findings at relevant medical conferences or seminars.

EXPERIMENTAL WORK :

Comparative case study between Allopathic and Ayurvedic treatment

Case study No.1	Case study No.2
• Age:47	• Age:43
• Gender:Male	• Gender:Male
Occupation:Government Job	Occupation:Teacher
• Diagnosed with AVN of femoral head with pyrogenic arthritis right hip.	• Diagnosed with Grade three bilateral AVN of the hip .
Treatment taken: Allopathic	• Treatment taken: Ayurvedic

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C	•	J
Surgery	IS	aone.

No surgery is done. ٠

CasestudyNo.3	CasestudyNo.4			
• Age:57	• Age:52			
• Gender:Male	• Gender:Male			
Occupation:Police(ASI)	Occupation:Teacher			
Diagnosed with Grade three	Diagnosed with Grade two			
bilateral AVN of the hip .	bilateral AVN of the hip .			
• Treatment taken: Ayurvedic	• Treatment taken: Ayurvedic			
• No surgery is done.	• No surgery is done.			

CasestudyNo.5	CasestudyNo.6		
Age:56	• Age:26		
Gender: Male	• Gender: Male		
Occupation: Business	Occupation: Business		
Diagnosed with grade two AVN of right hip and grade three AVN of left hip.	• Diagnosed with grade two AVN of right hip.		
Treatment taken: Ayurvedic	• Treatment taken: Ayurvedic		
No surgery is done.	• No surgery is done.		

Case study No. 1:

- Age : 48 •
- Age at the time of diagnosis : 47 •
- Gender : Male •
- Occupation : Government Job ٠

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- A 47 year male patient was diagnose with AVN of femoral head with pyrogenic arthritis of right hip.
- It is a traumatic osteonecrosis.
- The patient slipped and fell about a year ago which resulted into a fracture.
- The patient fell two times in a span of fifteen days.
- The patient experienced sharp pain.
- The movements were very painful.
- The pain was so severe that the usual duties and daily life of patient was affected.
- All the symptoms and pain worsened over time from mild to severe.
- The patient once suffered from Covid-19.
- The patient was probably given corticosteroids during treatment of Covid-19.
- The patient has no chronic medical conditions.
- The patient occasionally drinks alcohol.
- The patient has no blood clotting disorder.
- The patient did not take any anti-coagulants except for the time of surgery.
- The patient also tried homeopathic treatment for a while which helped a little but eventually worsened after sometime.
- The patient was prescribed painkillers ,multivitamins ,antacids and medicines used to treat osteoarthritis and rheumatoid arthritis for treatment.
- When these medicines did not help much the patient was suggested surgery.
- He had to use walking assistives few days prior and post the surgery.
- Surgery was done in two parts with time window of 1.5 months between both the surgeries.
- The patient showed signs of swelling and redness post surgery for few days.
- After 4-5 days of surgery the patient had to use a walker for about fifteen days.
- After 15 days the patient used a stick for about 8-10 days.
- In about 30 days post surgery the patient was able to walk without any support.
- The doctor has suggested daily exercise and walk for about 2-3 kms to the patient.
- The patient is now living normally and is able to do everything on his own.
- However, the patient can not climb stairs post surgery as it is very difficult and painful.
- The patient can not ride a bike post surgery as he is advised not to by doctor and also as ti is very painful.
- The patient was prescribed medications for up to two months post surgery.
- And currently the patient has no prescribed medications.
- The patient is asked for a follow up checkup after a year of surgery.
- The patient has a slight limp while walking post surgery.

Before treatment	After treatment		
Movements were painful.	Could move without pain.		
Had to use walking assistives.	No need of walking assistives.		
Could not walk freely.	Can walk freely but has as slight limp.		
Very difficult to ride a bike.	Prohibited to ride a bike.		

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Case study No. 2:

- Age : 43
- Age at the time of diagnosis : 41
- Gender : Male
- Occupation : Teacher
- Diagnosed with Grade three bilateral AVN of the hip.
- It is a Atraumatic osteonecrteosis.
- It all started with leg pain.
- Later on the patient had complaints about the pain in the legs especially the soles.
- The pain was so severe.
- The patient could not stand or walk.
- The patient even had to crawl sometimes.
- The patient was suggested surgery.
- The patient also visited some for allopathic treatment of the same but it did not help much significantly.
- After about an year the patient started ayurvedic treatment.
- Where he was given ताप्यादि लोह tablets, पंचदतक्त घृत गुगुल tablets, गोक्षुरादि गुग्गुल tablets, अश्वगंधा, पुनननवा, गोक्षुरा, शतावरी चुर्न for treatment.
- He was also given वातघ्न तेल for the local application for pain management.
- The patient also undergoes अदिकर्न and दवद्धकर्न treatment for three consecutive days after every fifteen days.



Fig.No.1Agnikarma



Fig.No.2Viddhakarma

- The patient experienced no improvements for the first six months into the treatment.
- After six months into the same, the patient started feeling better and the pain was managed effectively.
- The oil (वातघ्र तेल) for the local application was prescribed to the patient two times a day along with a hot water bag.
- According to the patient, this has helped him a lot.
- According to the patient , he has now recovered up to 80% .
- While the patient believes that he will be all better without having to opt for a surgery in about 4-6 months.







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Before treatmentAfter treatmentCould not walk, sometimes even had to
crawl.Can walk easily without
crawling.Severe pain while riding a bike.Pain Is minimized to a much greater extent.Movements were very painful.Pain is reduced upto 80%.

Ingredients of Tapyadi Loha: Table 1:

Dravya	Quantity	Dravya	Quantity
Haritaki	5gm	Tvak	10gm
Vibhitaki	5gm	Chavya	10gm
Amalaki	5gm	Shuddh Shilajeet	20gm
Trikatu	5gm	Swarn Makshik Bhasma	20gm
Chitrakmoola	5gm	Rupya Bhasma	20gm
Vidanga	5gm	Loha Bhasma	20gm
Pippalimoola	10gm	Mandura Bhasma	200gm
Devadaru	10gm	Sharkara	320gm
Daruharidra	10gm		

Ingredients of Panchtikta Ghrita guggulu: Table 2 :

Dravya	Quantity	Dravya	Quantity	Dravya	Quantity	Dravya	Quantity
Nimba	480gm	Vidanga	12gm	Mishreya	12gm	Agni	12gm
Patola	480gm	Suradaru	12gm	Chavya	12gm	Rohini	12gm
Vyaghri	480gm	Gajopakulya	12gm	Kushta	12gm	Arushkara	12gm
Guduchi	480gm	Vavakshara	12gm	Tejovati	12gm	Vacha	12gm
Vasa	480gm	Sarjikshara	12gm	Maricha	12gm	Kandamoola	12gm
Ghrita	768ml	Nagara	12gm	Vatsaka	12gm	Yukta	12gm
Patha	12gm	Nisha	12gm	Deepyaka	12gm	Manjishta	12gm
Ativisha	12gm	Vishani	12gm	Yavani	12gm	Shuddha Guggulu	12gm
						Sugguiu	

Ingredients of Gokshuradi guggulu: Table 3

Dravya	Quantity	Dravya	Quantity
Gokshura	10gm	TrikatuPippali	6gm
Guggulu	40gm	Mustak	5gm
Haritaki	3.33gm	Ela	2gm
Vibhitaki	3.33gm	Vidanga	2gm
Amalaki	3.33gm	Svarnapatri	5gm





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Question 1 2 3 4 5 6 7 8 9 10 ~ 1 Improvementin jointmovement 2 Abilitytoperform daily activities \checkmark 3 ~ Overallenergyand well being 4 Satisfactionwith ~ Ayurvedic treatment Tolerabilityof 5 ~ treatment(side effects) Perceived improvement 6 ~ compared to beforetreatment 7 Balance and stabilitywhile standing or ~ walking 8 Sleepqualityafter treatment ~ 9 Confidence in avoidingsurgery ~ afterAyurvedic treatment √ Weight bearing 10 abilityonaffected limb 11 Easeofsittingand standing √

Scoring System(Total out of 110)

,	
TotalScore	Interpretation
90-110	Excellent outcome
80-89	Good outcome
60-79	Moderate outcome
40-59	Minimal improvement
Lessthan40	Poor outcome, need further intervention

According to the above table we get the score 97 so we can interpret that the outcome of Ayurvedic treatment is **Excellent**.



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Case study No. 3:

- Age : 57
- Age at the time of diagnosis : 54
- Gender : Male
- Occupation : Police (ASI)
- Diagnosed with Grade three bilateral AVN of the hip .
- It is a atraumatic osteonecrteosis.
- It all started with leg pain.
- The pain was so severe.
- The patient was suggested surgery.
- The patient also visited some for allopathic treatment of the same but it did not help much significantly.
- After few months the patient started ayurvedic treatment.
- The patient is undergoing ayurvedic treatment for about 3 years now.
- Where he was given योगराज गुगुल tablets, पंचदतक्त घृत गुगुल tablets, लाक्षािी गुगुल tablets, अश्वगंधा, बला, प्रवाळ दपष्टी, गोक्षुरा, शतावरी चुर्न for treatment.
- He was also given वातघ्न तेल for the local application for pain management.



Fig.No.1Agnikarma



Fig.No.2Viddhakarma

- The patient also undergoes अदिकर्न and दवद्धकर्न treatment for three consecutive days after every fifteen days.
- According to the patient , he has now recovered up to 85%.

Dravya	Quantity	Dravya	Quantity	Dravya	Quantity	Dravya	Quantity
Nimba	480gm	Vidanga	12gm	Mishreya	12gm	Agni	12gm
Patola	480gm	Suradaru	12gm	Chavya	12gm	Rohini	12gm
Vyaghri	480gm	Gajopakuly a	12gm	Kushta	12gm	Arushkara	12gm
Guduchi	480gm	Vavakshara	12gm	Tejovati	12gm	Vacha	12gm

Ingredients of Panchtikta Ghrita guggulu: Table 1:

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Vasa	480gm	Sarjikshara	12gm	Maricha	12gm	Kandamoola	12gm
Ghrita	768ml	Nagara	12gm	Vatsaka	12gm	Yukta	12gm
Patha	12gm	Nisha	12gm	Deepyaka	12gm	Manjishta	12gm
Ativisha	12gm	Vishani	12gm	Yavani	12gm	Shuddha Guggulu	12gm

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Ingredients of Yograj guggulu: Table 2:

Dravya	Quantity	Dravya	Quantity	Dravya	Quantity	Dravya	Quantity
Chitrak	5.4mg	Devdaru	5.4mg	Dhania	5.4mg	Maricha	5.4mg
Pippali	5.4mg	Chavya	5.4mg	Haritaki	5.4mg	Yavakshar	5.4mg
Yavani	5.4mg	Ela	5.4mg	Bahera	5.4mg	Talis Patra	5.4mg
Kala Jeera	5.4mg	Sendha Namak	5.4mg	Amla	5.4mg	Tejpatta	5.4mg
Vidanga	5.4mg	Pratinidhi Dravya of Kuth	5.4mg	Nagarmotha	5.4mg	Guggulu	135mg
Ajmoda	5.4mg	Rasna	5.4mg	Sonth	5.4mg		
Safed Jeera	5.4mg	Gokshura	5.4mg	Dalchini	5.4mg		



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	Question	1	2	3	4	5	6	7	8	9	10
1	Improvementin jointmovement									~	
2	Abilitytoperform daily activities									~	
3	Overallenergyand well being									~	
4	Satisfaction with Ayurvedic treatment										~
5	Tolerability of treatment(side effects)										~
6	Perceived improvement compared to beforetreatment								~		
7	Balance and stabilitywhile standingor walking								~		
8	Sleepqualityafter treatment										✓
9	Confidence in avoidingsurgery										~

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	after Ayurvedic treatment					
10	Weight bearing abilityon affected limb				~	
11	Easeofsittingand standing				✓	

Scoring System (Total out of 110)

Total Score	Interpretation							
90-110	Excellent outcome							
80-89	Good outcome							
60-79	Moderate outcome							
40-59	Minimal improvement							
Lessthan40	Poor outcome, need further intervention							

According to the above table we get the score 101so we can interpret that the outcome of Ayurvedic treatment is **Excellent**.

Case study No. 4:

- Age : 52
- Gender : Male
- Occupation : Teacher
- Diagnosed with Grade two bilateral AVN of the hip

	Question	1	2	3	4	5	6	7	8	9	10
1	Improvement in joint movement								~		
2	Abilitytoper form daily activities								✓		
3	Overallenergyand well being								~		
4	Satisfactionwith Ayurvedic treatment										~
5	Tolerabilityof treatment(side effects)										~
6	Perceived improvement compared to beforetreatment								~		
7	Balance and stabilitywhile standing or walking								~		
8	Sleepqualityafter										

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	treatment					~
9	Confidence in avoidingsurgery					\checkmark
	afterAyurvedic					
	treatment					
10	Weight bearing					
	abilityonaffected limb				\checkmark	
11	Easeofsittingand					
	standing				~	

Scoring System(Total out of 110)

TotalScore	Interpretation
90-110	Excellent outcome
80-89	Good outcome
60-79	Moderate outcome
40-59	Minimal improvement
Lessthan40	Poor outcome, need
	further intervention

According to the above table we get the score 96 so we can interpret that the outcome of Ayurvedic treatment is **Excellent**

Case study No. 5 :

- Age : 56
- Age at the time of diagnosis : 53
- Gender : Male
- Occupation : Business
- Diagnosed with grade two AVN of right hip and grade three AVN of left hip.

	Question	1	2	3	4	5	6	7	8	9	10
1	Improvementin										
	jointmovement							\checkmark			
2	Abilitytoperform										
	daily activities									✓	
3	Overallenergyand										✓
	well being										
4	Satisfactionwith										
	Ayurvedic										✓
	treatment										
5	Tolerabilityof										
	treatment(side effects)										✓



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6	Perceived improvement compared to beforetreatment				~			
7	Balance and stabilitywhile standing or walking						~	
8	Sleepqualityafter treatment						~	
9	Confidence in avoidingsurgery afterAyurvedic treatment							~
10	Weight bearing abilityonaffected limb						\checkmark	
11	Easeofsittingand standing					~		

Scoring System(Total out of 110)

TotalScore	Interpretation
90-110	Excellent outcome
80-89	Good outcome
60-79	Moderate outcome
40-59	Minimal improvement
Lessthan40	Poor outcome,need further intervention

According to the above table we get the score **98** so we can interpret that the outcome of Ayurvedic treatment is **Excellent.**

Case study No. 6 :

- Age : 26
- Age at the time of diagnosis : 25
- Gender : Male
- Occupation : Business
- Diagnosed with grade two AVN of right hip .

	Question	1	2	3	4	5	6	7	8	9	10
1	Improvementin										✓
	jointmovement										
2	Abilitytoperform									~	
	daily activities										
3	Overallenergyand										
	well being									✓	

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4	Satisfactionwith						
	Ayurvedic						~
	treatment						•
_							
5	Tolerabilityof						
	treatment(side effects)						\checkmark
6	Perceived						
	improvement						\checkmark
	compared to						
	beforetreatment						
7	Balance and					✓	
'	stabilitywhile						
	standing or						
	walking						
8	Sleepqualityafter						
	treatment						\checkmark
9	Confidence in						
	avoidingsurgery						\checkmark
	afterAyurvedic						
	treatment						
10	Weight bearing				 	✓	
10	abilityonaffected limb					•	
11	•						
11	Easeofsittingand						
	standing						✓

Scoring System(Total out of 110)

Total Score	Interpretation
90-110	Excellent outcome
80-89	Good outcome
60-79	Moderate outcome
40-59	Minimal improvement
Lessthan40	Poor outcome,need further intervention

According to the above table we get the score 106 so we can interpret that the outcome of Ayurvedic treatment is Excellent.

Patient consent :

Written permission for publication of this case study has been obtained from the patients.

Patient's perspective :

The patient was satisfied with the given treatment.

II. DISCUSSION

Avascular Necrosis is considered as degenerative condition primarily involving Asthi and Majja dhatu often due to aggravated Vata dosha. The use of Tapyadi loha tablet. Guggulu tablet and medicated enemas supports tissue regeneration and balance vata. Herbal formulation such as panchtikta ghrita guggulu and Gokshuradi guggulu and Ashwangandha are known for their adaptogenic and anti inflammatory properties. Vataghna tel for the local application for pain management.

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III. CONCLUSION

The Ayurvedic approaches to AVN demonstrated promising clinical outcomes in term of pain relief, functional improvement and potential halting of disease progression, and the results were encouraging and improving during follow up. The therapy is cost effective as compared to Allopathic treatment. Management and treatment of AVN through Ayurvedic principles provides significant relief and improve quality of life.

IV. RESULT

- The Patient who received allopathic treatment had to undergo surgery.
- Out of 6 patients diagnosed with AVN, 5 received a standardized ayurvedic treatment including Agnikarma, Viddhakarma & internal Medication along with life style changes.
- Pain score reduced from an average of 9.5 to 3.2 over ten months.
- No adverse effect were Reported.
- Improvement in movement and daily activities.

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CONFLICT OF INTREST

The authors confirm that there are no competing interests with any institutions, organizations, or products that may influence the findings or conclusions of this manuscript.

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