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Hirudotherapy In The Modern World-An Updated Review

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ABSTRACT
Hirudotherapy is one of the best and most successful instances of the use of invertebrates for therapeutic purposes. While the practice dates back to the ancient ages, where it was employed as an alternative to phlebotomy; its recognition as a modern therapeutic device came after the discovery of several medicinal substances in its saliva which have been demonstrated to have anti-inflammatory, analgesic, antiseptic, anti-metastatic, anesthetic, anti-coagulant and several other effects. Although the procedure is known to have certain adverse effects, yet no serious outcomes have been reported in the majority of studies. Given the popularity of hirudotherapy, scientists are on the verge of developing a mechanical device, which could act as a leech. This is a comprehensive review on leech therapy, with special emphasis on recent developments in the field. The search was completed on all major scientific search engines (i.e. Pubmed, Scientific Commons, Google Scholar etc) and classical textbooks of Unani medicine.

KEY WORDS: Hirudotherapy, talīq, hirudin, leech therapy.

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INTRODUCTION

The practice of alleviating diseases using leeches, the natural blood suckers, dates back to the ancient ages. Hirudotherapy (also known as leech therapy) is a latin word which pertains to a non-invasive treatment involving the application of medicinal leeches on the surface of the body as therapy for certain diseases. The word 'leech' is supposed to be derived from an old English word for physician, 'laece'[1]. Leeches are known as 'Alaq in Arabic language, and zalawcha in Persian. Leech therapy was a very popular treatment in ancient India, and most of the ancient Unani physicians were strong proponents of talīq (leech therapy) due to the many benefits it offered; and it was used in a range of local and systemic diseases with remarkable outcomes.[2] The benefits of leech therapy were earlier thought to be derived from the amount of blood lost, hence relieving congestion and removing the noxious humors. Recent researches have demonstrated that more than the blood loss, it is the salivary secretions of the leech which contain an amazing cocktail of medicinal substances.1 This has led to its therapeutic use in numerous medical and surgical conditions like varicosities, various skin diseases like scabies, psoriasis, ringworm etc; phlebitis, to reduce post-operative swelling in plastic and reconstructive surgery and pain relief in various arthritic diseases.[3] In July 2004, the FDA approved leeches as a medical device in the area of plastic and reconstructive sur-gery.[4] Recently, scientists are on the verge of developing a mechanical leech that could do the job of the real leech so as to reduce some of the risks.[5] Such a device is presently under trial for its therapeutic effects.[6]

MEDICINAL LEECHES AND LEECH SALIVA:

The Unani physicians described that leeches should be put in clean water before examination. For medicinal purpose, they recommend to use the leech which has been obtained from fresh water and which is small in size; having a small anterior sucker and a broader posterior sucker. The leech should be greenish on the dorsal aspect and reddish colored on the ventral aspect.[2] Modern scientific knowledge had further added to this existing information. The mostly used medicinal leech (Hirudo medicinalis) belongs to phylum Annelida, class Clitellata and subclass Hirudinea. Among Indian leeches, Hirudinaria granulose has got medicinal properties. It is a fresh water leech and is found abundantly in the states of Tamil Nadu, Kerala, Madhya Pradesh, Uttar Pradesh and Punjab.[1,7,8] In addition, *Hirudo decora, Hirudinaria mannilensis, Hirudo nipponia, Hirudo verbena, Hirudo orientalis* and *Haementaria depressa* have also been considered as medical devices.[6]

The leech is a segmented annelid without an exoskeleton, with pronounced ability to extend and contract its body. Medicinal leeches grow upto 12 cms in length, with their resting length being about one-third of their maximum length. It crawls using its larger posterior sucker, and bites with the smaller anterior sucker, which has a mouth and three jaws arranged in tri-radiate fashion; each jaw having about 100 teeth each.[1,7,8] Leeches can survive in the temperature range of 0ºC to 30ºC; however, rapid temperature changes may stress these animals to death. They breathe water dissolved or atmospheric oxygen through their general body surface. Oxygen requirements are little and they do not suffocate even in nearly completely closed containers. However, they are readily killed by harmful substances, like chlorine in water, even in low doses. Leeches secrete a layer of mucous on their body surface under stressful conditions; thus it may be used as an indicator of stress.[1]

Leech saliva contains an amazing cocktail of about a hundred biologically active substances, ranging from analgesics, anesthetics, vasodilators, antiseptics, antithrombotic substances and even several hormones.[7] Hirudin, a thrombin inhibitor, was the first compound to be isolated in 1884.[9] Eglins and Gelin isolated from *Hirudinaria mannilensis* have a similar action. Hirudin has later been isolated in almost all species of leeches. However, its activity demonstrates significant seasonal variation and has been found to be practically absent in the month of July.[10] A 'spreading substance', Hyaluronidase, is also found in leech saliva, which modifies the permeability of connective tissue through the hydrolysis of endoglucoronidic linkages of hyaluronic acid, thus helps in the absorption of saliva. Another important compound is a serine protease inhibitor, Hirustatin. A protein similar to it has been isolated from *Haementeria ghiliani* and was named ghilanten. These are potent specific Factor Xa inhibitors. A similar acting compound, Piguamerin, has been isolated from the Korean medicinal leech, *Hirudo nipponia*. Bdellins are also proteinase inhibitors found in leech saliva. Apyrase and Decorsin are platelet inhibitors, the latter derived from the American medicinal leech, *Macrobdella decorsa*. Another important compound present in leech saliva is Destabilase, which has a combined enzymatic, non-enzymatic and antibacterial activity. Calin found in leech saliva is a collagen inhibitor and especially inhibits collagen induced platelet aggregation.[11] In addition, newer studies on leech saliva have revealed the presence of histamine, serotonin and also certain

steroid hormones including cortisol, progesterone, testosterone, estradiol, and dehydroepiandrosterone. Certain kininases have also been isolated from the leech saliva, which are possibly responsible for the analgesic action.[10] However, many studies have reported a significant variation in the antithrombotic activity of leech saliva depending upon environmental conditions (temperature, humidity etc) and starvation period.[12] whether similar effect is seen on other actions is a matter of speculation. The combined benefits obtained from these substances are resolvent effects, anti-oedematous, correction of certain micro-vascular disorders, increased immune system activity, antiseptic and bacteriostatic activity, elimination of hypoxia, pain relief, promotion of healing and many other effects.[1] There is also evidence that leech saliva may have a role in stimulating erythropoiesis.[13]

Procedure:

The procedure of leech therapy includes application of one or more leeches on the surface of skin. For this purpose, medicinal leeches grown under strict quarantine are used for treatment and preferably discarded after single use to prevent infection.3 When properly attached, the leeches continue sucking blood for about 30 minutes to 2 hours, until completely satiated, and suck about 5-50 ml of blood; then drop off spontaneously. The resulting wound continues to ooze for the next 24 hours or so.[1] Leeches should never be detached by force, nor by application of alcohol. If the leech has to be detached manually, application of 5% topical cocaine is helpful, as it paralyses the leech. Upon removal, the leeches should be disposed off as bio-hazardous waste after sacrifice in 70% alcohol.[8] If the leech crawls into any orifice like throat, and attaches there, the ancient Unani physicians recommended to feed lehsun (Allium sativa bulb) to the patient or to give him ferrous sulphate (3.5 gm) orally in a single dose. Then ask the patient to open his mouth over a vessel filled with water. The drugs were thought to make the leech thirsty and it drops into the water as a result.[14] Recently, a study reported that garlic methanolic extract (600µg/ml) could paralyze and kill leeches in around one hour time.[15] However, it is better to plug nearby orifices with sterile gauze before applying leeches.

CLINICAL USES OF LEECH THERAPY IN GRECO-ARABIAN MEDICINE

Leech therapy was also a popular means of treatment in the Meidieval ages, when the Greco-Arabian medicine, better known as Unani medicine, was at its zenith.[16] The scientists of the Medieval Ages described various therapeutic uses of leeches in numerous disorders ranging from skin diseases, nervous disorders, pains, fissures, certain infections etc.

For the treatment of sanguineous diphtheria, Azam Khan advised that if the patient is not weak or malnourished, then application of leeches on the neck and below both the ears is beneficial. If the patient had difficulty in swallowing, then he advised the application of leeches on the nape of the neck in young age patients.[17] Likewise, certain disorders of the nervous system were also thought to benefit from application of leeches. According to Razi, application of leeches on the post-auricular region is beneficial in migraine.[18] Tabri advised that in psychosis caused due to combustion of sanguineous humors, leech application on the scalp is beneficial.[14] Application of leeches on the hip joint has been described by Razi for relieving sciatic pain.[19] According to Hkm. Azam Khan, this helps in expelling the noxious matter from the joints.[20] Similarly, Razi advised the application of leeches on the affected area in a patient of backache.[19]

Leech therapy was also believed to benefit tissues bereft of nutrition. According to Ibn Sina, application of leeches on a gangrenous part may help in restoring the tissues.[21] This action may be attributed to its vasodilating effect; and also the hyaluronidase present in leech saliva which promotes tissue penetration.[1] The blood-sucking activity of leeches was also employed for relieving piles by Azam Khan. The best results, according to him, are obtained by applying leeches on the lowest part of the sacrum, or they may be applied around the swellings or even on the pile mass. It was also used in the treatment of anal fissure. Application of leeches on the affected area was also thought to be beneficial in orchitis. Leech therapy was even employed in certain pediatric diseases. According to Azam Khan, if an abscess is caused on the umbilicus, then application of leeches around the lesion may be done if the child is more than two months old. For drainage of stagnant secretions in filariasis, leech therapy may be done on the heels after the patient had undergone istafragh (evacuation of morbid humors) with medicines.[20]

RECENT DEVELOPMENTS IN HIRUDOTHERAPY

Cancers: Leech therapy has also been employed for relieving severe pain in advance stage cancers.22 Antimetastatic activity has been studied in the salivary extract of Haementaria ghilianii [23] and Haementaria officinalis.[24] Synthetic hirudin similar to the hirudin obtained from H. medicinalis has also been demonstrated to have efficacious antimetastatic activity in a wide range of malignant tumor cells, such as pulmonary carcinoma, osteo-carcinoma, breast carcinoma, leukemia, etc.[25] Of late, there have been attempts at identifying the active principle responsible for the antimetastatic activity of leech saliva. Ghilanten from the salivary gland secretions of H. ghilianii has been found to suppress metastasis of melanoma, lung cancer, breast cancer, and prostate cancer in animal models.6 In a recent study, salivary extract from *H. manillensis* demonstrated significant cytotoxic activity against human small cell lung cancer and also a synergistic

action with the anticancer drugs Irinotecan and Carboplatin.[26]

Diabetes and its complications: In a recent study, the salivary extract of H. mannilensis was found to have anti-hyperglycemic activity on subcutaneous injection in animal models of diabetes. A synergistic effect was also observed with insulin use.[27] Recently, it was reported that leech saliva from the tropical leech H. manillensis possessed an anti-hyperglycemic activity against alloxan-induced DM.[6] Gangrene of the foot, usually seen on the planter surface is a much dreaded complication of Diabetes, which occurs in about 15% of the patients and frequently leads to amputation. The underlying cause is a combination of neuropathy, ischemia and infection, of which neuropathy plays the maximum role.[28] Leech therapy has been found to be effective in many studies for healing diabetes-related foot ulcers and improving circulation to the tissues.[29] The underlying mechanism is thought to be a combination of removal of metabolic substrates, acceleration of tissue remodeling, changing the states of temperature, tissue oxygenation, pH, fluid dynamics and microorganisms in the ulcers.[30]

Antibiotic activity: The search for an appropriate agent for management of infections is a continuous challenge for health care personnel, largely due to the emergence of resistant species, and also due to the adverse effects of various antibiotics on the human system. Previously, anti-bacterial property was demonstrated in leech saliva extract. Four anti-bacterial peptides were later found in the central nervous system of leeches. Similar substances have also been demonstrated in leech saliva in recent studies.[31] Furthermore, a protease-inhibitor, Gelin has been identified in leech saliva having specific anti-bacterial activity specifically against A. hydrophilia. The action of gelin is attributed to inhibition of human and porcine leucocyte elastase and chymotrypsin.[32] Also, destabilase present in leech saliva has a lysozyme-like activity and can destroy bacterial cell wall.[6]

Micro and reconstructive surgery: Venous congestion leading to occlusion, thrombus formation, stasis and tissue necrosis is the most dreaded complication following microsurgery for replantation of amputated parts or plastic surgery; which cannot be drained surgically. The 'annelids of medicine' come to the rescue at such an occurrence, since they not only drain the stagnant blood, but also create a wound that continues to bleed for a few hours. Also, the vasodilatation and increased tissue perfusion lead to enhanced healing, while the tri-radiate wound created by the bite heals without significant scarring, promoting all-round healing. However, no precise guidelines exist for the frequency and duration of the therapy, and the applications are mainly based on case reports. The average duration of treatment is about 7 days and ranges from 2-14 days in different studies.[6]

Arthritis: Pain and limitation of movement is the most common manifestation in all types of arthritis, whether infectious or non-infectious. Leech therapy has demonstrated better efficacy in the treatment of osteo-arthritis when compared with diclofenac. Some studies also proved that leech therapy can reduce synovial inflammation, leading to decreased pain and improvement in disability. Similar effects have been observed in iliosacral joint pain and cervicobrachialgia syndrome.[6]

Other less common applications of hirudotherapy: Leech Therapy has also been used for the treatment of macroglossia caused by mandibular fracture and secondary bleeding. Another researcher described the successful use of Hirudotherapy in forearm compartment syndrome, although the observations are yet to be further evaluated. Another possible application of hirudotherapy may be evaluated in non-microsurgical penile replantation. Such procedure is usually complicated by skin loss, stricture of urethra, and sensory loss and leech therapy may prevent these complications by maintaining post-operative venous outflow.[8] In recent studies, extracts obtained from leeches have also been demonstrated to have anti-narcotic, anti-stress and anti-psoriatic activity, which may further pave the way for the use of leeches in several other disorders.[31] Some researchers have also outlined the use of leeches in oral diseases like inflammation and abscesses of the gums. A study also reported the efficacy of leech therapy in acute deafness, and also acute and chronic tinnitus, though the mechanism of action is not known presently. Owing to the anti-coagulant and antithrombotic actions of leech saliva, it has also been used in the treatment of certain cardiovascular disorders like thrombophlebitis, hypertension, varicose veins, hemorrhoids, gonarthritis, and secondary ischemia related dermatosis.[6]

POSSIBLE COMPLICATIONS AND PRECAUTIONS:

Several potential complications have been reported following leech therapy, most of which are preventable if the procedure is done as per guidelines. Infection with several bacterial species, especially Aeromonas hydrophilia and Aeromonas veronii is the most commonly reported complication in many studies, with the prevalence ranging from 2.4% to 20% cases in the literature,[33] while a severe case of Aeromonas meningitis has been reported in a case where leech therapy was used to salvage a skin flap after central nervous system surgery.[34] These microorganisms are present in the intestinal flora of the leech and can cause wound infection, and in the most severe cases even sepsis.[35] Leech application can also cause infection with Mycobacterium marinum, a parasitic bacteria usually hosted by salt water fish, which may result in erysipelas

and submucosal abscesses.[36] Vibrio fluvialis infection has also been reported after hirudotherapy.[37] For preventing infections, it has been recommended to restrict the use of leeches on tissues with arterial perfusion, to minimize contamination of necrotic tissue; and use of prophylactic antibiotics.38 Environmental surveillance cultures and antibiotic susceptibility testing on water collected from leech tanks is a novel method for deciding on the appropriate antibiotic to be used.[39] Researchers have also attempted to use antibiotic-fed leeches with positive results. Such treatment did not affect the lifespan or feeding behavior of the leeches, and also eliminated the risk of infection for extended periods.[40] The only possible drawback is that it may induce allergic reactions in sensitive patients.31 However, since leeches are a potential vector of blood-borne infections, including HIV and viral hepatitis, they should be procured from appropriate sources.[8]

As the bite lesion heals, ecchymosis and scarring are also common occurrences. Another commonly reported complication is the occurrence of excessive bleeding following leech therapy, which may be alarming at times. The saliva of the leech contains hirudin, which inhibits thrombin in the clotting process, and histamine-like substances which may cause continuous bleeding by preventing closure of capillaries. The bleeding may be controlled by applying pressure bandages after removal of the leeches, and applying sterile gauze dipped in thrombin solution if the bleeding persists.[36] The bleeding caused is believed to be primarily due to inhibition of platelet aggregation by leech saliva, and not due to coagulation changes.[13] Further, it is also established that to produce an anticoagulant effect in an average patient, about 256000 thrombin inhibitory units per hour are required, the equivalent of total extractable hirudin from 1280 H. Medicinalis.[41]

A rare complication of leech therapy is thrombotic microangiopathy leading to acute renal failure.[42] Other possible complications are anaphylaxis, local allergic reactions and mucosal synechiae.[43] Both irritant and allergic contact dermatitis has been rarely reported. An uncommon complication is the development of sensitization to leech saliva after several sessions of leech therapy. Contact dermatitis to an extract of Hirudo medicinalis has been reported in a patient by a patch test, although the patient reported negative to the same test by recombinant hirudin.[34] Anaemia of the hypochromic type is a rare complication which has been reported in a few cases and is mostly caused by unrestrained application of leeches for long periods of time,[44] or due to leech infestation.[45] There is also an instance wherein uncontrolled leech therapy with upto 130 leeches at a time was carried out by the patient himself, which resulted in coagulation disorders, intractable hemorrhage and anemia requiring blood transfusion.[46]

CONCLUSION

Leech therapy has come a long way from the ancient concept of blood-letting to its development as a full-fledged means of treatment in numerous disorders. The discovery of several bio-active substances having curative properties have turned the modest annelid into a valued medical and surgical device. Numerous controlled and uncontrolled observations on the therapy over centuries have definitely established that it is also one of the safest treatments available, in addition to being effective and relatively cheap. A big challenge taken up in the recent years is the development of artificial leeches. It remains to be observed whether such a device can entirely replace the natural leech, especially because all components of the saliva have not been identified yet, nor are their mutual interactions fully studied.

REFERENCES

1.Abdullah S, Dar LM, Rashid A, Tewari A. Hirudotherapy /Leech therapy: Applications and Indications in Surgery. Arch Clin Exp Surg 2012;1(3):172-80.

2.Jurjani AH. Zakhira Khwar-zam Shahi (H. H. Khan, trans). India, Lucknow: Munshi Nawal Kishore. 1903. vol. 1 (3). p. 225-6.

3.Ahmad T, Anwar M. Clinical importance of Leech Therapy. Indian J Traditional Knowledge 2009 Jun;8(3):443-5.

4.Whitaker IS, Izadi D, Oliver DW, Monteath G, But¬ler PE. Hirudo medicinalis and the plastic surgeon. Br J Plast Surg 2004;57:348-353.

5.Adam R, Zakrzewski P. Therapeutic Use of Leeches: From the "Annelids" of Medicine. University of Toronto Medical Journal 2001:79(1):65-67.

6.Abualkader AM, Ghawi AM, Alaama M, Awang M, Merzouk A. Leech Therapeutic Applications. Indian J Pharma Sci 2013 Mar-Apr; 75(2): 127–137.

7.Lone AH, Ahmad T, Anwar M, Habib S, Sofi G, Imam H. Leech Therapy-A Holisitic Approach of Treatment in Unani (Greeko-Arab) Medicine. Ancient Sci Life 2011:31(1);31-5.

8.Porshinsky BS, Saha S, Grossman MD, Beery PR, Stawicki SPA. Clinical uses of the medicinal leech: A Practical Review. J Postgrad Med 2011:57;65-71.

9.Yeung CJL. Leeches: The Resurgence of an Anachronism. In: Whitelaw WA. The Proceedings of the 12th Annual History of Medicine Days. Calgary: Health Sciences Centre; 2003. p. 318.

10.Baskova IP, Yudina TG, Zavalova LL, Dudkina AS. Protein-lipid particles of medicinal leech salivary gland secretion; their size and morphology. Biochemistry (Mosc). 2010 May;75(5):585-9.

11.Zaidi SMA, Jameel SS, Zaman F, Jilani S, Sultana A, Khan SA. A systematic overview of the Medicinal Importance of Sanguivorous Leeches. Altern Med Rev 2011;16(1):59-65.

12.Ghawi AM, Abdualkader AM, Merzouk A, Alaama M. Season Variation and Starvation Period Influence on the Antithrombotic Activity of Leech Saliva Extract From the Medicinal Malaysian Leech, Hirudinaria Manillensis. J Bioequiv Availab 2012, S14.

13.Chmiel H, Anadere I, Moser K. Hemorheological changes after leeching. Clinical Hemorheology 1989;9:569-76.

14.Tabri R.Firdaus Al-Hikmat (Md. A. S. Sambhali, trans.) Pakistan, Lahore: Sheikh Mohd. Basheer and Sons. 1996. p.147, 221.

15.Bahmani M, Abbasi J, Mohsenzadegan A, Sadeghian S, Ahangaran MG. Allium sativum L.: the anti-immature leech (Limnatis nilotica) activity compared to Niclosomide. Comp Clin Path. 2013 Mar;22(2):165-168. Epub 2011 Dec 15.

16.Zaid H, Rayan A, Said O, Saad B. Cancer Treatment by Greco-Arab and Islamic Herbal Medicine. The Open Nutraceuticals Journal 2010; 3: 203-12.
17.Khan A. Al-Ikseer (Md. Siddiqui, trans.) New Delhi: Daftar-ul-Masih;1904. vol. 1. p. 385, 386.
18.Razi Z. Kitab Al-Hawi (CCRUM, trans) New Delhi: CCRUM. 1997. vol. 1. p. 218.
19.Razi Z. Razi Z. Kitab Al-Hawi (CCRUM, trans) New Delhi: CCRUM. 2004. vol. 11. p. 139, 179.
20.Khan A. Al-Ikseer (Md. Siddiqui, trans.) New Delhi: Daftar-ul-Masih;1904. vol-2. p. 1123, 1152, 1321, 1439.
21. IBN Sina. Al-Qanoon Fit-Tibb (G.H. Kinturi, trans.) Pakistan, Lahore: Book Printers; 1992. vol 4&5. p. 156, 178.
22.Kalender ME, Comez G, Sevinc A, Dirier A, Camci C. Leech therapy for symptomatic relief of cancer pain. Pain Med. 2010 Mar;11(3):443-5.
23.Gasic GJ, Viner ED, Budzynski AZ, Gasic GP. Inhibition of lung tumor colonization by leech salivary gland extracts from Haementeria ghilianii. Cancer Res. 1983 Apr;43(4):1633-5.
24. Gasic GJ, Iwakawa A, Gasic TB, Viner ED, Milas L. Leech salivary gland extract from Haementeria officinalis, a potent inhibitor of cyclophosphamide- and radiation-induced artificial metastasis enhancement. Cancer Res. 1984 Dec;44(12 Pt 1):5670-6.
25.Wallis RB, Fidler IJ, Esumi N. Hirudin for the inhibition of cancer metastasis. E.P. Application.1992
26.Merzouk A, Ghawi AM, Abdualkader A, Abdullahi AD, Alaama M. Anticancer Effects of Medical Malaysian Leech Saliva Extract (LSE). Pharm Anal Acta2012;S15:001
27.Mohammed AA, Mohammad GA, Mohamed A, Mohamed A, Ahmed M.In vivo anti-hyperglycemic activity of saliva extract from the tropical leech Hirudinaria manillensis. Chin J Nat Med. 2013 Sep;11(5):488-93.
28.Dwivedi AP. case study of leech application in diabetic foot ulcer.IJRAP 2012;3(5):748-51.
29.Zaidi SA. Unani treatment and leech therapy saved the diabetic foot of a patient from amputation. Int Wound J. 2014 May 8. [Epub ahead of print]
30.Na HJ. The Effects of Live Leech (Hirudo medicinalis) Therapy on Diabetic Foot: a Clinical Case Report. Korean Journal of Oriental Medicine 2003;24(4):136-8.
31.Tasiemski A, Salzet M. Use of Extract of leeches as an Anti-bacterial agents. US Patent 1986. US 2012/0251625 A1.
32.Atkinson A, Electricwala A, Sawyer AT, Sicard N, Voerman G. Protease Inhibitor. US Patent 1995. US 005937694 A.
33.Maetz B, Abbou R, Andreoletti JB, Bruant-Rodier C. Infections following the application of leeches: two case reports and review of the literature. J Med Case Rep. 2012 Oct 25;6(1):364.
34.Ouderkirk JP, Bekhor D, Turett GS, Murali R. Aeromonas meningitis complicating medicinal leech therapy. Clin Infect Dis. 2004 Feb 15;38(4):e36-7. Epub 2004 Jan 23.
35.Pietrzak A, Kanitakis J, Tomasiewicz K, Wawrzycki B, Kozłowska-Łój J, Dybiec E, Chodorowska G. Cutaneous complications of improper leech application. Ann Agric Environ Med. 2012;19(4):790-2.
36.Ikizceli I, Avsarogullari L, Sözüer E, Yürümez Y, Akdur O. Bleeding due to a medicinal leech bite. Emerg Med J. 2005 Jun;22(6):458-60.
37.Verghese MR, Farr RW, Wax MK, Chafin BJ, Owens RM. Vibrio fluvialis infection associated with leech therapy. Clin Inf Dis 1996;22:709-10.
38.Lineaweaver WC, Hill MK, Buncke GM, Follansbee S, Buncke HJ, Wong RK, Manders EK, Grotting JC, Anthony J, Mathes SJ. Aeromonas hydrophila infections following use of medicinal leeches in replantation and flap surgery. Ann Plast Surg. 1992 Sep;29(3):238-44.
39.Wilmer A, Slater K, Yip J, Carr N, Grant J. The role of leech water sampling in choice of prophylactic antibiotics in medical leech therapy. Microsurgery. 2013 May;33(4):301-4.
40.Mumcuoglu KY, Huberman L, Cohen R, Temper V, Adler A, Galun R, Block C. Elimination of symbiotic Aeromonas spp. from the intestinal tract of the medicinal leech, Hirudo medicinalis, using ciprofloxacin feeding. Clin Microbiol Infect 2010 Jun;16(6):563-7.
41.Blackshear JL, Ebener MK. Leeching, Hirudin and Coagulation Tests. Ann Intern Med 1994;121(2):151-2.
42.Etemadi J, Ardalan MR, Motavali R, Tubbs RS, Shoja MM. Thrombotic microangiopathy as a complication of medicinal leech therapy. South Med J. 2008 Aug;101(8):845-7.
43.Karadag AS, Calka O, Akdeniz N, Cecen I. A case of irritant contact dermatitis with leech. Cutan Ocul Toxicol. 2011 Sep;30(3):234-5.
44.Glick S, Ritz ND. Hypochromic anemia secondary to leeching. N Engl J Med. 1957 Feb 28;256(9):409-10.
45.Krüger C, Malleyeck I, Olsen OH. Aquatic leech infestation: a rare cause of severe anaemia in an adolescent Tanzanian girl. Eur J Pediatr. 2004 Jun;163(6):297-9.
46.Kose A, Zengin S, Kose B, Gunay N, Yildirim C, Kilinc H, Togun I. Leech bites: massive bleeding, coagulation profile disorders, and severe anemia. Am J Emerg Med. 2008 Nov;26(9):1067.e3-6.