

Haematological Alterations in Human Blood after Leech Application

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Abstract

Hirudotherapy (application of leeches for medical purpose) is a new modality of treatment employed in the advanced surgical and medical sciences. US FDA has approved the use of Leeches for medicinal purpose in 2004.

Indian leech (*Poecilobdella viridis*) was applied to the human volunteers and the haematological alterations after the procedure were studied.

Keywords: Leech, Haematology, Thrombin

Introduction

Blood Leeching is an ancient method of bloodletting which has been used extensively in treatment of various disorders since centuries in the natural medicine. *Hirudo medicinalis* is the well known sanguivorous annelid. A substance extracted from the saliva of the leech is a powerful anticoagulant. Major known enzymes in the saliva of the leech are anticoagulant, anti-inflammatory, anti- oedematous and analgesic in nature.

The purpose of the present study was to see if apart from the bloodletting benefit, the leech treatment effects the hemorrheological properties. The blood was investigated before and after application of leeches.

Materials and Methods

Recommended method for Leech Application: FIG.1

Healthy male and female volunteers were taken in the study. Their average weight

was 70 kg, range 54 to 82 kg. All the volunteers were healthy and had normal biochemical and haematological profiles. Application of leech was carried out under the supervision of expert doctor in the field.

Disinfected and starved adult *Poecilobdella viridis* (sterile) of similar size were applied to the forearm volar surface of drug free male and female volunteers (n=7)

Experimental Setup: Blood sample from volunteers before and immediately after leech application were collected.



Figure:-1:-Leech Application to Volunteer

First set of Experiment

In the first set of experiment, one leech to each volunteer was applied to study total serum protein, immunological proteins, haematological parameters and antigen-antibody reaction to know the amount of hirudin injected. The venous blood was removed immediately after the full meal of leech.

Second set of Experiment

In this set of experiment immediately four leeches were applied to each volunteer until sated and there after immediately all haematological parameters were studied.

Blood Smear

Smears from peripheral blood oozing from leech wounds. By using Leishman stain each smear was examined by light microscopy for direct evidence of platelet aggregation.

The Methodologies used for the Present Study are Given Below

Sr.	Parameters,	Method,	Reference
1.	Haemoglobin %	Sabli's acid hematin	Dacie & Lewis (1995)
2.	Red Blood Cell Count	Hemocytometer	Dacie & Lewis (1995)
3.	Haematocrit Value	Macro hematocrit	Dacie & Lewis (1995)
4.	Erythrocyte	Wintrobe method	Dacie & Lewis (1995)
		Sedimentation Rate	
5.	White Blood Cell Count.	Hemocytometer	Dacie & Lewis (1995)
6.	Platelet Count	Neubauer counting	Dacie & Lewis (1995)
7.	Bleeding Time	Duke's method	IVY et al., (1940)
8.	Clotting Time	Capillary method	IVY et al., (1940)
9.	Prothrombin Time	Quick method	Quick (1942)
10.	Protein	Eluret	Rosenthal et al.(1956)
11.	Immunological Protein	Paper	Bier (1959)
		electrophoresis	

Observations and Results

Alterations in Haemoglobin (gm %) and Mean Cell Hb Conc. (gm %) in Male and Female Volunteers Before and After Application of one and More than Two Leeches (Table 1.)

Five healthy volunteers (both male and female) were applied leeches for one hour to know the changes in haemoglobin content in volunteers due to application of leeches.

No significant, but slight alteration in Hb content was seen in all the volunteers who were applied with one leech for one hour. However, in volunteers having applied more than two leeches significant ($p < 0.001$) decrease in Hb conc. (gm %) was seen

Table 1:-Alterations in Haemoglobin (gm%) and Mean Cell Hb Conc. (gm%) in Male and Female Volunteers before and After application of more than Two Leeches.

No. of volunteers	Haemoglobin (gm%)		Mean cell Hb Conc. (gm%)	
	Before application	After application	Before application	After application
1	10.2±0.26	10.2±0.32	31.8±1.20	32.7±0.92
2	13.2±0.15	10.4*±0.11	32.5±2.16	33.8±2.11
3	14.0±0.18	13.4*±0.12	35.5±1.33	35.4±1.09
4	13.0±0.26	12.8*±0.33	35.3±1.66	35.4±1.50
5	14.0±0.25	13.0*±0.18	36.0±0.99	35.8±1.66

Alterations in Some Blood Parameters Like Red Blood Cell (Count/cmm), Haematocrit (%) Erythrocyte Sedimentation Rate (mm), Mean Cell Volume (fl) and Red Cell Distribution width (fl) in Male and Female Volunteers Before and after Application of one and More than Two leeches. (Table 2a&b)

Blood samples from normal healthy male and female volunteers immediately after 1 hr. leeching were collected to study the changes in Red Blood Cell Count, ESR, Haematocrit, Mean Cell Volume, and Red Cell Distribution Width.

Slight increase in red blood cell count was noted in some volunteers when one leech was applied for one hour. However, decrease in red blood cell count was noted after application of more than two leeches in all the volunteers under study.

No significant changes in hematocrit values were observed after application of one leech in all volunteers. But moderately significant decrease in hematocrit values was observed in volunteers after application of more than two leeches.

Moderate increase in ESR was noted in all the volunteers.

Mean cell volume reveals the size of the cell. There was slight decrease in mean cell volume after application of one or more than two leeches in all volunteers. However, this decrease was insignificant. Red cell distribution width reveals the size differences of RBC. No significant alterations were seen in red cell distribution width after application of one and more leeches.

Table 2 a: Alterations in some Blood Parameters in Male and Female Volunteers Before and After Application of One Leech.

No. of volunteer	Red Blood Cell Count/ cmm		Haematocrit (%)		Erythrocyte sedimentation rate mm/1 st hr		Mean cell volume (fl)		Red cell distribution width fl/%	
	Before	After	Before	After	Before	After	Before	After	Before	After
1	4.04 x 10 ⁶	4.55 x 10 ⁶	34.9	38.8	6	14**	86.4	85.3 ^{NS}	42.4	40.5
2	4.78 x 10 ⁶	4.88 x 10 ⁶ NS	44.4	43.4	5	7*	92.9	92.7 ^{NS}	46.8	40.0
3	4.44 x 10 ⁶	4.57 x 10 ⁶ NS	34.8	34.3	3	7**	78.4	78.5 ^{NS}	41.5	42.8
4	4.36 x 10 ⁶	4.49 x 10 ⁶ NS	35.3	36.9	4	5 ^{NS}	32.2	32.2 ^{NS}	13.2	13.2
5	5.29x 10 ⁶	5.73 x 10 ⁶ *	46.2	47.1	8	8 ^{NS}	32.7	32.2 ^{NS}	12.4	11.5

NS- Not significant; * $p < 0.1$; ** $p < 0.05$

Table 2b: Alterations in some Blood Parameters in Male and Female Volunteer Before and after Application of more than Two Leeches.

No. of volunteer	Red Blood Cell Count/ cmm		Haematocrit (%)		Erythrocyte sedimentation rate mm/1 st hr		Mean cell volume (fl)		Red cell distribution width fl/%	
	Before	After	Before	After	Before	After	Before	After	Before	After
1	4.78 x 10 ⁶	4.64 x 10 ⁶ *	32.0	31.1*	4	10**	67.0	67.2 ^{NS}	16.1	16.10
2	5.74 x 10 ⁶	4.36 x 10 ⁶ *	40.5	30.7*	3	11**	70.6	70.6 ^{NS}	15.6	14.60
3	4.17 x 10 ⁶	4.03x 10 ⁶ ^{NS}	39.4	34.8*	5	7*	94.5	93.9 ^{NS}	16.1	15.70
4	4.15 x 10 ⁶	4.10 x 10 ⁶ ^{NS}	36.8	36.3 ^{NS}	4	8*	88.9	88.7 ^{NS}	14.4	13.8
5	4.68 x 10 ⁶	4.60 x 10 ⁶ ^{NS}	41.7	40.4*	6	12**	82.5	82.4 ^{NS}	13.2	12.5

* $p < 0.05$; ** $p < 0.01$; NS- Not significant..

Alterations in Total Leucocyte Count (Cmm) and Differential Leucocyte Count in Male And Female Volunteers Before and After Application of One and More Than Two Leeches. (Table 3a And B)

Five healthy male and female volunteers were applied one or more than two leeches for one hour to know the changes in total leucocyte count (cmm) and differential leucocyte count.

Slight increase in leucocyte count was noted in all volunteers when one leech was applied to each volunteers for one hour. However, decrease in leucocyte count was recorded after application of more than two leeches in all volunteers.

Lymphocyte percent was found to be significantly increased after application of one leech in the volunteers. Where as in volunteers having applied more than two leeches lymphocyte percent was decreased and this decrease was highly significant

Moderate ($p < 0.05$) to significant ($p < 0.01$) decrease in granulocyte was experienced in volunteers who were treated with one leech for one hour. However, there was significant increase ($p < 0.05$) in the granulocyte number in all the volunteers who were applied more than two leeches.

Table 3a: Alterations in TLC and DLC in Male and Female volunteers Before and After Application of one Leech.

No. of volunteer	White blood cells count/cmm		Lymphocyte (%)		Monocyte		Granulocyte	
	Before	After	Before	After	Before	After	Before	After
1	7.5×10^3	$8.6 \times 10^3^*$	23.8	48.9**	4.2	4.0 ^{NS}	48.5	44.2*
2	10.2×10^3	$11.9 \times 10^3^*$	22.5	36.3**	6.4	6.2 ^{NS}	60.1	51.7*
3	9.9×10^3	$11.6 \times 10^3^*$	20.6	33.5**	7.9	7.8 ^{NS}	51.3	48.7*
4	10.2×10^3	$10.2 \times 10^3^{\text{NS}}$	37.2	52.0*	4.6	4.9 ^{NS}	57.2	43.1**
5	6.2×10^3	$8.5 \times 10^3^{**}$	23.9	35.5**	6.9	6.9 ^{NS}	64.2	53.5**
6	9.3×10^3	$11.9 \times 10^3^{**}$	23.2	47.6**	3.9	3.9 ^{NS}	62.9	43.5**

NS- Not significant; * $p < 0.1$; ** $p < 0.05$

Table 3b: Alterations in TLC and DLC in Male and Female Volunteers Before and After Application \ of More than Two Leeches.

No. of volun-teeer	White blood cell		Lymphocyte		Monocyte (%)		Granulocyte (%)	
	Before	After	Before	After	Before	After	Before	After
1	7.4×10^3	6.7×10^3	26.3	21.3*	5.9	5.7	60.80	67.40*
2	9.7×10^3	6.7×10^3	20.1	14.8*	7.2	7.3	70.80	80.20*
3	9.2×10^3	8.9×10^3	29.5	22.5*	6.4	7.0	55.30	60.90*
4	9.7×10^3	$9.2 \times 10^3^{\text{NS}}$	17.3	15.4*	4.4	4.2	71.40	79.90*
5	9.6×10^3	$8.8 \times 10^3^{**}$	22.7	19.7*	7.1	6.9	62.50	65.20*

* $P < 0.01$; ** $p < 0.05$; NS- Not significant

Alterations in Platelet (Cmm), Mean Platelet Volume (Fl), Platelet Distribution Width (Fl), Platelet Crit (%) In Male And Female Volunteers Before And After Application Of One And More Than Two Leeches. (Table 4a&B)

Marked decrease in platelet count was seen after application of one leech to the volunteers. At the same time increase in mean platelet Volume and increased platelet distribution width was observed. Platelet crit was significantly decreased after application of one leech in all the volunteers. Increased platelet count was observed after application of more than two leeches however no significant change in mean platelet volume was observed in the volunteers.

Increased platelet distribution width was observed after application of more than two leeches it was moderate to highly significant. Platelet crit values were also formed to be decreased ($p < 0.05$) except in volunteer No. 1

Table 4a Alterations in Platelet parameters of Male and Female Volunteers Before and After application of one Leech.

No. of volunteer	Platelet/ cmm		Mean platelet volume (fl)		Platelet distribution width (fl)		Platelet Crit (%)	
	Before	After	Before	After	Before	After	Before	After
1	154x 10 ³	135 x 10 ³ *	10.4	12.5**	16.1	18.2*	180	171**
2	270 x 10 ³	142 x 10 ³ **	9.4	10.3**	12.9	13.0**	211	209*
3	212 x 10 ³	197x 10 ³ **	10.1	11.0*	15.7	20.2*(169	158*
4	275 x 10 ³	164x10 ³ **	7.3	09.0*	14.7	19.4**	201	147*
5	155 x 10 ³	99 x 10 ³ **	9.4	08.3*NS	14.5	20.1**	160	97*
6	157x 10 ³	120x 10 ³ *	10.3	10.3 ^{NS}	13.2	17.55**	133	129*

* $p < 0.05$; ** < 0.01 , NS- Not significant

Table 4b: Alterations in Platelet Parameters of Male and Female Volunteers Before and after Application of More Than Two Leeches.

No. of volunteer	Platelet/ cmm		Mean platelet volume (fl)		Platelet distribution width (fl)		Platelet Crit (%)	
	Before	After	Before	After	Before	After	Before	After
1	213 x 10 ³	216x 10 ⁶ *	10.4	10.7 ^{NS}	15.7	17.8*	0.227	0.221 ^{NS}
2	208x10 ³	218x 10 ³ *	10.4	10.4 ^{NS}	15.2	19.4**	0.222	0.183*
3	207 x 10 ³	213x 10 ³ *	7.8	8.0 ^{NS}	16.2	17.2*	0.171	0.149*
4	202x10 ³	211x 10 ³ *	8.5	8.5 ^{NS}	16.0	18.8*	0.171	0.149*
5	190x10 ³	211x10 ³ *	7.9	7.8 ^{NS}	15.5	16.9**	0.169	0.157*

* $p < 0.05$; ** < 0.01 , NS- Not significant

Alterations in Bleeding Time (Min /Sec) and Clotting Time (Min/Sec) in Male and Female Volunteers Before and After Application of One and More Than Two Leeches.(Table 5a&B)

In all the healthy male and female volunteers, before application of leech, bleeding time was measured by Duke's Method. All the volunteers were having normal bleeding time i.e. in the range of 1 to 5 minutes but after leeching significant prolonged bleeding time was noted in all volunteers. In all the volunteers, it was more than two hours with a maximum of four hours

The clotting time of all the volunteers before leeching exhibited normal clotting time. (in the range of 5 to 7 min) whereas after leeching highly significant increase in coagulation time was observed in all male and female volunteers under investigation

Prothrombin time was found to be unaltered. In all the volunteers after application of more than two leeches the bleeding time was more than three hour. Whereas highly significant increase clotting time was observed in all male and female volunteers. The prothrombin time was found to be unaffected.

Table 5a : Alterations in Bleeding time (min/sec), Clotting time (min/sec) and prothrombin time (sec) in Male and Female volunteers Before and after Applications of one Leech.

No. of volunteers	Bleeding time (min/sec)		Clotting time (min/sec)		Prothrombin time (min/sec)	
	Before	After	Before	After	Before	After
1	3/15	180/0**	7/10	15/20*	11	9
2	2/20	120/0**	5/10	8/30*	10	10
3	3/00	120/15**	7/20	12/00**	9	10
4	2/00	120/25**	5/10	13/00**	10	10
5	3/20	180/25**	6/25	10/12**	12	12
6	2/20	240/17**	5/50	15/00**	11	10
7	2/25	180/0**	6/30	12/00**	12	12

* $p < 0.005$; ** $p < 0.01$

Table 5b: Alterations in Bleeding Time (Min/Sec), Clotting Time (Min/Sec) and Prothrombin Time (Sec) In Male and Female Volunteers Before and after Application of More Than Two Leeches.

No. of volunteer	Bleeding time (min/sec)		Clotting time (min/sec)		Prothombin time (sec)	
	Before	After	Before	After	Before	After
1	2/40	240/20	4/25	18/15	11	09
2	3/20	340/50	6/30	19/20	13	11
3	2/10	380/35	5/10	18/0	10	10
4	2/18	340/10	5/30	16/10	12	10
5	3/40	300/20	7/00	19/12	12	11

Alterations in Serum Protein and immunological Proteins Before and After Leeching in Healthy Male and Female Volunteers.(Table 6)

Blood samples from normal healthy male and female were taken before and after application of leech to know the alterations in total protein (g/dL), Albumin (%), α_1 globulin (%), α_2 globulin (%), β globulin, γ globulin and A/G ratio. The analysis was done by paper electrophoresis and then by using densitometer.

Decrease in protein values and albumin (%) was observed after leeching in all volunteers. This decline in proteins was moderately significant. ($p < 0.05$)

Significant decrease in A/ G ratio was obtained in all the volunteers after leeching.

Table 6 Alterations in total protein, Albumin and Immunological Proteins in male and female volunteers before and after Application of one Leech.

No. of volunteers	Total protein		Albumin (%)		α_1 Globulin %		α_2 Globulin %		β Globulin %		γ Globulin %		A/G Ration	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	6.43	5.92	56.01	46.62	3.53	3.46	9.67	9.74	6.60	6.36	24.19	31.32	1.27	0.20
2	6.21	6.00	54.33	45.00	6.22	6.01	8.88	9.26	8.00	8.12	22.57	31.61	1.18	0.82
3	6.55	6.49	58.09	50.99	4.50	4.44	9.06	9.19	6.32	5.18	22.30	30.20	1.38	1.04
4	6.96	5.02	48.65	43.25	3.18	3.05	18.15	18.46	6.55	6.15	23.47	29.09	0.947	0.76
5	7.02	5.44	50.15	40.11	4.16	4.10	11.25	11.44	5.50	6.08	28.94	38.27	1.06	0.68

Discussion

Disinfected leeches *Poecilobdella viridis* (Blanchard) were fed on the blood of healthy human (male and female) volunteers to know the immediate biochemical alterations in human blood.

Hirudo medicinalis suck polling blood. The calcite teeth of the leech jaw rip up the skin tissue of the host. The contraction of the injured blood vessels is blocked by histamine. Damas (1) reported gelatinolytic activity to be present in the salivary glands.

Hirudin forms a complex with thrombin inhibiting its activity, resulting into bleeding for long time affecting the coagulation process. We have observed increased bleeding time and clotting time in all the volunteers who were applied with one and more than two leeches. However, prothrombin time remained unaffected.

The findings outlined above are compatible with the interpretation that residual hirudin is present and is capable of inhibiting thrombin-sensitive parameters but that the hirudin is washed out by the end of this period and thereafter cannot account for excessive prolongation of bleeding times.

Most of the volunteers exhibited decreased haemoglobin after leeching and this decrease was significant after application of more than two leeches. These results imply that leech saliva contain proteases and lipases which might have digested the RBC membrane proteins and lipids and also the globulin from haemoglobin.

Application of one leech has also resulted into increase in RBC count, which indicates a factor in leech saliva having capacity to activate the erythropoiesis. The

decrease in RBC count was recorded in case of volunteers who were applied more than two leeches. This decrease in RBC count must be due to the entry of bacteria in blood flow along with leech saliva, which might have secreted the lipases resulting into breaking down of the RBC membrane. .

The anticoagulant effect of hirudin is highly specific for a thrombin without affecting other closely related serine proteinase or further enzymatic activities due to its high affinity for thrombin. Relatively low inhibitor concentrations are necessary to prevent coagulation. In the hirudin-thrombin complex, all proteolytic functions of the enzyme are blocked. Thus hirudin presents not only fibrinogen clotting but also further thrombin catalysed hemostatic reaction such as activation of clotting factor V, VII, XIII and the thrombin induced platelet reaction. Therefore, by instantaneous inhibition of the small amount of thrombin generated after activation of the coagulation system, the positive feedback on prothrombin activation is prevented that otherwise would lead to accelerated generation of further thrombin. (4).

Hoffmann (5) stated that depending on the hirudin concentration in blood, coagulation is retarded or completely inhibited. Accordingly, the coagulation variables, thrombin time, partial thromboplastin time and prothrombin time are prolonged. He further confirmed that after complexing with hirudin, thrombin loses its effect on platelet i.e. the thrombin induced platelet aggregation and release reaction are prevented. Glusa (1990) observed that no influence is exerted on the platelet reactions induced by other activators like ADP, collagen and adrenaline or platelet adhesion.

US FDA has approved Leeches in the treatment of Plastic and reconstructive surgery (2). Plastic and reconstructive surgeons have used leeches in the complex plastic surgeries(3,7). Present study shows the haematological alterations in the mammalian blood after the application of the leeches.

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