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Comparison of ATP Values on Meat and Fish Cutting Boards before and after Alcohol Disinfection

By Akemi Ito, Naomi Katayama, Mayumi Hirabayashi, Natuki Sasaki & Moe Inuzuka

Nagoya Women's University

Abstract- Sanitary control of cutting boards in the kitchen is important to prevent food poisoning. Using ATP and microbiological tests, we investigated the cleaning and 70% alcohol spraying effects of cutting boards for meat and fish. As a result, the ATP value and the number of microbial bacteria decreased after washing the cutting board but decreased more after spraying with 70% alcohol. The ATP value was 100 or less after spraying with 70% alcohol. The number of microbial bacteria decreased after spraying with 70% alcohol. However, not all bacterialeliminated even after spraying with 70% alcohol. If the cutting boardleft in a moist state at room temperature, microorganisms could grow again.

Keywords: *Gender: ATP wiping test, Microbial stamp test, Cutting board, alcohol disinfection.*

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Comparison of ATP Values on Meat and Fish Cutting Boards before and after Alcohol Disinfection

Akemi Ito [α], Naomi Katayama [σ], Mayumi Hirabayashi [ρ], Natuki Sasaki [ω] & Moe Inuzuka [¥]

Abstract- Sanitary control of cutting boards in the kitchen is important to prevent food poisoning. Using ATP and microbiological tests, we investigated the cleaning and 70% alcohol spraying effects of cutting boards for meat and fish. As a result, the ATP value and the number of microbial bacteria decreased after washing the cutting board but decreased more after spraying with 70% alcohol. The ATP value was 100 or less after spraying with 70% alcohol. The number of microbial bacteria decreased after spraying with 70% alcohol. However, not all bacterialeliminated even after spraying with 70% alcohol. If the cutting boardleft in a moist state at room temperature, microorganisms could grow again.

Keywords: Gender: ATP wiping test, Microbial stamp test, Cutting board, alcohol disinfection.

I. Introduction

Sanitary control of cutting boards in the kitchen is important to prevent food poisoning. In the past, we reported the results of hygiene management by repeatedly cleaning the cutting board with detergent and running water for 30 seconds or more[1)]. Currently, the COVID-19 epidemic requires stricter hygiene control. To control invisible microorganisms, it is necessary to take measures to avoid the risk of food poisoning accidents due to familiarity with cooking work; as the O-JT education, it is necessary to create a hygiene management manual and protect it with all the cooks[2,3,4,5)]. However, if the procedure is complicated and difficult, it will not last long. We need easy and reliable procedures and methods that anyone can do. The ATP tests[6,7,8)] and HACCP-based microbiological tests[9)] are useful in hygiene management to transform invisible bacteria into visible forms and educate them. Therefore, in this study, and the cutting board cleaning method we performed last time, a step of spraying 70% alcohol added. The effects of this alcohol disinfection compared by adding a stamping test (General bacteria, Staphylococcus aureus, Escherichia coli, Salmonella, Vibrio parahaemolyticus) in addition to the same ATP test as in the previous report.

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II. Materials and Methods

a) Kitchen cutting board

The six kitchen meat or fish thick cutting board (cutting board 1) and the six kitchen meat for the fish thin cutting board (cutting board 2) prepared in the kitchen were stored in the sterilization storage the day before cooking.

b) ATP inspection procedure

Each of the 12 cooks carried a kitchen cutting board for meat or fish at the start of their work and brought it to the cooking table. The work start time depends on the working conditions of the cooks. Still, the inspector always performed an ATP inspection before using meat or fish with a kitchen cutting board. Then, each cook finished the work, washes the cutting board firmly with detergent and sponge, rinse with running water for 30 seconds or more. Then, each cook repeated this process twice (as same as the last report[1)]). The inspector performed an ATP inspection after using meat or fish with a kitchen cutting board, again. Then, each cook sprayed 70% alcohol on the cutting board after washing. At last, the inspector performed an ATP inspection after using meat or fish with a kitchen cutting board. The ATP test kit used manufactured by KIKKOMAN.

c) Stamp test inspection procedure

Five types of stamp test (General bacteria, Staphylococcus aureus, Escherichia coli, Salmonella, Vibrio parahaemolyticus) used. The stamp test conducted by the inspector at the same time as the ATP. The stamp test was colony-counted after culturing in an incubator at 38 degrees for three days. The stamp test made by NISSUI.

d) Statistical processing

The results obtained compared using statistical methods. The data were statistically processed, was subjected to an F test to determine whether to use a parametric test or nonparametric test. When there is no difference in the F test, the presence or absence of a significant difference was confirmed using the student t-test with or without a correspondence. If there was a difference in the F test, the presence or absence of a significant difference was confirmed using the Wilcoxon

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test with a pair or the Mann-Whitney test without correlation.

III. Results

a) ATP value results before and after alcohol disinfection

The table 1 and 2 shows the results of ATP wiping tests on cutting board before and after alcohol disinfection. It can see that the average value of the ATP values measured after washing before and after cleaning, after cleaning, the ATP value is low. The ATP value after 70%alcohol spraying was statistically significantly lower than that before alcohol spraying. The ATP value dropped below 100 for both cutting boards.

Table1. ATP test value and statistical processing result of cutting board 1.

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	8414	50121	50121	13
2	210	56	56	31
3	132205	103	103	20
4	59141	62	62	31
5	30814	272	272	18
6	76010	70	70	72
Average value	51132.3	8447.3	8447.3	30.8
SD	49166.2	20416.0	20416.0	21.4
F test	P-0.025*		P=0.0001**	
Student-t*				
Wilcoxon	P=0.116		P=0.046*	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

Table2. ATP test value and statistical processing result of cutting board 2.

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	4817	3828	3828	38
2	1302	12	12	58
3	99080	456	456	16
4	61864	33	33	56
5	161792	293	293	17
6	243	85	85	50
Average value	54849.7	784.5	784.5	39.2
SD	66022.3	1500.9	1500.9	18.9
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.028*		P=0.173	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

b) Stamp test results before and after alcohol disinfection

Tables 3,4,5,6,7,8.9.10.11 and 12 show the results of ATP wiping tests on cutting board before and after 70% alcohol disinfection. Results of general bacteria show in Tables 3 and 4. Results of E. coli show in Tables 5 and 6. Results of Staphylococcus aureus shown in Tables 7 and 8. Result of Salmonella show in Tables 9 and 10. Result of Vibrio parahaemolyticus show in Tables 11 and 12. The number of all microbial bacteria was lower after washing than after cooking and after spraying 70% alcohol. However, there was no statistically significant difference in the number of microbial bacteria.

Table 3 Number of general bacteria on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	82	40	40	0
2	4	0	0	0
3	200	46	46	0
4	13	0	0	0
5	200	9	9	0
6	60	0	0	14
Average value	93.2	15.8	15.8	2.3
SD	87.7	21.4	21.4	5.7
F test	P=0.002**		P=0.003**	
Student-t*				
Wilcoxon	P=0.028*		P=0.273	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 4 Number of general bacteria on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	270	7	7	0
2	1	18	18	8
3	200	8	8	0
4	2	7	7	0
5	200	23	23	0
6	61	20	20	0
Average value	122.3	13.8	13.8	1.3
SD	115.6	7.3	7.3	3.3
F test	P=0.0001**		P=0.035*	
Student-t*				
Wilcoxon	P=0.116		P=0.028*	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.075			

*Paired Student-t test * P<0.05, ** P<0.01

Table 5 Number of E. coli on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	0	0	0	0
2	2	1	1	0
3	200	8	8	0
4	5	0	0	7
5	200	0	0	0
6	6	0	0	16
Average value	68.8	1.5	1.5	3.8
SD	101.6	3.2	3.2	6.6
F test	P=0.0001**		P=0.052	
Student-t*			P=0.518	
Wilcoxon	P=0.043*			
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.418			

*Paired Student-t test * P<0.05, ** P<0.01

Table 6 Number of E. coli on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	3	2	2	0
2	0	0	0	1
3	200	23	23	0
4	0	0	0	0
5	15	0	0	0
6	35	0	0	0
Average value	42.2	4.2	4.2	0.2
SD	78.5	9.3	9.3	0.4
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.068		P=0.285	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.080			

*Paired Student-t test * P<0.05, ** P<0.01

Table 7 Number of Staphylococcus aureus on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	0	0	0	0
2	5	1	1	0
3	0	0	0	7
4	0	0	0	3
5	152	2	2	0
6	212	0	0	0
Average value	61.5	0.5	0.5	1.7
SD	95.3	0.8	0.8	2.9
F test	P=0.0001**		P=0.004**	
Student-t*				
Wilcoxon	P=0.109		P=0.465	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.345			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 8 Number of Staphylococcus aureus on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	0	0	0	0
2	0	1	1	16
3	24	0	0	0
4	0	1	1	0
5	200	0	0	0
6	432	0	0	0
Average value	109.3	0.3	0.3	2.7
SD	176.3	0.5	0.5	6.5
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.225		P=0.655	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.144			

*Paired Student-t test * P<0.05, ** P<0.01

Table 9 Number of Salmonella on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	508	0	0	0
2	80	0	0	0
3	1	0	0	2
4	168	0	0	5
5	1	9	9	0
6	26	15	15	5
Average value	130.7	4.0	4.0	2.0
SD	195.4	6.5	6.5	2.4
F test	P=0.0001**		P=0.016*	
Student-t*				
Wilcoxon	P=0.075		P=0.465	
F test	P=.0001**			
Student-t*				
Wilcoxon	P=0.059			

*Paired Student-t test * P<0.05, ** P<0.01

Table 10 Number of Salmonella on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	0	1	1	0
2	0	5	5	0
3	34	1	1	2
4	0	23	23	0
5	21	1	1	0
6	55	0	0	0
Average value	18.3	5.2	5.2	0.3
SD	22.8	8.9	8.9	0.8
F test	P=0.019		P=0.0001**	
Student-t*				
Wilcoxon	P=0.463		P=0.138	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.109			

*Paired Student-t test * P<0.05, ** P<0.01

Table 11 Number of Vibrio parahaemolyticus on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	21	0	0	0
2	6	0	0	0
3	1	7	7	0
4	119	0	0	0
5	0	38	38	2
6	18	0	0	0
Average value	27.5	7.5	7.5	0.3
SD	45.7	15.2	15.2	0.8
F test	P=0.009**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.402		P=0.180	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.075			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 12 Number of Vibrio parahaemolyticus on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For meat	Before washing	After washing	After washing	After alcohol
1	0	0	0	0
2	0	4	4	2
3	5	0	0	0
4	0	0	0	0
5	200	0	0	0
6	256	0	0	0
Average value	76.8	0.7	0.7	0.3
SD	118.4	1.6	1.6	0.8
F test	P=0.0001**		P=0.68	
Student-t*			P=0.363	
Wilcoxon	P=0.144			
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.144			

*Paired Student-t test * P<0.05, ** P<0.01

IV. Discussion

To manage the hygiene of meat and fish cutting board that has a high risk of causing secondary contamination in cooking. We tried to verify using the ATP test and microbial stamp test by spraying 70% alcohol after cleaning instead of controlling only by the cleaning method[1)]. The ATP value decreased after washing then after cooking and after spraying 70% alcohol than after washing. The ATP value was a statistically significant decrease, which was less than 100 after 70% alcohol spraying. However, the microbial stamp test results were not statistically significant reductions in bacterial counts. The cutting board inspected by spraying 70%alcohol after cleaning. But if 70% of alcohol not sprayed after sufficiently wiping off the water, the alcohol may be dilute, and the bactericidal effect may weakened. In the future, we would like to verify the sterilization of microorganisms by spraying 70% alcohol on the cutting board by thoroughly wiping off the water after cleaning and then spraying 70% alcohol. Not all microorganisms are killed even after spraying 70% alcohol, so when using a cutting board left at room temperature (with moist), it is better to wash repeatedly and cook after spraying 70% alcohol.

V. Conclusions

The effects of 70% alcohol spraying investigated using cutting boards for meat and fish. Both cutting boards had high ATP and microbiological test values after cooking. However, although the value of the cutting board decreased after cleaning, the ATP value did not fall below 100. Microbial test values were also high in many cases. After spraying with 70% alcohol, the ATP value was 100 or less, and the value decreased statistically significantly. Microbial test values were decreasing with or without statistically significant reductions. Providing safe and secure meals by further spraying 70% alcohol after cleaning the cooking utensils helps prevent food poisoning. However, since the microorganisms are present even after spraying with 70% alcohol, the bacteria may grow again if the cooking utensils left for a long time. It is advisable to clean and spray 70% alcohol before using the equipment.

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Study of Biochemical and Anthropometric Variables among Pancagavya and Non-Pancagavya Diet Population: A Cross-Sectional Comparative Study

By Neeraj, Itagi Ravi Kumar, Dwivedi Krishna & Pandey Mangesh

Deemed-to-be University

Abstract- *Background:* Diseases negatively affect people's work performance, joy in life, emotional and physical health, quality of life, and spiritual well-being. The pancagavya (five cow derivatives) diet based on the consumption of five cow derivatives like milk, curd, ghee, urine and dung from Bos indicus cow not only helps to provide physical health but also useful in other aspects of life.

Objective: To study the biochemical and anthropometric variables among the pancagavya diet population.

Materials and method: Both male and female sample size 80 with an age range between 20 to 80 years were recruited from different states of India. The current study is between the pancagavya diet and non-pancagavya diet groups and had more than two years in their diet.

Keywords: *pancagavya diet, non-pancagavya diet, bhramari time, health.*

GJMR-K Classification: *NLMC Code: QT 235*



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Study of Biochemical and Anthropometric Variables among Pancagavya and Non-Pancagavya Diet Population: A Cross-Sectional Comparative Study

Impact of Panchagavya Diet on Health

Neeraj [α], Itagi Ravi Kumar [σ], Dwivedi Krishna [ρ] & Pandey Mangesh [ω]

Abstract- ***Background:*** Diseases negatively affect people's work performance, joy in life, emotional and physical health, quality of life, and spiritual well-being. The pancagavya (five cow derivatives) diet based on the consumption of five cow derivatives like milk, curd, ghee, urine and dung from Bos indicus cow not only helps to provide physical health but also useful in other aspects of life.

Objective: To study the biochemical and anthropometric variables among the pancagavya diet population.

Materials and method: Both male and female sample size 80 with an age range between 20 to 80 years were recruited from different states of India. The current study is between the pancagavya diet and non-pancagavya diet groups and had more than two years in their diet.

Result: Pancagavya diet group had significantly less random blood glucose levels and had no change in hemoglobin level compared to the non-pancagavya diet group. Pancagavya diet group have more bhramari (humming honey bee sound produced at the back of the throat during the practice time) and respiratory rate with exponential significance, less pulse rate with highly significant, and was having lesser blood pressure compared to the non-pancagavya diet group.

Conclusion: In the biochemical and anthropometric variables studied pancagavya diet group had a better healthy lifestyle compared to the non-pacagavya diet group.

Keywords: pancagavya diet, non-pancagavya diet, bhramari time, health.

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I. Introduction

Health is a positive concept accentuating social and personal assets as well as physical and psychological aptitudes. In a healthy condition, an individual can satisfy needs and deal with interpersonal, social, biological, and physical environments. Therefore, it is a resource for every day [1]. In the perspective of understanding health, it is required to focus on the concept of global health. It is an area for study, investigation, and practice that places precedence on refining health and attaining justice of health for all people internationally. Global health emphasizes worldwide health issues, causes, and solutions, includes numerous corrections within and beyond the health sciences and indorses interdisciplinary association [2]. In addition to health, quality of life involves the standard of living, the quality of housing, and the neighborhood in which one lives, job satisfaction, and many other factors. According to the World Health Organisation (WHO), health is defined as a "state of complete mental, physical, and social well-being and not only the absenteeism of disease or disability. Health brings "broadness"- nothing is missing from the person; it brings "proper functions"-everything is working proficiently [3]. Biochemical and anthropometric variables are directly connected to health. For Cardiovascular Disease (CVD), diabetes is one of the cause. Recent decades have seen a striking rise in diabetes dominance across the globe [4].

According to the World Health Organization (WHO), the global prevalence of anemia is 24.8%, which means about 1.62 billion people worldwide [5]. A decrease in the level of hemoglobin is associated with reduced health-related quality of life, congestive heart failure, and increased mortality in chronic kidney disease [6]. The previous studies has described that bhramari (humming bee breath) is a yoga practice, in which subjects should sit in any meditative posture, inhale through both nostrils, and while exhaling, produce the sound of a humming bee. More the bhramari timing, the more will be the lungs capacity [7]. The effect of pulse rate is also measured at the time of diagnosis. High pulse rate, at the time of diagnosis, is strongly associated with cardio related risks [8]. For the person's

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health condition and physiological stability, respiratory rate also provides information. An abnormal respiratory rate is a strong pointer that a health crisis is about to happen [9]. Blood pressure is the pressure exerted by blood on the walls of blood vessels while flowing. Globally, the cause of death is high blood pressure, and also it is the second foremost cause of debility next to childhood malnutrition. More than 80% of the adults are at threat from their blood pressure [10].

According to modern science, the gross (physical) body is made up of packets of energy. The ancient indian scripture taittiriya Upanishad has mentioned that the physical body is made of Annam, and the Annam is called food, which consists of five elements (earth, water, space, air, and fire). The yogic diet mentioned in Katha Upanishad and Hatha yoga scriptures consists of cow milk, cow ghee, sprouts, fruits, which is easy to digest and helps to maintain the physical and mental health. The Bhagavadgita highlights three categories of food tamasika (which is stale, tasteless, stinking, cooked overnight and impure), rajasika (that are bitter, sour, saline, over-hot, pungent, dry and burning), and satvika (that increase vitality, energy, vigour, health, joy and cheerfulness) based on the characteristics of food and its influence on human personality. The quantity of food, place, time, the mental state also contributes equally to maintain positive health [11]. Medical research centers emphasize lifestyle modification consisting of diet, normalization of body weight, and aerobic exercise as factors in treating non-insulin-dependent diabetes mellitus (NIDDM). Diet and lifestyle modification can be in controlling non-insulin-dependent diabetes mellitus (NIDDM) and reducing risk factors linked with macrovascular complications [12].

The other diet known as the pistachio diet also improved endothelial function, blood glucose level, some indices of inflammation, and oxidative status in healthy young men. Studies have also shown that frequent nut consumption decreases the risk of coronary artery disease [13]. A low-carbohydrate ketogenic diet (LCKD) has also shown beneficial effects in patients with type 2 diabetes, including reducing anti-diabetic medication dosage [14]. Hemoglobin determination is considered as a screening index valuable in describing various degrees of iron deficiency anemia. Dietary factors play a role in the growth of iron deficiency [15]. A Diet of calorie consisted of moderate carbohydrate, high protein, and rich in vitamins with a high amount of vegetables and fruits can increase the hemoglobin level [16]. The study also shows; changes in anthropometric variables like body weight, hip circumference, and waist circumference due to specific dietary intake [17]. There is an intensive investigation of the relationship between diet and blood pressure in recent years. A vegetarian diet shows lesser BP values in hypertensive subjects [18]. The diet approach is recommended to lower blood pressure. The diet improves cardiovascular risk factors and beneficial in increased cardiometabolic risk [19]. The dietary approach to stop hypertension shows a high reduction in blood pressure and improvement in autonomic and vascular functions [20].

Pacagavya, as given in Ayurveda, consist of five substances obtained from cow namely, urine, dung, milk, ghee, and curd [21], and this diet is called as a pancagavya diet (PD). The Bos indicus (Indian) cow is known as kamadhenu (divine bovine-goddess/cow of plenty), signifying its nourishing nature, similar to a mother. According to the Indian scripture, The Indian sage maharshi vashistha served the divine kamadhenu cow, and Indian sage maharshi dhanavantari offered a wonderful medicine pancagavya to humanity [22]. Many formulations mentioned in Ayurveda describe the use of pancagavya components either as a single ingredient or in combination with drugs of herbal, animal, or mineral origin [21]. The cow milk consists of essential nutrients that are good for health, such as vitamins A, B, C, carotenes, and proteins. It contains the low calorific value and less cholesterol. It is a good animator for human health, easily digestible, and it also plays a bio-protective role [22].

Cow curd is the removal of three humors of the body and a blood purifier. It is beneficial for gastrointestinal disorders, piles, and blood-related problems. It is one of the most health-giving among all food items. In a non-drug manner, it helps to manage infections as it is an efficient anti-infection. Buttermilk and cow curd helps to control the growth of harmful microorganisms [23]. Cow's ghee enhances the body's resistance to infections, intelligence, eyesight, voice quality, and memory. It is for cholesterol and a heart patient as well as it is an anti-aging agent. It purifies the blood to an extent, and it also improves physical and mental health [23]. Ayurveda mentioned the formulation of pancagavya ghee, which is useful against anemia, fever, inflammations, and liver disorder [21]. Cow urine is used to remove the blockage in arteries, used for arthritis, psoriasis, eczema, diabetes, heart attack, prostrate, fits, piles, migraine, ulcer, acidity, constipation, gynecological problems, nose and ear problems [24]. Recently cow urine has been granted U.S. Patents (No. 6896907 and 6410059) for its use along with antibiotics for the fight against cancer and to control bacterial infections [25]. Cow urine helps to enhance immune responses in the body. Several elements in the body can be balanced by cow urine. Total salts present in cow urine are 24 in numbers [23]. In treating diseases like respiratory diseases, chronic renal failure, hepatitis A, B, and C, urological disorders, asthma, and cancer, cow urine plays an important role. It also acts as a disinfectant against many diseases like various kinds of allergies, acne vulgaris, scabies, eczema, and psoriasis [26]. In ancient times cow dung was widely used as fertilizer. Goumayarash is used as a skin tonic and useful in many skin related disorders like

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gangrene, psoriasis, eczema. The properties which cow dung includes are antibacterial, antifungal, and antiseptic [23].

II. Materials and Methods

Pancagavya diet (PD) group and non-pancagavya diet (NPD) group were recruited from Delhi, Haryana, and Rajasthan states of India, and its demographic details are given in table 1. The data for both the groups were collected between the period of January 2020 and February 2020. The sample size was calculated by using G-power software; based on; the previous study, the sample size was 76 with alpha 0.05, power 0.95, effect size 0.84 [27]. The assessments of the two groups were based on people adhering to PD and NPD for more than two years were considered. People with psychiatric ailments underwent any recent surgery, infectious disease, and female under menstruation and pregnancy were excluded from the study. Group of PD was directly or indirectly consumers of Bos indicus cow's products mainly of milk, curd, and clarified butter (ghee), cow urine, and cow dung. Non-pancagavya diet group was consumers of NPD diet, including buffalo, jersey cow, or any other animal's milk, ghee, curd, and grains produced by UREA/DAP and other pesticides more than two years are considered. In the present study biochemical variables, blood glucose, hemoglobin, and anthropometric variables bhramari time, pulse rate, respiratory rate, and blood pressures are measured. Bhramari (breath-holding time is a yoga practice, in which subjects sits in any meditative posture, inhale through both nostrils, and exhale, produce the sound of a humming bee) [7]. Data analysis was done by using JASP software with Shapiro–Wilk test for normality, and independent sample t-test was performed.

III. Result

The result showed that there was a significant difference between the pancagavya diet group compared to the non-pancagavya diet group for blood glucose ($p<0.05$), but there is no significant difference between both groups ($p<0.975$) in levels of hemoglobin as shown in table 2. For anthropometric variables, there is a significant difference in bhramari time ($p<0.001$), pulse rate ($p=0.02$), respiratory rate ($p<0.001$), systolic blood pressure ($p<0.01$), and for diastolic blood pressure ($p<0.05$).

IV. Discussion

The random blood glucose level used as a bio-marker showed a significantly lower in the pancagavya diet subjects than the subjects consuming non-pancagavya diet. The bhramari time with exponential significance, pulse rate with the highly significant and respiratory rate with exponential significance in PD group compared to the NPD group. The Subjects following PD had less measurement in both systolic and diastolic blood pressure compared to NPD. These indicate that the PD group have an opportunity for a better life-style, which comprises physical, mental, and spiritual well-being. The pancagavya have much application like in treating many diseases and to increase the body resistance to fight diseases medicines prepared from panchagavya are effective [22], therapeutic benefits of cow urine in managing cancer [27], practical application of pancagavyha products in the field of agriculture, to rejuvenate the soil health [24], based on the synergistic and systematic harnessing of energies from cows, plants, and earth [22], pancagavya ghrita, is also one of the formulations mentioned in Ayurveda which is prepared with all five components of panchagavya viz cow milk, ghee, urine, dung and curd in equal proportions useful for rejuvenation [21]. As the side effects of antibiotic medicine have harmful, one need to look into new therapeutic approach like panchagavya to remove diseases and to control infections.

V. Conclusions

Pancagavya diet group had less random blood glucose level, and have more bhramari time, less pulse rate, respiratroy rate, and blood pressure. Pancagavya diet had shown a more positive impact on health compared to the non-pancagavya diet.

Sources of funding
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Conflict of interest
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Table 1: Demographic details

Particulars		PD	NPD
Number of subjects		40	40
Age(year) mean±SD value		42.12±13.66	42.22±16.17
Gender	Male	29	29
	female	11	11
Occupation	Agriculture	29	24
	Job	1	4
	Business	5	3
	Student	1	5
	Housewife	2	3
	Others	2	1
Diet (from years)	2 years	5	0
	3 years	6	1
	More than 5 years	29	39

Legend: PD- Pancagavya diet.

NPD-Non-pancagavya diet.

Table 2: Result of statistical analysis

Domain	PD Mean±SD	NPD Mean±SD	T-value	*p*-value	Cohen's d
Biochemical variables					
Blood glucose (mg/dl)	110.63±20.08	135.34±63.08	2.361	0.021*	0.528
Hemoglobin (gm/dl)	13.55±1.90	13.52±2.08	0.062	0.975	0.014
Anthropometric variables					
Bhramari time (sec)	20.17±6.49	12.92±5.67	5.315	<0.001***	1.188
Pulse rate (bpm)	76.92±4.99	83.37±11.65	3.218	0.002**	0.720
Respiratory rate (cpm)	13.97±3.39	18.22±3.97	5.141	<0.001***	0.720
Sys. BP(mmhg)	118.65±7.67	127.35±15.67	3.152	0.002**	0.705
Dia. BP(mmhg)	78.40±6.93	82.45±10.20	2.077	0.041*	0.464

Legend: PD- Pancagavya diet.

NPD-Non-pancagavya diet

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Comparison of ATP Values on Vegetables Cutting Boards before and after Alcohol Disinfection

By Naomi Katayama, Akemi Ito, Mayumi Hirabayashi, Natuki Sasaki & Moe Inuzuka

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Abstract- Sanitary control of cutting boards in the kitchen is important to prevent food poisoning. To preventing secondary and tertiary contamination of food poisoning bacteria, it is necessary to know the hygiene status of cooking utensils. Therefore, in this study, we compared the values after cooking, washing, and spraying 70% alcohol on cutting boards for vegetables using the ATP test and microbiological test. As a result, the ATP value after spraying with alcohol was 100 or less, which was better than that after washing. Microbial test results showed that microorganisms were present on the vegetable cutting board even after spraying with 70% alcohol. Since microorganisms are present even after spraying with alcohol, it is possible that the growth of microorganisms will occur again if the vegetable cutting boardleft in a moist state at room temperature. When using a vegetable cutting board left unattended, it is necessary to wash repeatedly and spray it with alcohol.

Keywords: ATP wiping test, cutting board, hygiene education, double wash.

GJMR-K Classification: NLMC Code: QV 250



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Comparison of ATP Values on Vegetables Cutting Boards before and after Alcohol Disinfection

Naomi Katayama [α], Akemi Ito [σ], Mayumi Hirabayashi [ρ], Natuki Sasaki [ω] & Moe Inuzuka [¥]

Abstract- Sanitary control of cutting boards in the kitchen is important to prevent food poisoning. To preventing secondary and tertiary contamination of food poisoning bacteria, it is necessary to know the hygiene status of cooking utensils. Therefore, in this study, we compared the values after cooking, washing, and spraying 70% alcohol on cutting boards for vegetables using the ATP test and microbiological test. As a result, the ATP value after spraying with alcohol was 100 or less, which was better than that after washing. Microbial test results showed that microorganisms were present on the vegetable cutting board even after spraying with 70% alcohol. Since microorganisms are present even after spraying with alcohol, it is possible that the growth of microorganisms will occur again if the vegetable cutting boardleft in a moist state at room temperature. When using a vegetable cutting board left unattended, it is necessary to wash repeatedly and spray it with alcohol.

Keywords: ATP wiping test, cutting board, hygiene education, double wash.

I. Introduction

Care should taken when cleaning vegetable cutting boards, as compared to meat and fish cutting boards, it may not be possible to wash them carefully due to the lack of sliminess[1)]. In this study, we used the ATP test and microbial test to compare the ATP value and the number of microbial bacteria immediately after cooking, washing, and spraying 70% alcohol on cutting boards for vegetables. The ATP value is preferably 100 or less, and the number of microbial bacteria is preferably free. We reported the results of the actual ATP test and the microbiological test.

II. Materials and Methods

a) Kitchen vegetable cutting board

The twelve vegetables cutting board prepared in the kitchen were stored in the sterilization storage the day before cooking. Cooking done in two places, and six cutting boards used for each.

b) ATP inspection procedure

Each of the twelve cooks carried a vegetable cutting board for the vegetable of their work and brought it to the cooking table. Still, the inspector always performed an ATP inspection before using vegetables with a cutting board. Then, each cook finished the work, washes the cutting board firmly with detergent and sponge, rinse with running water for 30 seconds or more. Then, each cook repeated this process twice (as same as the last report[1)]). The inspector performed an ATP inspection after using vegetable with a cutting board, again. Then, each cook sprayed 70% alcohol on the cutting board after washing. At last, the inspector performed an ATP inspection after using vegetables with a kitchen cutting board. The ATP test kit used manufactured by KIKKOMAN.

c) Stamp test inspection procedure

Five types of stamp test (General bacteria, Staphylococcus aureus, Escherichia coli, Salmonella, Vibrio parahaemolyticus) used. The stamp test conducted by the inspector at the same time as the ATP. The stamp test was colony-counted after culturing in an incubator at 38 degrees for three days. The stamp test by MISSUI.

d) Statistical processing

The results obtained compared using statistical methods. The data statistically processed, was subjected to an F test to determine whether to use a parametric test or nonparametric test. When there is no difference in the F test, the presence or absence of a significant difference confirmed using the student t-test with or without a correspondence. If there was a difference in the F test, the presence or absence of a significant difference was confirmed using the Wilcoxon test with a pair or the Mann-Whitney test without correlation.

III. Results

a) ATP value results before and after alcohol disinfection

Tables 1 and 2 show the results of ATP wiping tests on vegetables cutting board before and after alcohol disinfection. The ATP value was statistically significantly lower after washing than after cooking. However, the ATP value did not fall below 100. The ATP

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value after70% alcohol spraying was 100 or less. The ATP value was statistically significantly lower after 70% alcohol spraying than after cooking.

Table1. ATP test value and statistical processing result of cutting board 1.

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	176205	863	863	10
2	909793	68	68	10
3	6543	39	39	44
4	15	42	42	26
5	38244	283	283	11
6	14200	5790	5790	17
Average value	190833.3	1180.8	1180.8	19.7
SD	358322.3	2279.9	2279.9	13.4
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P0.046*		P-0.046:	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.046*			

*Paired Student-t test * P<0.05, ** P<0.01

Table2. ATP test value and statistical processing result of cutting board 2.

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	9194	2630	2630	18
2	1103	56	56	35
3	48126	449	449	45
4	3168	52	52	22
5	136610	259	259	3
6	3983	616	616	23
Average value	33697.3	677.0	677.0	24.3
SD	53435.9	981.9	981.9	14.4
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.28*		P-0.028*	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

b) Stamp test results before and after alcohol disinfection

Tables 3,4,5,6,7,8.9.10.11 and 12 show the results of ATP wiping tests on vegetables cutting board before and after alcohol disinfection. The result of common bacteria, Staphylococcus aureus and Vibrio parahaemolyticus was that microorganisms could be present on the cutting board even after 70% alcohol spraying. However, the number of microorganisms reduced compared to after cooking. In the case of E. Coli and Salmonella, the number of microorganisms decreased statistically significantly after spraying with 70% alcohol.

Table 3 Number of general bacteria on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	298	110	110	0
2	8	10	10	13
3	22	3	3	0
4	50	0	0	3
5	7	42	42	0
6	8	1	1	0
Average value	65.5	27.7	27.7	2.7
SD	115.1	43.3	43.3	5.2
F test	P=0.016*		0.0001**	
Student-t*				
Wilcoxon	P=0.249		P-0.345	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.046*			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 4 Number of general bacteria on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	87	120	120	0
2	1	2	2	18
3	9	1	1	0
4	90	14	14	0
5	200	47	47	0
6	3	0	0	3
Average value	65.0	30.7	30.7	3.5
SD	78.0	47.3	47.3	7.2
F test	P=0.124		P=0.0001**	
Student-t*	P=0.273			
Wilcoxon			P=0.345	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.138			

*Paired Student-t test * P<0.05, ** P<0.01

Table 5 Number of E. coli on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	14	3	3	0
2	3	3	3	2
3	6	1	1	0
4	23	0	0	0
5	30	30	30	0
6	3	0	0	0
Average value	13.2	6.2	6.2	0.3
SD	11.3	11.8	11.8	0.8
F test	P=0.463		P=0.0001**	
Student-t*	P=0.110			
Wilcoxon			P=0.043*	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

Table 6 Number of E. coli on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	7	1	1	0
2	0	0	0	0
3	21	11	11	0
4	16	4	4	4
5	200	0	0	0
6	60	0	0	0
Average value	50.7	2.7	2.7	0.7
SD	76.1	4.4	4.4	1.6
F test	P=0.0001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.043*		P=0.075	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.043*			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 7 Number of Staphylococcus aureus on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	28	18	18	4
2	1	2	2	3
3	100	0	0	1
4	7	0	0	0
5	4	200	200	0
6	15	1	1	0
Average value	25.8	36.8	36.8	1.3
SD	37.6	80.2	80.2	1.8
F test	P=0.044*		P=0.0001**	
Student-t*				
Wilcoxon	P=0.463		P=0.345	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.046*			

*Paired Student-t test * P<0.05, ** P<0.01

8 Number of Staphylococcus aureus on cutting board 2. and statistical processing

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	2	2	2	0
2	0	0	0	2
3	0	0	0	0
4	7	0	0	0
5	250	56	56	0
6	33	0	0	0
Average value	48.7	9.7	9.7	0.3
SD	99.4	22.7	22.7	0.8
F test	P=0.001**		P=0.0001**	
Student-t*				
Wilcoxon	P=0.109		P=0.423	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.106			

*Paired Student-t test * P<0.05, ** P<0.01

Table 9 Number of Salmonella on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	210	3	3	0
2	48	0	0	27
3	3	100	100	2
4	240	35	35	8
5	1	9	9	0
6	175	0	0	0
Average value	112.8	24.5	24.5	6.2
SD	107.9	39.3	39.3	10.7
F test	P=0.013*		P=0.003**	
Student-t*				
Wilcoxon	P=-.173		P=0.281	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

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Table 10 Number of Salmonella on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	378	0	0	0
2	3	2	2	0
3	8	0	0	6
4	25	0	0	0
5	1	5	5	0
6	43	0	0	0
Average value	76.3	1.2	1.2	1.0
SD	148.6	2.0	2.0	2.4
F test	P=0.0001**		P=0.335	
Student-t*			P=0.914	
Wilcoxon	P=0.075			
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.028*			

*Paired Student-t test * P<0.05, ** P<0.01

Table 11 Number of Vibrio parahaemolyticus on cutting board 1. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	11	15	15	0
2	0	1	1	1
3	20	0	0	0
4	0	15	15	0
5	61	0	0	0
6	0	92	92	0
Average value	15.3	20.5	20.5	0.2
SD	23.8	35.8	35.8	0.4
F test	P=0.172		P=0.0001**	
Student-t*	P=0.811			
Wilcoxon			P=0.109	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.144			

*Paired Student-t test * P<0.05, ** P<0.01

Table 12 Number of Vibrio parahaemolyticus on cutting board 2. and statistical processing result

	No alcohol treatment		Alcohol treatment	
For vegetables	Before washing	After washing	After washing	After alcohol
1	192	31	31	0
2	40	0	0	0
3	0	0	0	0
4	0	40	40	2
5	0	100	100	1
6	28	3	3	0
Average value	43.3	29.0	29.0	0.5
SD	74.8	38.8	38.8	0.8
F test	P=-0.067		P=0.0001**	
Student-t*	P=0.706			
Wilcoxon			P=0.068	
F test	P=0.0001**			
Student-t*				
Wilcoxon	P=0.225			

*Paired Student-t test * P<0.05, ** P<0.01

IV. Discussion

On cutting boards for vegetables, hygiene tests performed on the ATP value and the number of microorganisms. For the microbiological test, a selective medium of general bacteria, Escherichia coli, Staphylococcus aureus, Salmonella, and Vibrio parahaemolyticus used. The ATP level and the number of microorganisms decreased after washing as compared with after cooking. Furthermore, after alcohol spraying, the ATP level, the number of E. Coli, and the number of Salmonella bacteria decreased statistically significantly. However, the bacteria did not disappear. Microorganisms are more likely to grow if they are moist, at the right temperature, and hove nutrients. If the cutting board is left unattended after cooking, it may be necessary to wash repeatedly and spray it with alcohol before use. The ATP test can show invisible

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microorganisms on the spot with visible numbers[1,2,3,4]. Therefore, it is used in many places and is useful for hygiene education and food poisoning prevention[5,6,7,8]. Although it takes time, it is useful for hygiene education to know the condition of food poisoning bacteria by conduction microbiological tests.

V. Conclusions

As a result of the ATP test and microbiological test performed on the cutting board for vegetables, there are surviving bacteria that even after spraying 70% alcohol, so spray 70% alcohol firmly, and the cutting board left for a while is washed repeatedly and sprayed with alcohol before cooking. We think it's better to use it.

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A New Medical Device for Platelet Rich Plasma Filler

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Introduction- Injectable fillers are used in aesthetic medicine to reduce visible signs of facial aging.

It involves loss of volume in the skin, muscle and superficial and deep fat compartments, superficial wrinkles and deep folds (1-2).

There are different products that vary on filling capacity, longevity, potentiality for causing allergic reaction, safety, indication.

Physicians select the most suitable agent for each patient by considering the advantage and disadvantage.

In fact, hyaluronic acid (HA) has the property to increase the skin's water-binding capacity (3-5).

Calcium hydroxylapatite (6) and poly-L-lactic acid (7) have been proved to stimulate autologous collagen (8-9).

Keywords: Growth factors, PRP, regenerative medicine, face wrinkles, perioral wrinkles, dermal matrix.

GJMR-K Classification: NLMC Code: WH 300



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A New Medical Device for Platelet Rich Plasma Filler

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I. Introduction

Injectable fillers are used in aesthetic medicine to reduce visible signs of facial aging.

It involves loss of volume in the skin, muscle and superficial and deep fat compartments, superficial wrinkles and deep folds (1-2).

There are different products that vary on filling capacity, longevity, potentiality for causing allergic reaction, safety, indication.

Physicians select the most suitable agent for each patient by considering the advantage and disadvantage.

In fact, hyaluronic acid (HA) has the property to increase the skin's water-binding capacity (3-5).

Calcium hydroxylapatite (6) and poly-L-lactic acid (7) have been proved to stimulate autologous collagen (8-9).

Although fillers have lower immunogenicity and a good safety profile, several scientific articles have showed side effects (10).

Those can vary from allergic reaction (11), inflammatory nodule formation (12), infection (13), vision loss (14) and the limited use in human immunodeficiency (15).

Platelet rich plasma (Prp) has proved to achieve regenerative capacity on human tissues (16) and positive effect on facial dermal fibroblasts (17-18).

Autologous plasma filler was first introduced by Krajcik et al. in 1999 (19) and used for the treatment of different types and grades of facial wrinkles (20-22).

The main issues of the clinical use of plasma filler is its poor filling effect and duration.

In fact, the volume achieved just after the injection lasts only few hours because the plasma is reabsorbed.

The goal of our study was the preparation of a new medical filler device able to fill soft tissues for a reasonable period of time and also containing the regenerative Prp property.

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Our first step was the formulation of a thermosensitive gelableto embed Prp, the second to evaluate the behavior of the formulation alone, in particular, its dispersibility and the homogeneity and the third the study of platelets and the growth factors behavior inside the formulation.

After a detailed research on the scientific literature (23,24) poloxamer 407 was chosen for our purpose.

Multiple reasons have corroborated this choice: first of all, the family of "poloxamer" polymers is listed in the US and European Pharmacopoeia (25) and is approved by FDA for parenteral use in humans and this ensure safety, biocompatibility and tolerability required for parenchymal use.

Furthermore, poloxamer 407, being a non-ionic block copolymer, does not negatively interact with the biomolecules embed in the gel, such as platelets and growth factors.

Again, it allows to develop thermosensitive formulations without the need of excipients in preparations.

II. Materials and Methods

1. *Preparation of poloxamer formulation*

Aqueous solutions of poloxamer 407, purchased from Sigma-Aldrich (Milano, Italy), were prepared by the so-called "cold method".

Poloxamer powder was dissolved in bidistillate water at 277.15 K (4°C) under gentle stirring to facilitate the copolymer dissolution (26).

Solutions at poloxamer concentration of 15% (w/v) were prepared with this method and stored at 277.15 K (4 °C).

Differential scanning calorimetry (DSC) was used to determine the micellization and the gelation point.

Measurements were performed using a calorimeter Mettler 821^e (Mettler-Toledo, Greifensee, Switzerland) equipped with a cooling system with liquid nitrogen.

Scans were recorded from 273.15 (0 °C) to 313.15 K (40 °C) at a heating rate of 5 K/min (27).

Measurements were performed in duplicate and the results were expressed as the mean of the two measurements.

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2. Analysis of the Platelet

The analysis of the platelet contained inside the formulation could not be address by normal hemochrome automatic analysis systems.

For this reason, platelet numbers were firstly estimated by using a Burker's chamber and the Breker – Cronkite method.

Observations were performed with a Nikon Eclipse 80i microscope provided of a digital camera Nikon Coolpix 8400.

Breker – Cronkite solution was prepared adding 0,01 g of brilliant cresyl blue to 100 mL of 1% (w/v) ammonium oxalate aqueous solution.

Medical Device with Prp sample was diluted with Breker – Cronkite solution (dilution 1/100) in a Thoma pipette.

After five minutes of pipette manual shaking, a drop of diluted solution was charged in the Burker's chamber and observed at microscope with magnification 40X.

We counted platelets presents in a big square of 1 mm^2 (Figure 1).

To obtain the number of platelets within 1 μL of PRP, the average of platelets counts in three squares was corrected for the initial dilution (100) and for the height of chamber (10) (28).

For this reason, the final formula is: Platelet number counted X 1000.

Prp platelet size and number were also determined using an AccusizerTM 770 Optical Particle Sizer (PSS Inc., Santa Barbara, CA, USA), using the technology "single particle optical counting", coupled with an auto-dilution system AccusizerTM 770A Autodiluter^{PAT} (PSS Inc., Santa Barbara, CA, USA).

100 μL of solution with Prp were injected in the system and measurements were performed for 30 minutes to allow the analysis of all the particles present in the sample.

Saline (0.9% NaCl) was used for the analysis and results were expressed as total particles size and mean volume diameter (Figure 2).

3. HPLC analysis of growth factors

We analyzed the Epidermal Growth Factor (EGF) which is centrally involved in the regulation of key processes of the epithelia.

Calibration curve for RP-UHPLC analysis was obtained using an HPLC Jasco LC-2000 plus, equipped with the quaternary gradient pump PU 2089 Plus, the UV-vis detector diode array MD 2010 plus and injector Rheodyne.

Controlled EGF was purchased from Sigma-Aldrich (Milano, Italy).

The column used was a C18 Ascentis Express Peptide 150 × 4.6 mm (L × I.D.), 2.7 μm and analysis were made combining two different mobile phases: H_2O/CH_3CN (95/5 %) + 0.1 % TFA (solvent A) and CH_3CN/H_2O (95/5 %) + 0.1 % TFA (solvent B), with a flow of 2.0 mL/min (29).

The mobile phases were degassed prior to use; cell and column temperature at 313.15 K (40 °C).

The calibration curve was performed in the concentration range of 0.086-0.4 mg/mL, with an injection volume of 5 μL.

4. Rheological analysis

Poloxamer rheological characterization was performed with the aim of better understanding the thermal behavior of poloxamer formulation.

A Stresstech HR Rheometer (Rheologia Instruments AB, Milano, Italy), equipped with a Peltier device for temperature control, was used for the rheological characterization.

Viscosity was measured with a cone-plate geometry (cone angle 1°, cone-plate diameter 40 mm).

Poloxamer formulation was analyzed in duplicate at different temperature (278, 283, 288, 293, 298, 303 and 308 K corresponding to 5, 10, 15, 20, 25, 30 and 35 °C).

For formulation in sol state, measurements were performed applying a stress range from $3.044 \cdot 10^{-3}$ to 1 Pa, while for temperatures at which the formulations were already at the gel state, measurements were carried out applying a stress range from $3.044 \cdot 10^{-3}$ to 10 Pa. 12.

5. Spreadability

The gel was tested by two independent persons for the spreadability.

The gels stored at 277.15 K (4 °C) were withdraw from the recipients and spread on the skin surface.

6. Preparation of Prp

For the preparation of the Prp, Plasma Active system (CE0373 – Medical Device s.r.l. via artigianato n.6, 52022 Meleto, Craviglia (AR) – Italy) was used.

It is composed of two vacutainer tubes (*BD Vacutainer® Brand SST II)* of 9 mm each with separator gel and anti-coagulant for harvesting 18 ml of peripheral blood.

Tubes were centrifugated for 5 minutes at 1800 rpm (*Sorvall Legend XTR Centrifuge - Thermo Fisher Scientific Robert-Bosch-Straße 1 D - 63505 Langenselbold Germany*) and 8 ml of Prp were collected from the upper part of the tube in a sterile matter and placed inside the Medical Device.

7. Preparation of poloxamer containing PRP

Five mL of Prp were added to 10 mL of thermosensitive gel.

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The ratio of Prp/gel was chosen by considering the future application of the thermosensitive gel and the size of the final device (30).

Results have shown that 5 mL of Prp could be easily dispersed within 10 mL of the hydrogel by stirring the cold gel (4 °C) or by agitation of the closed container.

III. Result

1. Differential scanning calorimetry (DSC) results

DSC data show broad enthalpic transitions in the formulation due to poloxamer micellization.

In fact, in aqueous solution, with increasing temperature, poloxamer aggregates in micelles to minimize the free energy of solution.

Micellization temperatures decrease with increasing poloxamer concentration (Figure 3).

Also, micellization energy transition (ΔH_{mic}) per amount of poloxamer increases with increasing concentration.

Data of micellization temperature and ΔH_{mic} of poloxamer preparation is reported (Table 1).

Very interesting, in DSC thermograms is possible to see also a little enthalpic transition at temperature slightly higher than micellization point.

This peak is more evident in solutions with higher poloxamer concentrations and it has been previously attributed to the gelation transition (Figure 4).

In agreement with this little peak, gelation energy (ΔH_{gel}) is very small (Table 2).

Gel temperatures found from this approach agree with results reported in the literature (27).

This DSC analysis give a first information about poloxamer solution thermal behavior.

In fact, the decrease of micellization and gelation temperature with increasing poloxamer concentration clearly shows how formulations with high poloxamer concentration (e.g. 25-30% w/v) pass to gel state at lower temperatures compared to the formulations with a low poloxamer concentration.

Micellization and gelation temperatures are plotted in Figure 5.

These results demonstrate that gelation point is strongly temperature and concentration dependent.

2. Rheological analysis result

In Figure 6 is shown the behavior of poloxamer formulation viscosities as a function of temperature.

In the first part of curves, solution viscosity decreases slightly on warming but, reached a certain temperature, is possible to see a steep increase in viscosity.

This viscosity increase can be attributed to the sol-to-gel transition.

Very interesting, results obtained with rheological characterization agree with gel temperatures obtained from the DSC data (Figure 3).

3. Spreadability results

The best spreading was individuated for the formulation containing 15% of poloxamer that did not gelify immediately after contact with the skin.

However, once the gel was spread it remained on surface as a thick gel.

4. Result of poloxamer containing PRP

Prp dispersed homogeneously into the matrix without forming lipid globules but changing the appearance of the gel from completely transparent to a light-yellow color (Figure 7A).

The formulation pH did not change with respect to the control formulation (blank gel).

The smell of the formulation containing PRP was slightly different from the blank gel, but it was still pleasant.

Prp loaded thermosensitive gel was stored at 4 °C for 1 month and its characteristics were evaluated 7 and 30 days after preparation.

After 7 days of storage, the gel color and smell were unchanged and, more important, Prp was still homogeneously dispersed (Figure 7B).

After 1 month of storage, the smell was unchanged while a slight phase separation was observed (Figure 7C).

This phase separation was reversible by simpler agitation.

In fact, by gently shaking the closed container, the gel assumed the same aspect and homogeneity of a fresh prepared formulation (Figure 7D).

This finding is of particular interest for the final use and we store the formulation at: 2-8 °C andshake if before use.

5. Platelet behaviour

We analyzed 10 different samples of solution of poloxamer containing PRP after 12, 23, 73, 96, 120, 144, 168 hours from the preparation and expressed the results as mean.

Platelet remained intact for 72 hours and then numbers dropped rapidly as shown on Figure 8.

6. HPLC results of growth factors

Analysis made on the 10 different samples of solution of poloxamer containing Prp after 12, 23, 73, 96, 120, 144, 168 hours from the preparation showed that the EGF concentration increased progressively and reached his peak after 4 days and then decreased progressively.

The Egf concentration remained above 2,5 μg/mL after 7 days (Figure 9).

IV. Discussion

Fillers are among the most performed cosmetic medicine procedures worldwide (31-32).

They are used mostly on the face for the reduction of superficial and deep wrinkles (32-34), for

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increasing the volumes of lips (35) and cheekbones (36) and for the redefinition of the mandibular profile (37).

There are different materials used for fillers including hyaluronic acid (38), collagen (39), calcium hydroxyapatite (40-41), and polycaprolactone (42), polymethylmethacrylate (43).

They vary depending on the filling capacity, due to their ability of recalling water (44), or even for the ability to stimulate collagen (45).

The advantages of commercial fillers are the simplicity of use, as they are supplied in single-use vials and the very low incidence of major complications as embolisms (46-48).

The disadvantages are the high cost, since to obtain appreciable results, several vials are required, and because of the limited duration, the treatment must be repeated periodically (49-50).

Again, possible side effects, although minor, create discomfort to the patients and stress to the doctors (51).

And definitely, fillers do not have regenerative activities.

Prp is used in dermatological clinic for its regeneration effects on dermal cells (17).

Different studies have already shown the positive effects on the treatment of facial wrinkles.

"Plasma filler" has always meant the simple injection of Prp into subcutaneous tissues (18, 20, 21).

But the main limitation of plasma fillers is the fact that it does not generate filling effects, if not for a few hours.

For this reason, we set up a new filler device able to combine volumetric and regenerative effects.

We faced different problems: first of all, the Prp is a fluid and to be well mixed inside the new filler device it is necessary that this is also in the liquid state.

Moreover, once injected into the tissues, this liquid would have to become a gel to guarantee the volumetric effect.

Furthermore, since both the platelets and the Prp have a low half-life outside the plasma, we had to guarantee the new filler device a chemical and physical composition that allows the survival of platelet and growth factors for several days.

So, we have chosen a polymer (poloxamer 407) that contains all these characteristics (23-24).

In fact, at low temperatures (2-8 C °) it is found in the liquid phase and this allows the simple mixing of the Prp inside it.

When the temperature reaches 32-37 ° C, the liquid passes to the gel state.

This ensures either the volumizing effect and the progressive release of growth factors within the tissues.

We performed precise measurements on both the number of platelets and EGF present in the new filler device at regular time intervals for 7 days.

EGF is known to be a potent stimulator of cell proliferation of various cells including keratinocytes, fibroblasts and vascular endothelial cells.

EGF stimulates the migration of keratinocytes and also stimulates fibroblasts and endothelial cells to promote the formation of granulation tissue (52-54).

These measurements were also difficult because platelets and the EGF could not be measured with the common automatic measurement systems.

The results found were encouraging for a clinical point.

In fact, the platelets were maintained vitality until 72 hours and then their number felled by freeing the granules containing the growth factors.

EGF, progressively increased its concentration with a peak after 72-96 hours and then slowly decreased until 7 days.

This new filler device could open new scenarios in facial rejuvenation and revolutionize the treatment of face wrinkles.

In fact, the advantages are evident: it would have volumizing and regenerative capacities.

This would allow wrinkles and grooves to be filled and regenerated at each application.

Furthermore, being autologous growth factors, there would be no side effects related to allergic reactions or inflammatory nodules.

Again, since the immune system is also present inside the plasma, this would help prevent the infections.

Finally, the cost of treatment would be cheaper than normal commercial filler, since for each session of treatment 8 ml of Prp are added to 16 ml of hydrogel to obtain a 24 ml filler.

The disadvantage lies on the Prp preparation.

V. Conclusions

Our study has shown that it is possible to obtain a new type of filler able to have both filling capacity, due to the gelling effect of the material used, and a regenerative effect due to the presence of Prp.

Therefore, new clinical trials will be necessary to assess the duration of the volumizing effect in the different areas of the face.

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Table 1: DSC data of micellization transition of poloxamer preparation

Poloxamer concentration (% w/v)	Micellization onset (K)			Micellization peak (K)			ΔH mic (-J/g)		
	Sample 1	Sample 2	Mean ± D.S.	Sample 1	Sample 2	Mean ± D.S.	Sample 1	Sample 2	Mean ± D.S.
15	287.02	286.22	286.62 ± 0.57	289.68	288.65	289.17 ± 0.73	3.58	3.79	3.68 ± 0.15

Table 2: DSC data of gelation transition of various poloxamer preparations

Poloxamer concentration (% w/v)	Gelation onset (K)			Gelation peak (K)			ΔH gel (-J/g)		
	Sample 1	Sample 2	Mean ± D.S.	Sample 1	Sample 2	Mean ± D.S.	Sample 1	Sample 2	Mean ± D.S.
15	295.95	-	295,95	296.36	-	296,36	0.00015998	-	0.00016

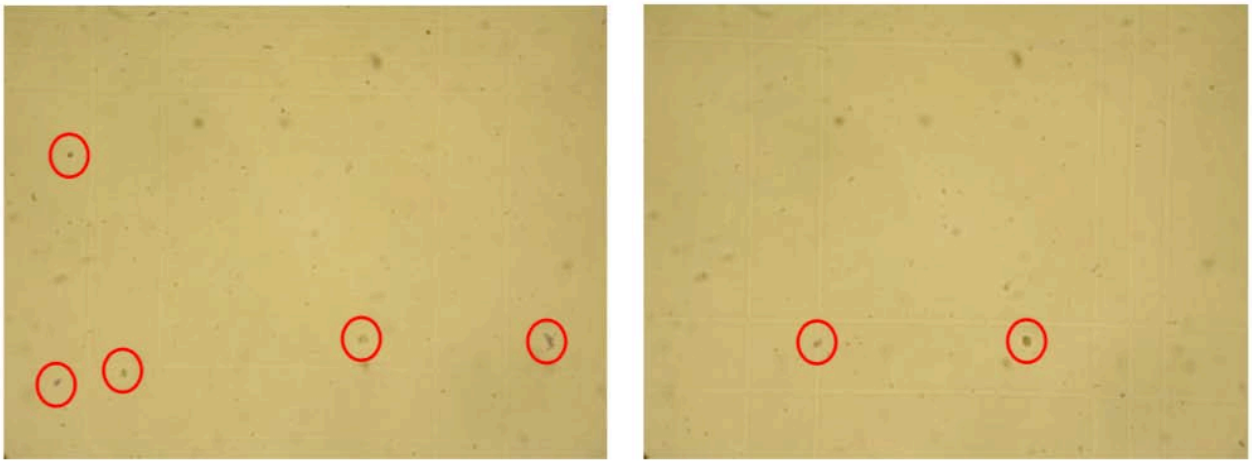


Figure 1: A schematic representation of Burker's chamber

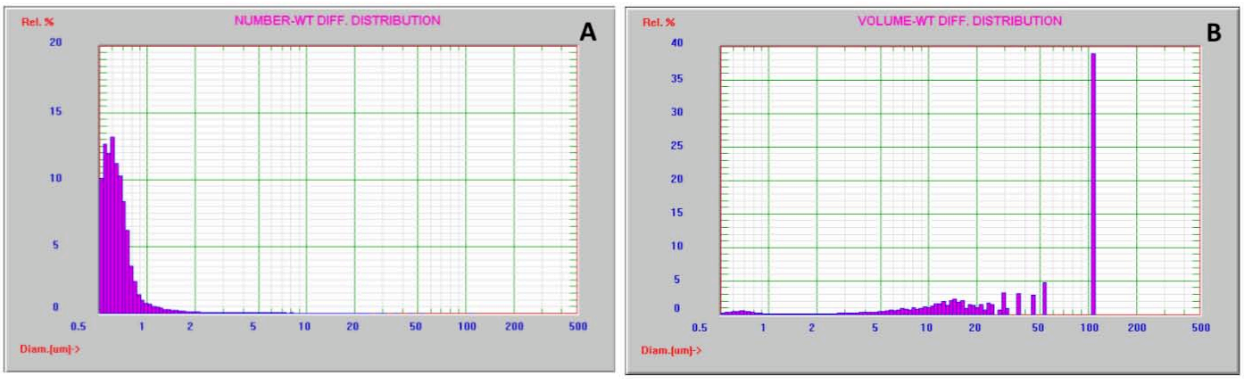


Figure 2: Optical Particle sizer.

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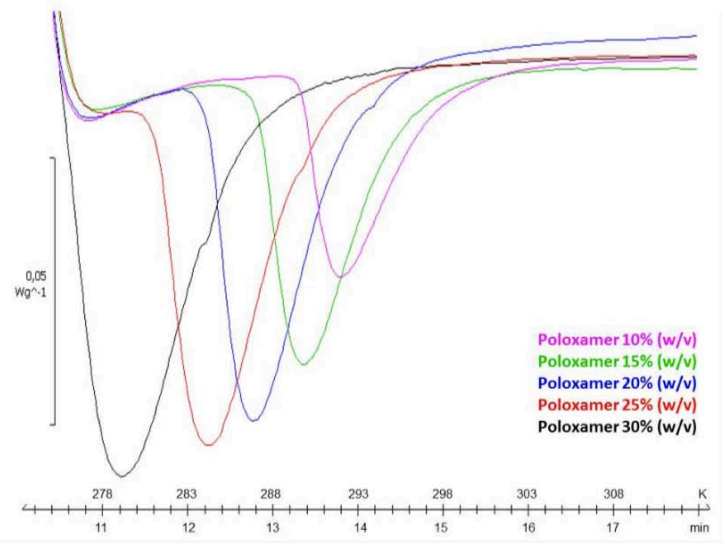


Figure 3: DSC thermograms of different poloxamer concentration samples. Micellization temperature (represented by the enthalpicpeak) decreases with increasing poloxamer concentration

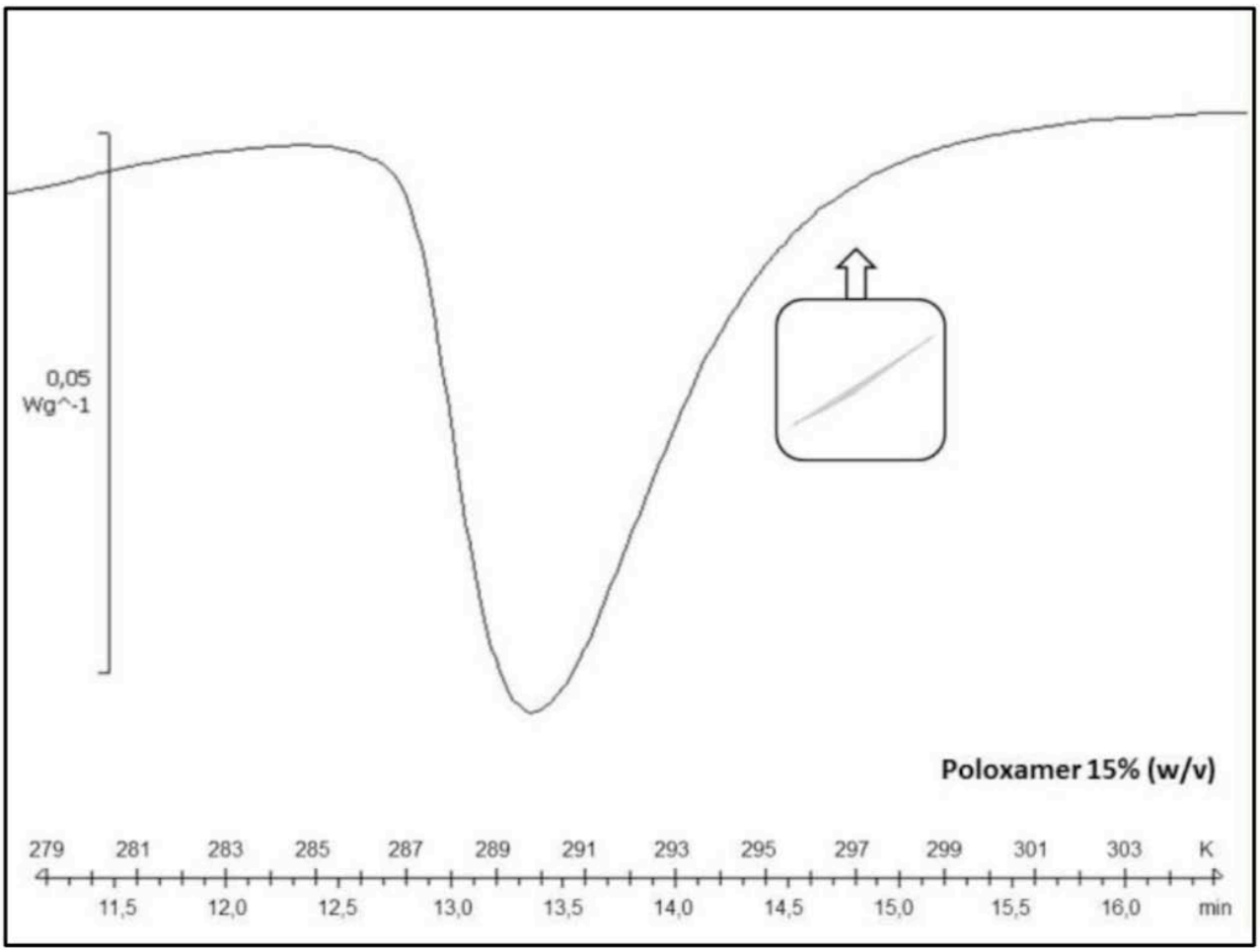


Figure 4: DSC thermogram of poloxamer concentration sample. In the pane there is an enlargement of gelation transition

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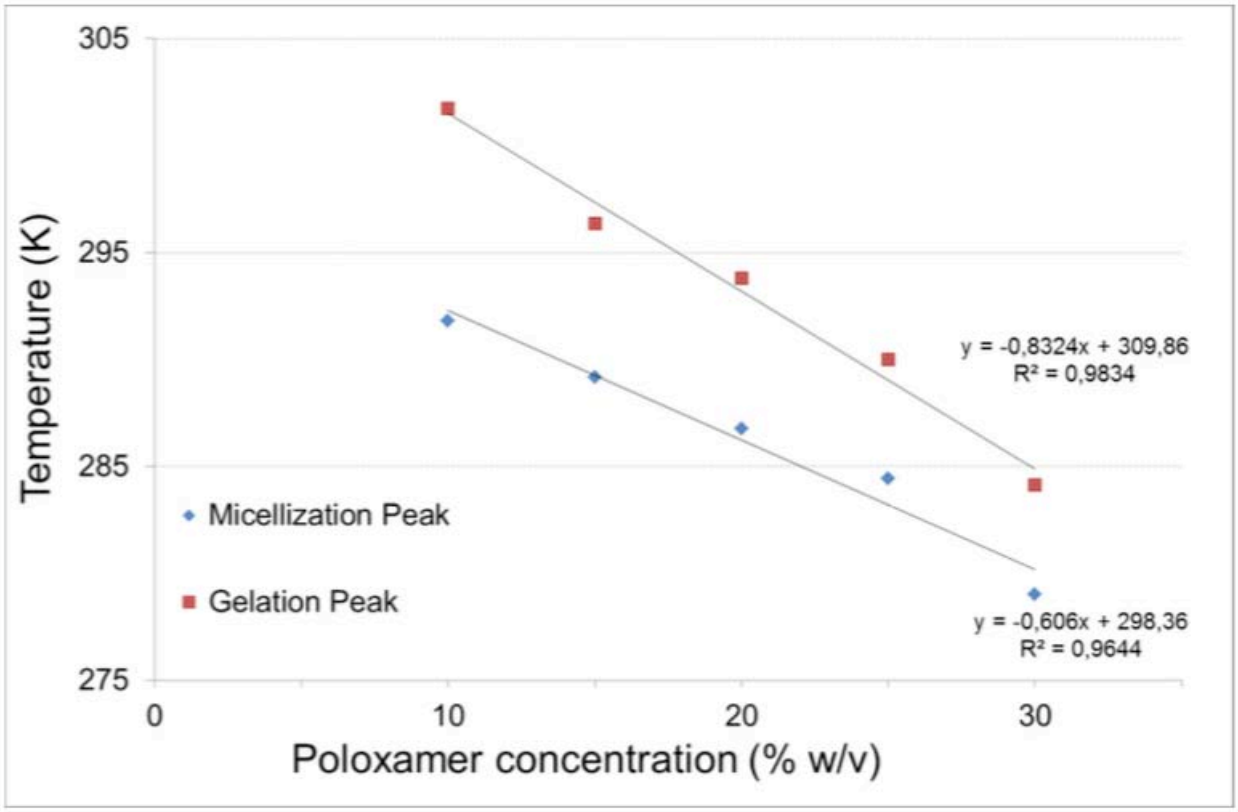


Figure 5: Micellization and gelation temperatures at different poloxamer concentrations

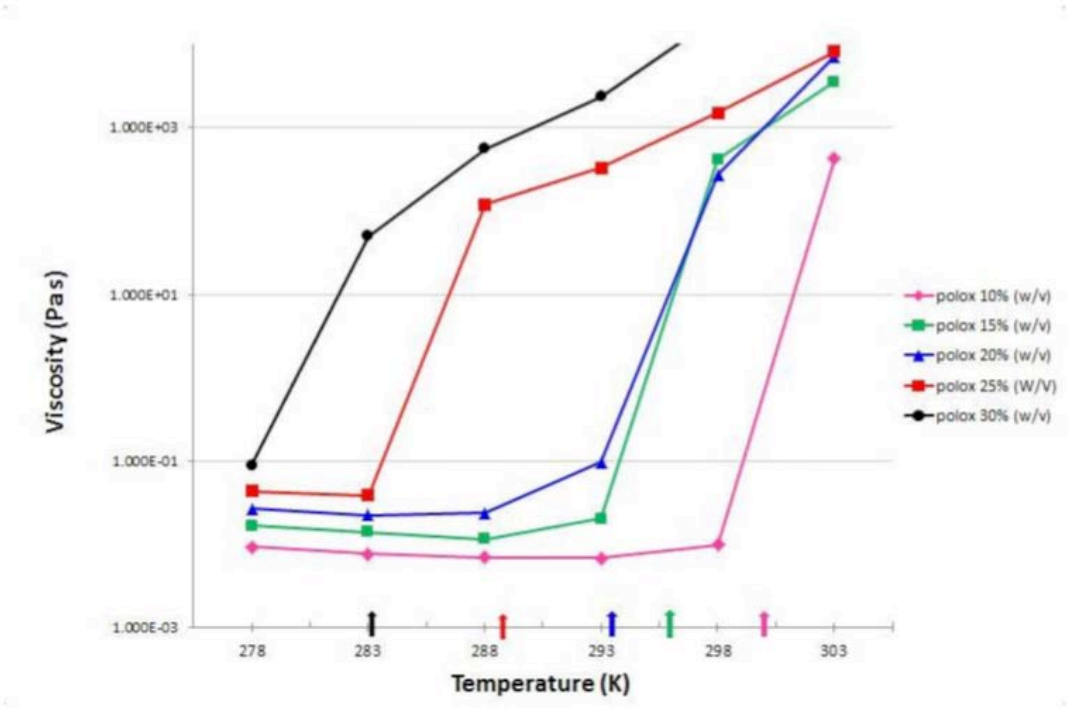


Figure 6: Trend of poloxamer formulation viscosity as a function of temperature. The arrows in the x-axis indicate the gel temperatures obtained from DSC measurements

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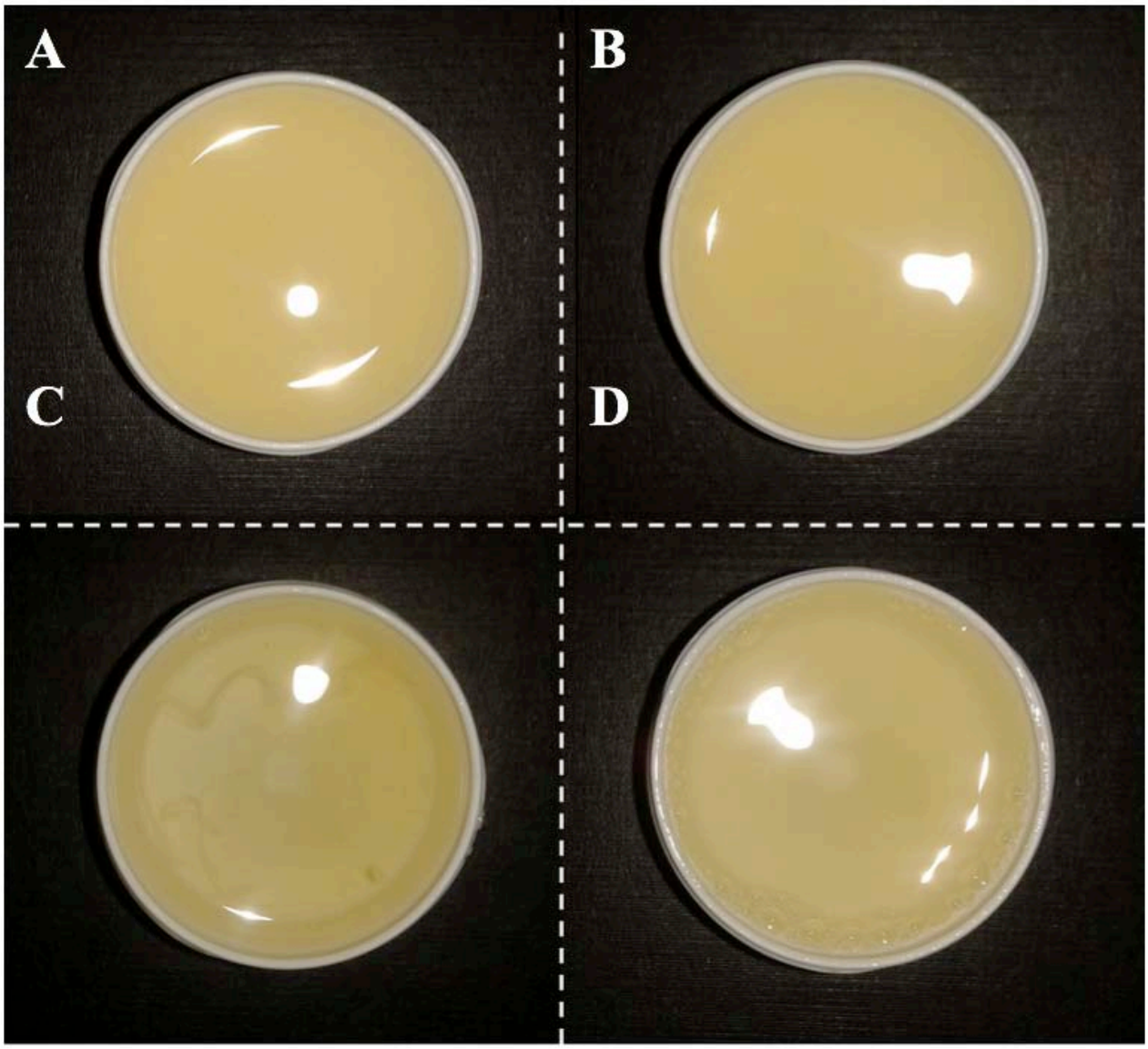


Figure 7: Thermosensitive gel containing PRP fresh prepared (A) and after 7 days from preparation (B). Formulation after 1 month of storage at ~ 4 °C before (C) and after (D) manual shaking

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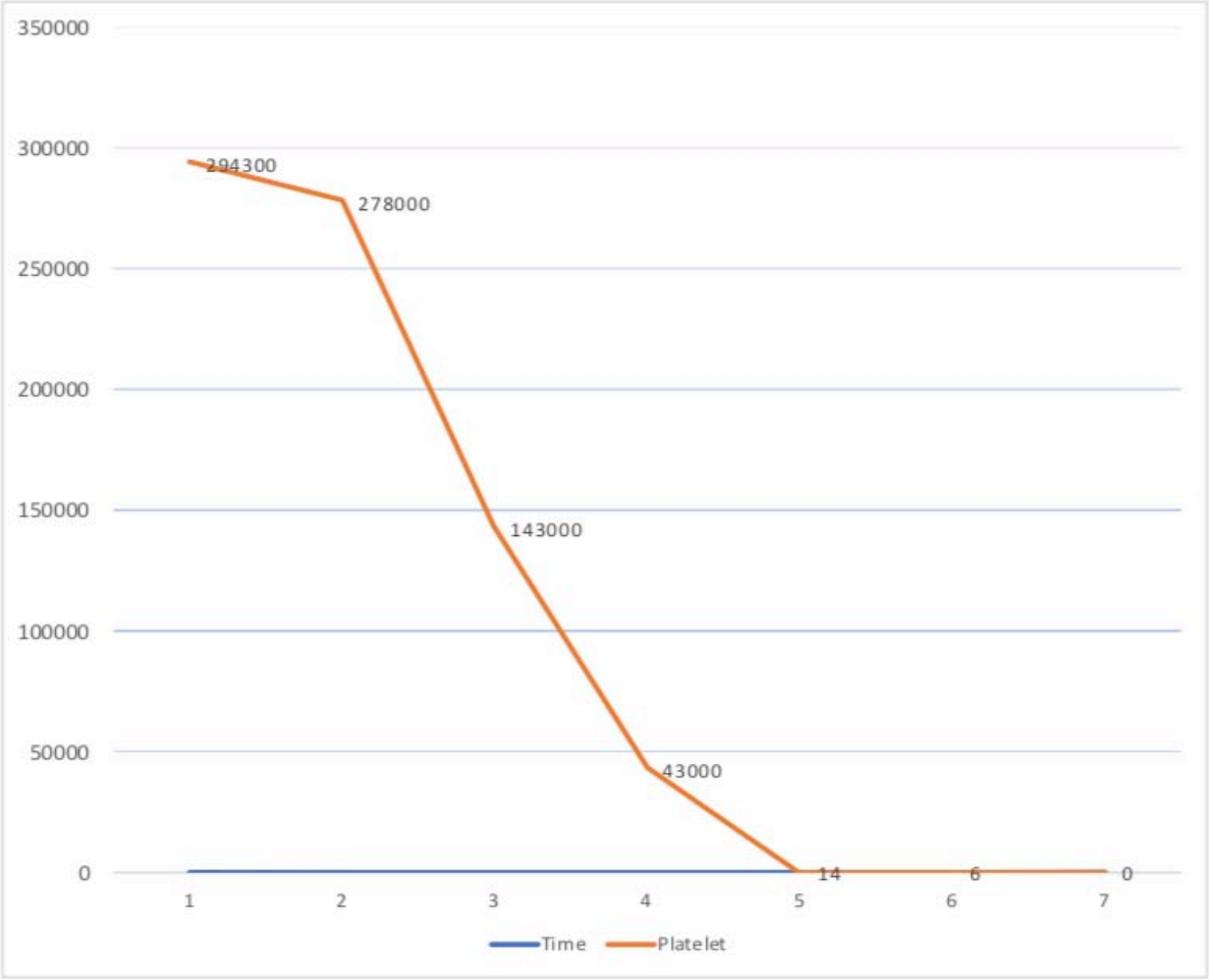


Figure 8: Platelet behaviour on preparation of poloxamer containing PRP

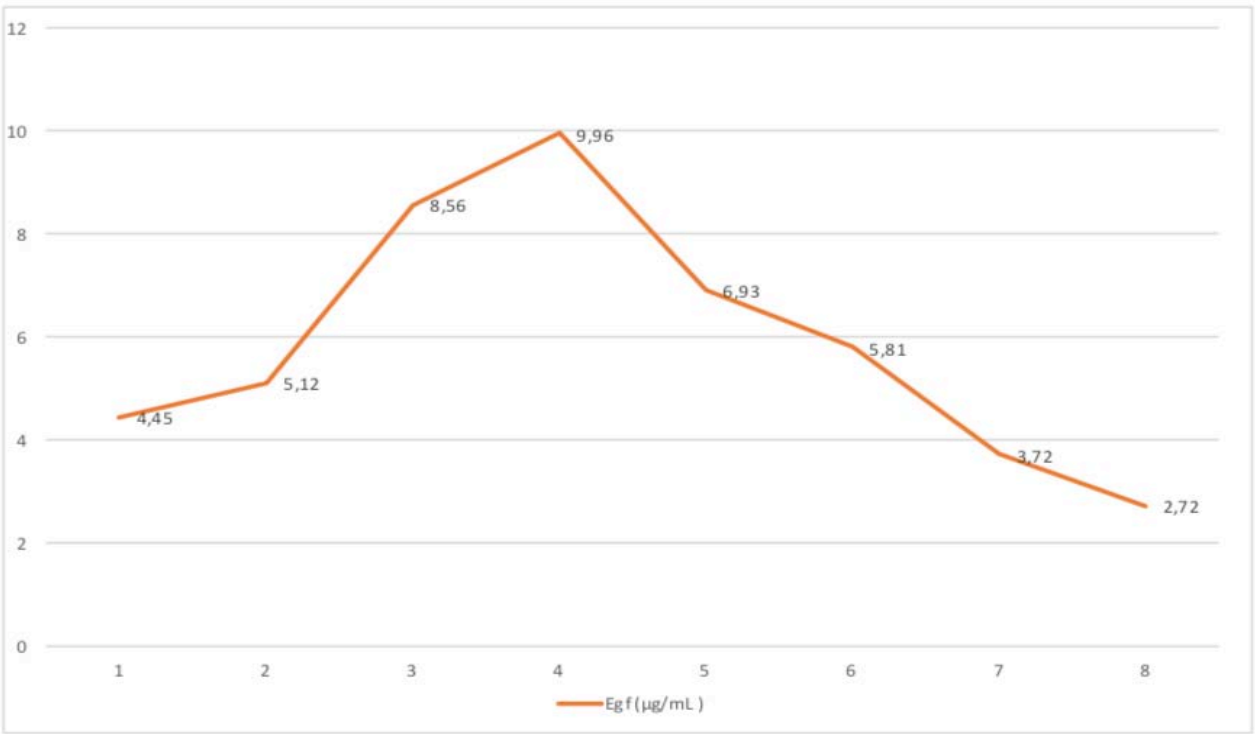


Figure 9: Egf behaviour on preparation of poloxamer containing PRP

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Deep Learning for Classification of Sleep EEG Data during the Epidemic of Coronavirus Disease

By Mingzhe E, Jinming Cao & Bin Zhao
Hubei University of Technology

Abstract- Sleep is an important part of the body's recuperation and energy accumulation, and the quality of sleep also has a significant impact on people's physical and mental state during the epidemic of Coronavirus Disease. It has attracted increasing attention on how to improve the quality of sleep and reduce the impact of sleep-related diseases on health during the Epidemic of Coronavirus Disease.

The electroencephalogram (EEG) signals collected during sleep belong to spontaneous EEG signals. Spontaneous sleep EEG signals can reflect the body's changes, which is also an basis for diagnosis and treatment of related diseases.

Therefore, the establishment of an effective model for classifying sleep EEG signals is an important auxiliary tool for evaluating sleep quality, diagnosing and treating sleep-related diseases.

Keywords: Sleep EEG; deep learning; softmax function; adam algorithm; multiple classifications problem.

GJMR-K Classification: NLMC Code: WC 532



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Deep Learning for Classification of Sleep EEG Data during the Epidemic of Coronavirus Disease

Mingzhe E [α], Jinming Cao [σ] & Bin Zhao [ρ]

Abstract- Sleep is an important part of the body's recuperation and energy accumulation, and the quality of sleep also has a significant impact on people's physical and mental state during the epidemic of Coronavirus Disease. It has attracted increasing attention on how to improve the quality of sleep and reduce the impact of sleep-related diseases on health during the Epidemic of Coronavirus Disease.

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Therefore, the establishment of an effective model for classifying sleep EEG signals is an important auxiliary tool for evaluating sleep quality, diagnosing and treating sleep-related diseases.

In this paper, outliers of each kind of original data were detected and deleted by using the principle of 3 Sigma and k-means clustering + Euclidean distance detection method. Then, using the Adam algorithm with adaptive learning rate constructs the Softmax multi-classification BP neural network the model, and relatively high accuracy and AUC values were finally obtained during the Epidemic of Coronavirus Disease.

Keywords: Sleep EEG; deep learning; softmax function; adam algorithm; multiple classifications problem.

I. Introduction

The sleep process is a complex process of dynamic changes. According to R&K, the international standard for the interpretation of sleep stages, there are different states during sleep.

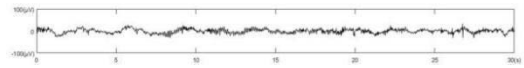
In addition to the awake period, the sleep cycle consists of two alternate sleep states, namely rapid eye movement(REM), and non-REM.

In non-REM, according to the gradual change of sleep state from shallow to deep, it is further divided into sleep, I, II, III, and sleep IV. Sleep stage III and sleep stage IV can be combined with a deep sleep stage.

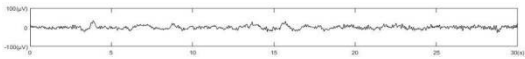
Author α ρ: School of Science, Hubei University of Technology, Wuhan, Hubei, China.
Author σ: School of Information and Mathematics, Yangtze University, Jingzhou, Hubei, China.
Corresponding Author ρ: School of Science, Hubei University of Technology, Wuhan, Hubei, China. e-mail: zhaobin@hbut.edu.cn

Figure 1 shows the time series of EEG signals corresponding to different sleep stages, from top to bottom, namely, wakefulness, sleep I, sleep II, deep sleep and REM.

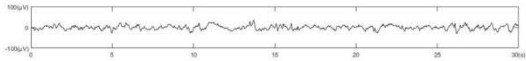
Figure 1 shows the characteristics of EEG signals vary in different sleep stages during the epidemic of Coronavirus Disease.



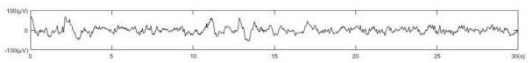
(a) Stage wake.



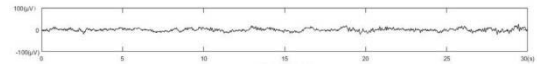
(b) Sleep stage I.



(c) Sleep stage II.



(d) Deep sleep stage.



(e) Rapid eye movement.

Figure 1: The sequence of sleep EEG signals in sleep stages.

Automatic staging based on EEG signals can reduce the artificial burden on expert physicians and it is a useful auxiliary tool for assessing sleep quality, diagnosing and treating sleep-related diseases. In this paper, Python is used to build a neural network, and design a sleep staging prediction model. Based on as few training samples as possible, it can obtain relatively high prediction accuracy.

II. Overview of BP Neural Network

An artificial neural network gets widely used in some aspects, including pattern recognition, function approximation, data compression, data classification, data prediction, etc. [1-6] BP neural the network is an

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algorithm in ANN. Figure 2 shows the basic structure of the BP neural network.

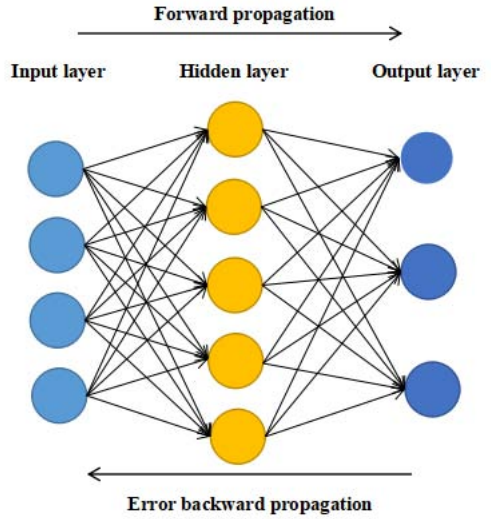


Figure 2: Basic structure diagram of BP neural network.

Introduction to activation function and algorithm:
ReLU function:

$$\mathrm{ReLU} = \max(0, x)$$

Softmax function:

$$S_i = \frac{e^i}{\sum_j e^j}$$

Figure 3 shows the operating principle.

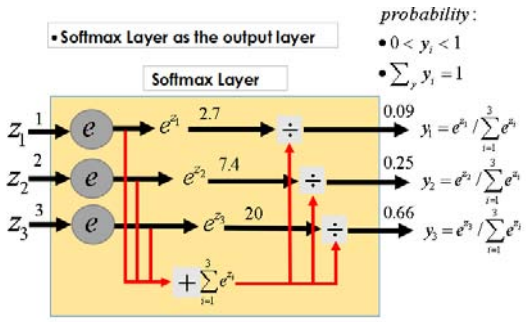


Figure 3: Principle of Softmax function.

Adam algorithm:

$m_0 v_0 t \leftarrow 0$ Initialize 1st, 2st moment vedor and time step do while:

$$t \leftarrow t+1$$

$$g_t \leftarrow \nabla_\theta f(\theta_{t-1})$$

Computing the gradient.

$$m_t \leftarrow \beta_1 \cdot m_{t-1} + (1-\beta_1) \cdot g_t$$

Update biased first moment estimate.

$$v_t \leftarrow \beta_2 \cdot v_{t-1} + (1-\beta_2) \cdot g_t^2$$

Upgrade biased second moment estimate.

$$\hat{m}_t \leftarrow \frac{m_t}{(1-(\beta_1)^t)}$$

Compute bias-corrected first moment estimate.

$$\hat{v}_t \leftarrow \frac{v_t}{(1-(\beta_2)^t)}$$

Compute bias-corrected second draw moment estimate.

$$\theta_t \leftarrow \theta_{t\text{-}1} - \alpha \cdot \frac{\hat{m}_t}{\sqrt{\hat{v}_t} + \varepsilon}$$

Upgrade parameters.

Where α is the step length, β_1, $\beta_2 \in [0,1)$ is the moment estimation of the exponential decay rate, and $f(\theta)$ is the random objective function of the parameter θ.

In this paper, the whole training process of the improved BP neural network model is:

Step 1: Parameter initialization. Determine the node number of the network input layer, hidden layer and output layer, and initialize the weight, bias between each layer, then initialize learning rate.

Step 2: Calculate the output of the hidden layer. The hidden layer output is calculated by the weight and bias between the input vector and the connection layer and the ReLU activation function.

Step 3: Calculate the output of the output layer. through the hidden layer output and connection weights and bias and the Softmax activation function calculate the predicted output.

Step 4: Calculate Softmax cross-entropy as cost function according to predicted output and real label.

Step 5: Back propagation, and this paper use the adaptive learning rate Adam algorithm [7] to update the weight and bias.

Step 6: Determine whether the cost reaches the error range or the number of iterations. If not, return step 2.

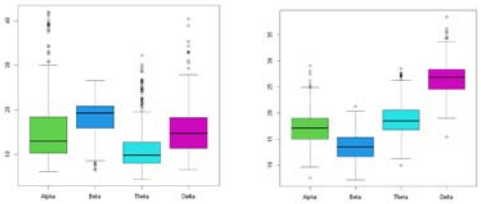
III. Data Description and Preprocessing

Data were collected from 3000 sleep EEG samples and their labels are taken from different healthy adults during overnight sleep. The first is a "known label," which represents the different sleep stages in digital form: stage wake (6), rapid eye movement (5), sleep I (4), sleep II (3), and deep sleep (2); The second to fifth columns are the characteristic parameters

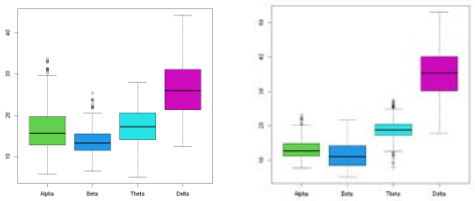
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calculated from the original time sequence, successively including "Alpha", "Beta", "Theta" and "Delta", which correspond to the energy proportion of EEG signals in the frequency range of "8-13Hz", "14-25Hz", "4-7Hz" and "0.5-4Hz" respectively. The unit of characteristic parameters is the percentage.

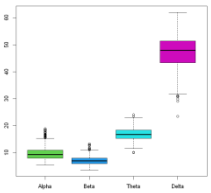
This paper gives raw data stage wake (6), and REM. (5), sleep I (4), sleep II (3), deep sleep (2), four brain electrical signal energy proportion of five sleep stages of brain electrical signal energy proportion, but the original data are generally given there are some abnormal data outliers or missing value, therefore we to each index of the five sets of data make a boxplot graph, the result is as follows in figure 4.



(a) Stage wake. (b) REM.



(c) Sleep I. (d) Sleep II.



(e) Deep sleep.

Figure 4: Boxplot of each sleeping period.

Five sleep period by Figure 4 shows, there are some outliers, namely, these all belong to the original data of abnormal points, this paper uses the principle of 3 sigmas [8] will each table of data deletion, then after the processing of five tables to merge, and then using the K-means clustering + Euclidean distance outlier test [9], to find and remove outliers, as shown in figure 5, a total of 2883 samples after pretreatment.

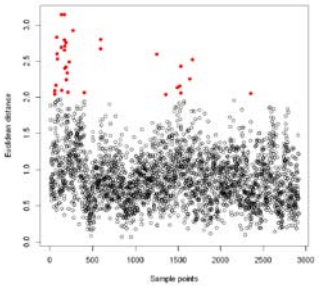


Figure 5: Diagram of outliers.

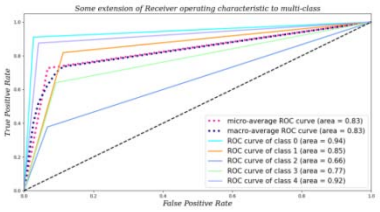
IV. Model Training and Prediction

We divided the data into a training set and test set in a ratio of 2:8. We trained and tested the data using the traditional decision tree model [10] (DT) and support vector machine model (SVM), and compared the classification effect with the accuracy rate and AUC value as evaluation indexes. The results are as follows:

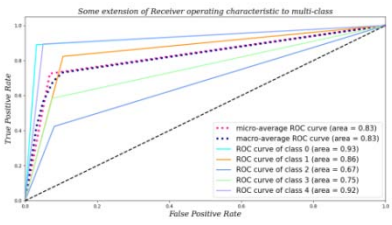
Table 1: Comparison table of several classifications accuracy rates.

Classifier	Accuracy rate
DT	0.59
SVM	0.68
Adam-BPNNet	0.73

As can be seen from Table 1, the accuracy of Adam-BPNNet in several traditional methods is relatively high. Figure 6 shows the ROC curve of each classification method.

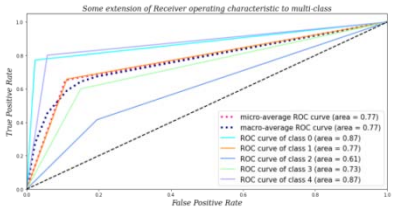


(a) Two-layer BPNNet-ROC.

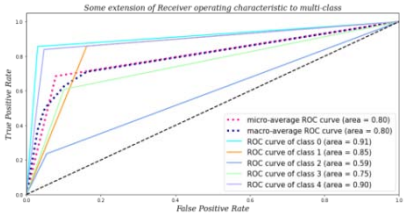


(b) Three-layer BPNNet-ROC.

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(c) DT-ROC.



(d) SVM-ROC.

Figure 6: ROC curve of each classification method.

Table 2: Comparison table of AUC values of different classification models.

Classifier	AUC
DT	0.77
SVM	0.80
Adam-Bennett	0.83

Table 2 shows that in the Adam-BPNNet model, fewer training sets will still have a better classification effect.

The prediction result is the best classification effect obtained after many experiments. In the early stage of experiment, the classification accuracy is low. After repeated debugging of the number of hidden layers and nodes, the best AUC value of this experiment is 0.83.

V. Conclusion

This study is mainly based on theoretical research and combines theory with practice. This paper uses BP neural network based on an adaptive learning rate Adam algorithm for data classification. Also, this paper selects Softmax as the activation function in the output layer, enabling the model to have good self-learning and self-adaptive ability. The most important thing is that the network has good generalization ability. When designing the classifier, it should consider whether the network can correctly classify the objects it needs to classify, and whether the network can correctly classify the unseen or noise-polluted patterns after training. The classification AUC value of this study is 0.83, which is scientific to a certain extent and can be used as auxiliary tool for the evaluation of sleep quality, diagnosis and treatment of sleep-related diseases.

Conflict of Interest

We have no conflict of interests to disclose and the manuscript has been read and approved by all named authors.

Acknowledgement

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Hidradenitis Suppurativa- The Imaging Spectrum

By Vasundhara Singh, Chitrangada Singh & Sharmila Patil

Norfolk and Norwich University

Abstract- This was a retrospective study including 10 cases of histologically proven Hidradenitis suppurativa to compare the Histological features with MRI findings and eventually derive a clinical classification including both parameters.

Keywords: *hidradenitis, MRI, follicular hyperkeratosis, sinus tract.*

GJMR-K Classification: *NLMC Code: WR 430*



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Hidradenitis Suppurativa- The Imaging Spectrum

Vasundhara Singh [α], Chitrangada Singh [σ] & Sharmila Patil [ρ]

Abstract- This was a retrospective study including 10 cases of histologically proven Hidradenitis suppurativa to compare the Histological features with MRI findings and eventually derive a clinical classification including both parameters.

Keywords: hidradenitis, MRI, follicular hyperkeratosis, sinus tract.

I. Introduction

Also known as Acne inversa or the Verneuil's disease, Hidradenitis suppurtiva is a chronic disease with recurrent abscess formation progressing to sinus tracts and resultant scarring. It was first described by Velpeau in 1839during his study involving the origin of abscess involving the sebaceous follicles in axillae [1]

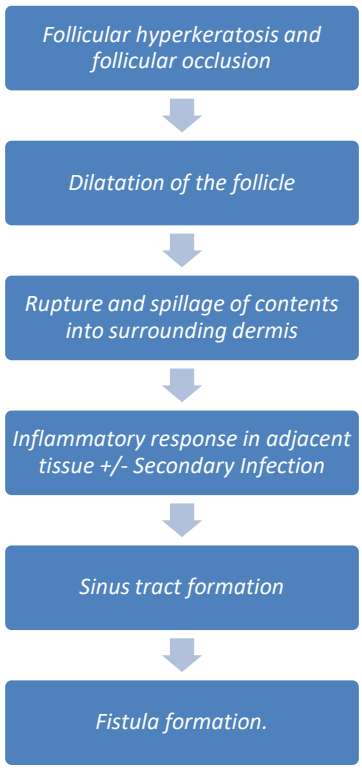
It is commonly seen in females and also incidence involving the axilla in both sexes is nearly equal.

Commonly seen in adolescents, it initially presents like a comedo and progresses with muco-purulent discharge eventually leading to scarring.

II. Epidemiology

- Prevalence: 1 in 300 adults
- Sex predilection: Females > Males
- Onset: Adolescent to middle age
- Family history: Positive family history with an autosomal-dominant mode of inheritance
- Associations: Crohn's disease, Dowling Dego's, Arthropathy (SAPHO), Smoking, Obesity, Hormonal influence [2]

III. Pathophysiology



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Clinical Classification: Hurley stages-3 well delineated stages have been described by Hurley emphasizing the clinical diagnosis.

Stage 1: Solitary or multiple isolated abscesses. No scarring or sinus tracts are seen- resembles an acne.

Stage 2: Recurrent abscess may be single or multiple widely separated lesions. Sinus tract may be present. Patient has significant movement restriction.

Stage 3: Diffuse or broad involvement across a regional area with multiple inter-connected sinus tracts and abscesses. Significant scarring is seen and fistula formation is present.

IV. Imaging

Imaging evaluation is initially indicated to evaluate the extent and the feasibility to obtain image guided aspirate for culture and sensitivity. A simple ultrasound guided aspiration of the involved part can be used to obtain sample for culture and sensitivity if secondary infections are suspected when there is no sinus tract to directly obtain swabs. [3]

MRI is the preferred modality for evaluating the extent of disease as well as follow up.

V. MRI Spectrum

The protocol

The MRI of the involved area is best suited to evaluate the extent of disease and also may help in monitoring treatment response. The MRI findings parallel with the clinical features therefore avoiding any clinical confusion. STIR (Short tau inversion recovery) and T2W sequences are recommended protocol.

It may initially just show thickening of the skin and subcutaneous tissue which soon progresses to induration best seen on routine T2W and STIR images as subdermal hyperintense signal extending upto skin. In a few days there is formation of subcutaneous abscesses however the disease is confined to the skin and subcutaneous tissue. MRI will show loculated T2W and STIR hyperintense pockets of collection with mild post contrast rim enhancement. This correlates to Stage 1 of clinical classification. (Refer figure A)

This may either heal by mild scarring or progress towards chronic skin involvement in the form of multiple raised subdermal pockets of pus which ultimately rupture to form sinus tract. (Refer figure B) This correlates to Stage 2 of clinical classification.

The stage 3 of clinical classification is includes extensive local involvement in terms of area as well as severity and often includes refractory cases with multiple interconnecting sinus tracts. The chronicity can be identified by thick walls of the sinus tract appearing hypointense on STIR due to scarring.(Refer figure C)

Rarely there is fistulous communication with bladder urethra or rectum etc. in patients with highly virulent infection or compromised immune status like diabetes.

Reactive inguinal lymph nodes are also seen in conjunction.

Post treatment cases demonstrate residual scarring as STIR hypointense tracts. (Refer figure D)

VI. Differential Diagnosis

Carbuncles, Lymphadenitis, Infected Bartholin's cyst, sebaceous cysts, Cellulitis/ erysipelas, Lumbo-sacral epidural abscess are few of the common differential diagnosis which can be easily ruled out combining the imaging and the clinical picture. [4]

VII. Sequelae

Various sequel can be strictures, disfiguring edema, arthropathy and chronic cases may undergo metaplasia and even lead to squamous cell carcinoma.

VIII. Treatment

Treatment of HS is directed according to disease severity. Aim is to alleviate symptoms and improve quality of life. Many a times combination therapy is resorted.

a) Anti-inflammatory Agent [5]

- Intralesional steroids: Triamcinolone acetonide 2-5mg/ml can be used for few lesions
- Anakinra: An IL-1 inhibitor (100mg SC/day for 12 weeks) showed reduction in severity of disease
- Antibiotics: Many topical and oral antibiotics like clindamycin(1%;300mg BD), tetracycline, rifampicin(300mg BD); have been used alone or in combination for their anti-inflammatory and immunomodulatory properties.[6] A study of hyperbaric oxygen therapy with antibiotic combination showed good improvement in sartorius and DLQI score.[7]

Antiandrogens: Although anecdotal in females, a double blinded study in women with Cyproterone acetate(100mg) and Ethinyl estradiol(50 micrograms) as per reversed sequential therapy laid down by Hammerstein and Cupceancu, showed reduced discharge and swelling. [8]

Finasteride(5-10 mg/day) used primarily for prostate cancer showed good results in pediatric patients. [9]

b) Retinoids

Isotretinoin worked in patients with mild disease when given in low doses 0.5-1.2 mg/kg/day over 4 to 12 months. [10]

A study of Acitretin (0.6mg/kg/day) over 6 to 12 in 12 patients with moderate to severe disease showed improvement. [11]

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The mechanism of action is through the keratolytic action thereby reducing ductal occlusions.

c) Immunosuppresive therapy

Cyclosporine (4.5mg/kg/day) showed raid relief in resistant cases to antibiotics and UVB therapy. [12]

TNFα inhibitors have been proven to be quite effective in Hurley's II and III stages of the disease.

Infliximab 5mg/kg IV at week 0, 2 and 6 were given to 33 patients; drug was well tolerated and showed good improvement in symptoms and severity of the disease. [13] Etanercept showed varied results when administered twice weekly 50mg for 12 weeks.

Adalimumab when given weekly instead of fortnightly showed superior results when 4 randomized control trials were analysed. [14,15]

Apremilast a selective phosphodiesterase 4 inhibitor (30 mg BD); used primarily for psoriasis; showed moderate results. [16]

d) Miscellaneous

Botulinum toxin reduces acetylcholine release and in turn reduces the sympathetic activation of apocrine glands. A dose of 40 to 50 Units per session for 3 to 4 times over 3 years reported remission in 4 cases. [17,18]

Metformin helps in decreasing androgen sensitivity by lowering circulating insulin and helps in managing the metabolic syndrome associated with disease. [19]

Others: Zinc, Cryotherapy and Photodynamic therapy

e) Surgical Intervention

This is the last resort to unresponsive cases. Deroofing is most effective in combination with antibiotics and anti-inflammatory. [20]

f) Laser therapy

Nd: YAG laser in 22 patients showed significant improvement in all (65%), axilla (62%), Inguinal (53%), Inframammary (51%). [21]

Carbon dioxide laser for lesions to heal by secondary intention has also been tried. [22]

HURLEY STAGE	TREATMENT
I	Topical antibiotics (clindamycin 1%, Benzoyl Peroxide gel 2.5/5%); Intralesional steroids; Oral Antibiotics: Tetracyclines; Retinoids; Nd YAG; cryotherapy; Botox
II	TNFα inhibitors Antibiotics Nd YAG Surgical/ CO_2 deroofing
III	Surgical with Oral antibiotics

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Figures and Legends



Figure A: Hurley Stage 1 MRI Axial STIR image of the perineum shows multiple isolated abscesses within the left natal cleft.

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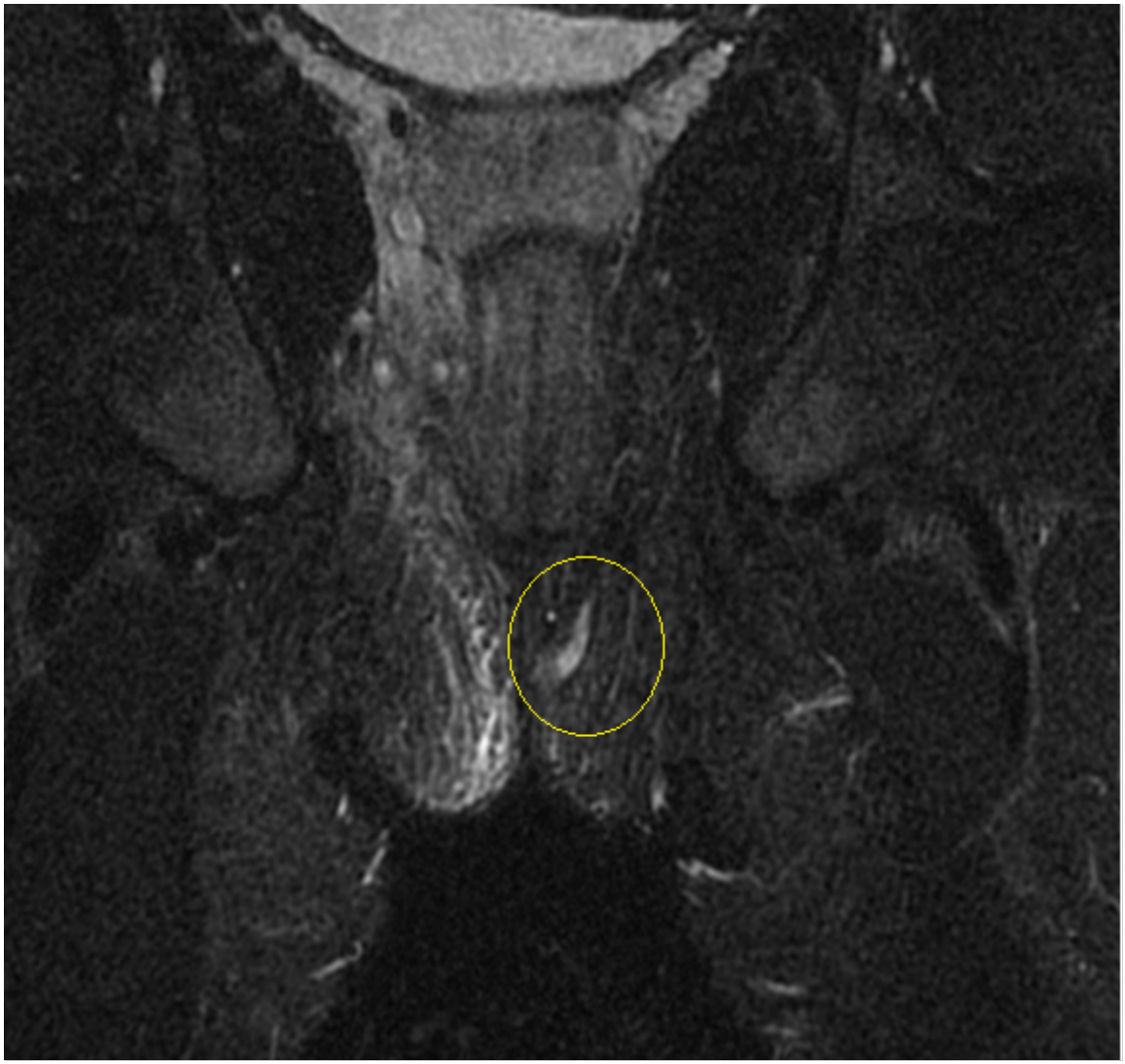


Figure B: Hurley Stage 2 MRI of the perineum: Coronal STIR image shows evolution of the abscess into a linear hyperintense tract/ sinus formation.

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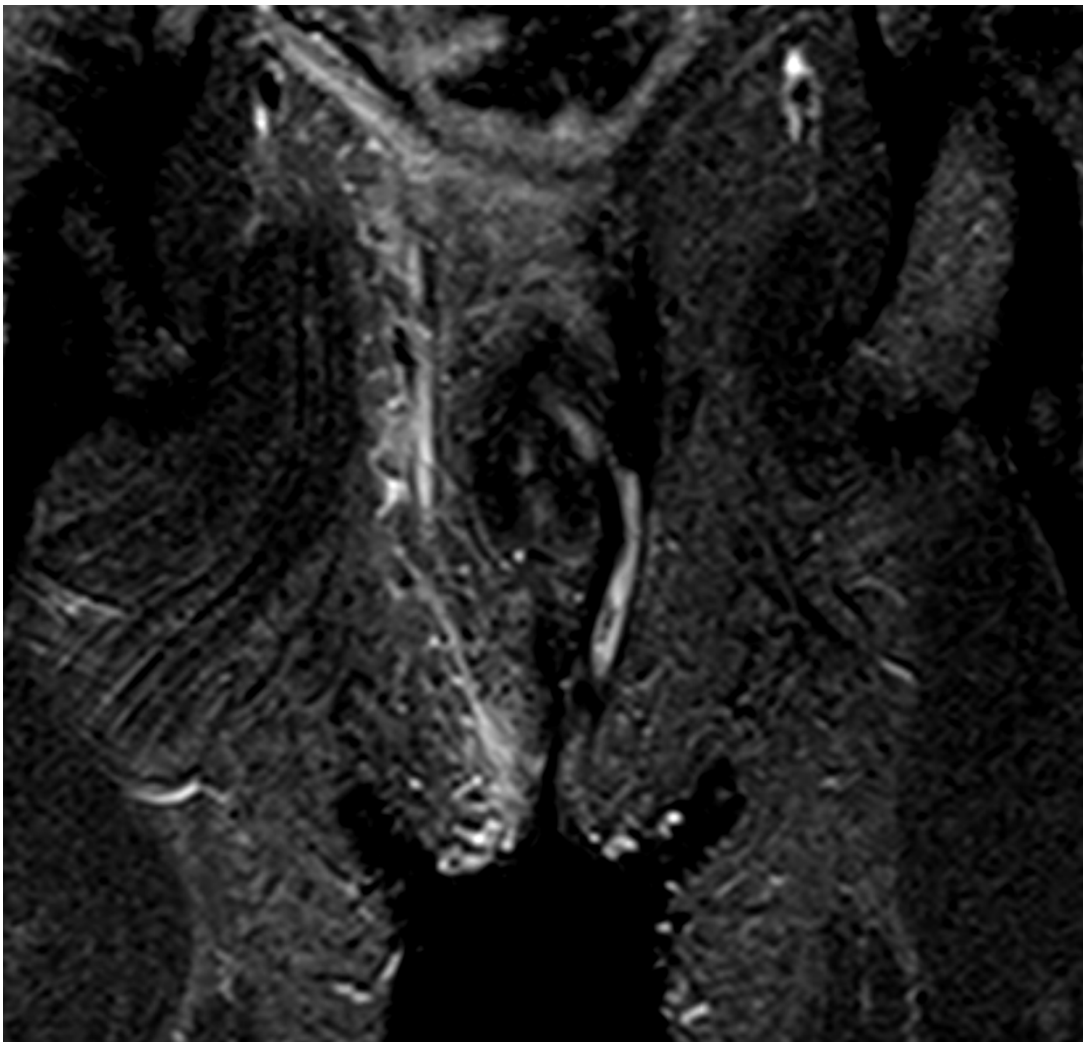


Figure C: Hurley Stage 3.

MRI of the perineum: Coronal STIR image from the selected slices shows evolution of the abscess into a linear hyperintense tract/ sinus formation. Due to chronicity of the sinus, the tract appears more fibrosed and hypointense.

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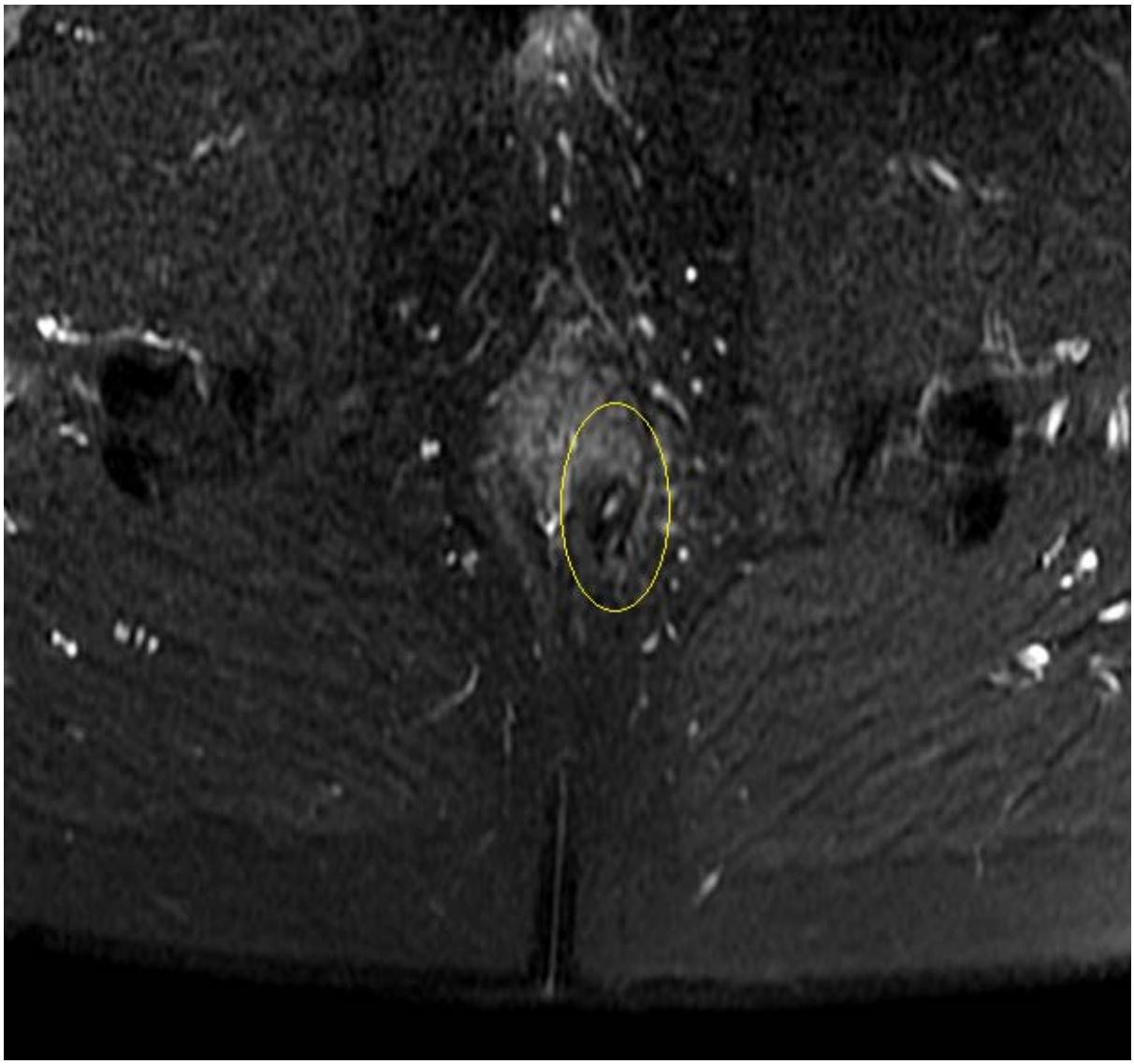


Figure D: Resolution post treatment

MRI of the perineum: Axial STIR image shows residual minimal inflammation with majority of fibrosis as hypointense scar.

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Acknowledgments

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Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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Author details

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Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

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One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

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Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

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18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

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23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

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The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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- Use paragraphs to split each significant point (excluding the abstract).
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- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

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Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

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The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

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Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

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Materials may be reported in part of a section or else they may be recognized along with your measures.

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- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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