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The Incidence of Lipoprotein Disorder in Patients with Psoriasis Attending in a Tertiary Care Hospital of Bangladesh

By Jamal S, Raihan SMR, Karim SA, Biswas R & Alam M

Chittagong Medical College

Abstract- *Objective:* In this study our main goal is to evaluate the incidence of lipoprotein disorder in patients with psoriasis attending tertiary care hospital in Bangladesh.

Method: This case control study was carried out in the Department of Dermatology & Venereology, Chittagong Medical College Hospital (CMCH), Chittagong from June 15, 2013 to May 14, 2014. In this study; 60 patients with Psoriasis (group-A) and 60 patient with skin disease other than Psoriasis (group-B) were included according to availability within the study period.

Results: During the study, it was found that; most of the patients were male among the psoriasis patients. Mean ±SD of weight was found 61.87±7.84 kg and height was found 1.62±0.05m among the psoriatic patients. There was a significant differences in Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) between group A (Psoriasis) and group B (Skin diseases other then Psoriasis).

Keywords: *psoriasis, dyslipidemia, diabetes, hypertension.*

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The Incidence of Lipoprotein Disorder in Patients with Psoriasis Attending in a Tertiary Care Hospital of Bangladesh

Jamal S [1], Raihan SMR [2], Karim SA [3], Biswas R [4] & Alam M [5]

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Conclusion: Our study indicates that the Psoriatic patients with Diabetes and Hypertension have increased risk of abnormal serum lipoprotein levels which may aggravate the Microvascular and Macrovascular end points. Further study is required to evaluate whether this lipoprotein abnormalities among Psoriatic patients having Diabetes Mellitus and Hypertension is due to other chronic co-morbid diseases or psoriasis plays the predominant role in the genesis of Dyslipidemia?

Keywords: psoriasis, dyslipidemia, diabetes, hypertension.

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I. Introduction

Psoriasis is a chronic inflammatory disease related to many diseases, especially cardiovascular disease. Among these diseases, atherosclerosis plays the most important role.[1] Atherosclerosis is caused by inflammation and an imbalance of the lipid metabolism. Psoriasis and atherosclerosis not only share the same cytokines involved in the immunological mechanism, such as interleukin-17 (IL-17), but also have common angiogenic factors and oxidative pathways.[2] In addition, both of them have similar lipid profiles, including decreased high-density lipoprotein (HDL) levels and/or increased low-density lipoprotein (LDL) levels.[3] In the pathological process of atherosclerosis, the accumulation of cholesterol triggers the production of pro-inflammatory cytokines, such as Tumor Necrosis Factor Alpha (TNFα), and also leads to the aggregation of monocytes and differentiation into foam cells. TNFα eventually induces an inflammatory cascade in blood vessels. [4]

In chronic inflammation TNFα may also influence the lipid profile, such as LDL-C levels, via a decreased concentration of a Apolipoproteins. Moreover, TNFα lowers the quality of lipoprotein by inducing the production of LDL and Oxidized LDL (oxLDL) and reducing the level of HDL-C at the same time. Oxidized LDL (oxLDL) not only exacerbates the inflammation but also promotes cholesterol accumulation in lysosomes, which eventually leads to cell death. On the other hand, HDL has a reverse cholesterol transport (RCT) function, anti-oxidative capacity, and anti-inflammatory properties by regulating dendritic cells' (DCs) differentiation, and reducing T cell activation and IL-12 production. However, these properties are reduced during chronic inflammation, such as psoriasis.[5]

In this study our main goal is to evaluate the incidence of lipoprotein disorder in psoriasis patients attending tertiary care hospital in Bangladesh.

II. Objective

To assess the incidence of lipoprotein disorder in psoriasis patients attending tertiary care hospital in Bangladesh.

III. Methodology

a) *Type of Study*
This study is a case control study.

b) *Place of study*
This study was carried out in Department of Dermatology & Venereology, Chittagong Medical College Hospital (CMCH), Chittagong from June 15, 2011 to May 14, 2012.

c) *Study Population*
Patient presented with psoriasis and presented with skin problem other than psoriasis.

d) *Sampling Technique*
Purposive / Judgment sampling.

e) *Sample Size*
Assuming the prevalence of Diabetes Mellitus and Hypertension among the Psoriasis patients is 35% and acceptable error is 10% of it (prevalence). we get required sample size:- $n=Z^2x\ p\ x\ q/e^2$. z=Standard normal deviation 1.96. p=Prevalence (Assumed) of the disease = 0.35.q=1-P=0.65. e= Acceptable error = 10% of p=0.035. So $n=(1.96)\ x\ 0.35\ x\ 0.65/(0.035)^2$ = 713.44 According to above formula, sample size was obtained but due to time limitation, in the present study 60 patients with Psoriasis and 60 patient with skin disease other than Psoriasis were included according to availability within study period.

f) *Selection criteria*

i. *Inclusion criteria*

- For case - Diagnosed case of Psoriasis, - Patients of both sexes Age group 18-65 years.
- For control - Age matched Patients without Psoriasis.

ii. *Exclusion criteria*
1) Pregnancy -2) Secondary causes of Diabetes Mellitus such as Cushing Syndrome, Acromegaly, Thyrotoxicosis, Pancreatitis, Ca-Pancreas 3) Drugs like Corticosteroid, Thiazide diuretics etc. 4) Secondary Causes of Hypertension such as Cushing Syndrome, Thyroid disorders, Acromegaly, Chronic Kidney Disease (CKD) Drugs: Corticosteroid, OCP 5) Patient unwilling to give consent. 6) Severely ill patients eg, patients with renal failure, myocardial infarction shock.

g) *Study Procedure*
Patients attending in the Dermatology Department were diagnosed case or psoriasis was included in the study. 60 patients with age matched control who were attending in the same Department with skin problem were also selected. These patients were selected after excluding the exclusion criteria, Psoriasis was diagnosed clinically attending in the CMCH. Secondary causes of Diabetes Mellitus and Hypertension were also excluded clinically. Selected patients were informed about the aims, objectives, significance and detail procedure of the study before examination. An informed written consent was taken from all the patients who was selected for the study and encouraged for voluntary participation and allowed freedom to withdraw from the study whenever they liked even after participation. All eligible subjects will be provided a structured questionnaire with direct supervision by the researcher herself to obtain socio-demographic and health related Then clinical examination was done. Blood pressure was recorded by a standard Sphygmomanometer in sitting position aller 30 minutes rest. At least two records of blood pressure of the patient were taken on two occasions. Then average blood pressure was noted. Patients were asked to come in fasting condition for at least 8 to 12 hours. Fasting blood sample was taken for fasting blood sugar and fasting lipid profile. Then patients were given 75gm glucose mixed in 300ml of plain water. After two hours second blood sample was also collected for post prandial blood sugar. Blood sample was collected by same laboratory technologist and analysis was done in the Clinical Pathology Department of CMCH.

h) *Data Collection Method*
All relevant information for each individual study subject was recorded on pre-tested data sheet. The data sheet was used for collection of information. Data was collected by the researcher.

i) *Data Processing Plan*
Data was processed and analyzed using computer software SPSS (Statistical Packages for Social Sciences Version-19). The test statistics was used for analysis of data are student's t test (for comparison of data presented in quantitative scale like blood glucose level), Chi-square test (for comparison of data presented in categorical scale like presence of DM and HTN in two groups). For any analytical test the level of significance is 0.05 and P-value <0.05 was considered significant.

IV. Results

Table-1: Distribution of the patients according to age group

		N	Mean	±SD	Median	Range	Significance
Age (years)	Group A	60	47.78	9.81	48.00	27-65	P=0.159^{NS}
	Group B	60	44.97	11.86	47.00	26-65	

Table 1:- shows that the mean age of patient in group A is 47.78±9.81 and in group B is 44.97±11.86 which is almost similar in both the groups"

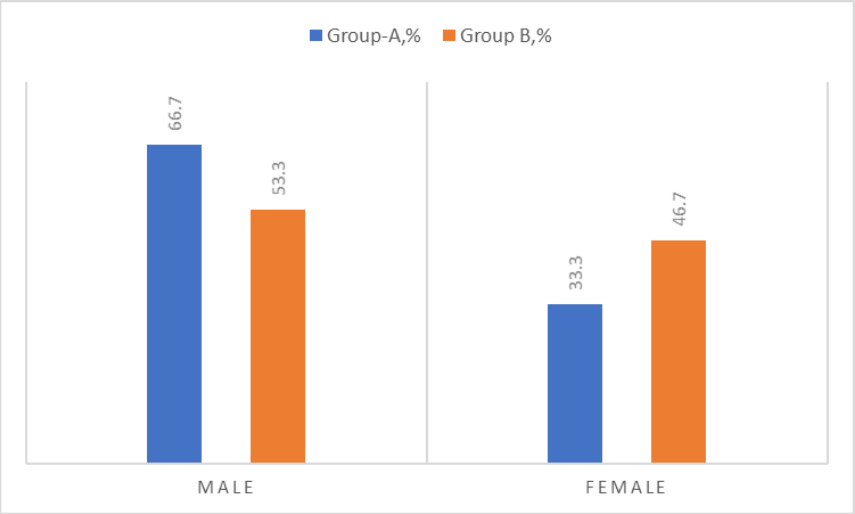

Figure-1: Gender distribution of the study

In figure-1 shows gender distribution of the study subjects where most of the patients were male in both groups. The following figure is given below in detail:

Table 2: Distribution of study groups (n=120)

Study Groups	Frequency	Percentage
GroupA (With Psoriasis)	60	50%
Group B (without Psoriasis)	60	50%
Total	120	100%

Table 2: among the total study subjects half of the patients included with diagnosed case of Psoriasis and the other half were with skin diseases other then Psoriasis.

Table-3: Socio Demographic status of patients.

Variables		Study group				X^2 Test of significance
		Group A, %		Group B, %		
		Number	% percent	Number	% percent	
Sex	Male	40	66.7	72	53.3	P= 0.136^{NS}
	Female	20	33.3	48	46.7	
Age group	≤30 years	6	10.0	20	23.3	P=0.121^{NS}
	31-40 years	8	13.3	21	21.7	
	41-50 years	24	40.0	38	23.3	
	51-60 years	18	30.0	33	25.0	
	>60 years	4	6.7	8	6.7	

In table-3 shows socio demographic status of patients. Among the study subject, two third was male. There was no significant difference in sex between group A and group B. In group A most of the patients belong to 4th decade and in group B was equal distribution of age group between 30-60 years. The following table is given below in detail

Table-4: Distribution of socioeconomic status among the study group A

Socio- economic status	Frequency	Percentage
Lower class	21	35
Lower Middle class	20	33
Upper middle class	16	26.7
Upper class	3	5

In table-4 shows distribution of socioeconomic status among the study group A. Among the psoriasis patients, most of them were from lower to upper middle-class family.

Table-5: Anthropometric findings of the patients with psoriasis

	N	Mean	±SD	Medium	Range
Weight (kg)	60	61.87	7.84	60.00	45-92
Height (m)	60	1.62	0.05	1.63	1.52-1.72
BMI (kg/M^2)	60	23.55	2.53	23.44	18.37-30.04

In table-5 shows anthropometric findings of the patients with psoriasis. Among the psoriasis patients mean ±SD of weight was found 61.87±7.84 kg and height was found 1.62±0.05m.

Table-6: Distribution of study groups according to blood pressure

Blood pressure		N	Mean	±SD	Medium	Range	Sign.
Systolic (mmHg)	Group A	60	130.08	18.42	130.00	100-210	P=0.002 highly significant
	Group B	60	120.50	13.74	120.00	100-140	
Diastolic (mmHg)	Group A	60	82.67	7.04	80.00	75-100	P=0.007 highly significant
	Group B	60	78.50	9.31	80.00	60-100	

In table-6 shows distribution of study groups according to blood pressure. There was a significant difference in systolic blood pressure and diastolic blood pressure between group A and group B.

Table-7: Distribution of study groups according to blood glucose level

Blood pressure		N	Mean	±SD	Medium	Range	Sign.
Fasting blood sugar	Group A	60	102.53	27.67	95.00	70-190	P=0.002 highly significant
	Group B	60	92.00	15.37	89.50	68-138	
Diastolic (mmHg)	Group A	60	82.67	49.98	130.00	90-360	P=0.007 highly significant
	Group B	60	78.50	27.27	124.50	85-198	

Table-7 shows distribution of study groups according to blood glucose level. There was significant difference in fasting blood sugar and post prandial blood sugar between group A and group B.

Table-8: Distribution of study groups according to lipid profile

Lipid profiles		N	Mean	±SD	Medium	Range	Sign.
Serum total cholesterol (mg/dl)	Group A	60	182.58	40.64	188.50	101-299	P=0.034 significant
	Group B	60	168.52	30.27	170.00	80-200	
Serum HDL (mg/dl)	Group A	60	37.97	6.68	37.50	20-62	P=0.032 significant
	Group B	60	35.73	4.38	36.00	30-42	
Serum LDL (mg/dl)		N	Mean	±SD	Medium	Range	Sign.
	Group A	60	120.47	29.63	125.00	65-185	P=0.022 significant
	Group B	60	109.52	21.15	110.00	68-144	
		N	Mean	±SD	Medium	Range	Sign.
Serum TG (mg/dl)	Group A	60	160.70	40.28	160.00	80-240	P=0.046 significant
	Group B	60	146.43	37.14	140.50	75-210	

Table-8 shows distribution of study groups according to lipid profile. There was significant difference in serum Total Cholesterol, HDL, LDL and TG between group A and B.

V. Discussion

Among the study subjects 60 (50% of the study Subjects) out of 120 patients were with Psoriasis and 60 (50% of the study subjects) out of 120 were without Psoriasis. The patients with Psoriasis were in the age group of 41-50 years. Among gender distribution 72(60% of the study subjects) were male. Group A and group B were statistically insignificant (p0.0).

In our study most of the Psoriasis patients were male (72%). The mean age of the Psoriatic patients are 44 years, which is supported by one other study.[6]In our country female are less conscious about their health. They have to do many households work. Economically they depend on males. For health related problems, they go to local village doctors for treatment. So, their attendance in tertiary level hospital like CMCH may be less than male.

In one large population-based study from the UK in which over 130,000 patients with psoriasis were included, showed higher incidences of Diabetes and Hypertension. Another study used the General Practice Research Database and found higher rates of Diabetes Mellitus, Hypertension, Hyperlipidemia, obesity and smoking in patients with psoriasis than in the controls subjects.[7-8] In one case control study conducted in USA in 2008 with data taken from the study of 1127 patients with Psoriasis and matched cohort of non-Psoriatic patients, Psoriatic patients were significantly more likely to develop Cardiovascular Co-Morbidities including Diabetes Hypertension, Hypercholesterolemia, compared with non-psoriasis patients.[9] In a hospital based case control study conducted in Italy, Metabolic Syndrome like Hyperglycemia, Hyperlipidemia, Hypertension was found significantly more common in Psoriatic patients than in controls (30.1% vs 20.6%, Odds ratio 1.65).[10] Another case control study conducted in Korea investigators also found a higher prevalence of metabolic syndromes (17.8%), Cardiovascular disease (4.6%), Hypertension (32.5%) and Hyperlipidemia (22.3%) in patients with Psoriasis, as compared with that of the controls.[11]

Regarding diastolic blood pressure mean±SD was found 82.67±7.04 mmHg in group A and it was 78.30±9.31 mmHg in group B. It was also found significant ($p<0.05$). Mean±SD of fasting biood sugar was found 102.53±27.67 mg/dl and post prandial blood sugar was found 144.75 ± 49.98 mg/dl among the group A patients whereas it was 92±15.37 mg/dl and 121±41.41 mg/dl in group B. Both the distribution is statistically significant ($p<0.05$). Among the study subjects DM was found in 26 patients (43.3) in group A and 10 patients (16.7%) in group B.[{OR(CI)}= 3.824 (1.635-R 8.942)]. p:0.051. HIN found 23(38.3%) in group A and 8(13.3%) in group B [{OR(CI)}= 4.041 (1.629-10020)]. $p<0.05$).

In the present study mean + SD of serum total cholesterol is 182.58±40.64 mg/dl, serum HDL is 37.97±6.68 mg/dl, serum LDL is 120.47±29.63 mg/dl and serum triglyceride is 160.70 40.28 mg/dl in group A and those are 168.52±30.27 mg/dl, 35.75±4.38 mg/dl, 109.52±21.15 mg/dl, 146.43±37.14 mg/dl respectively among group B patients. Distribution is statistically significant between both groups ($p<0.05$) which supports previous reports of an association between Psoriasis and Hyperlipidaemia. In a recent study among 1.3 million German health care recipients found that metabolic syndrome was 2.9 folds more frequent among Psoriasis patients and the most common diagnosis were Hypertension (35.6% in Ps Vs 20.6% in control) and Hyperlipidemia (29.9% Vs 17.1%).[12] Our study is nearly consistent with this study.

VI. Conclusion

1. Our study indicates that the Psoriatic patients with Diabetes and Hypertension have increased risk of abnormal serum lipoprotein levels which may aggravate the Microvascular and Macrovascular end points in patients with high risk groups.
2. Further study is required to evaluate whether this lipoprotein abnormalities among the patients with Psoriasis having Diabetes Mellitus and Hypertension is due to other chronic co-morbid diseases or psoriasis plays the predominant role in the genesis of Dyslipidemia?
3. Exploration of the relationship between Hyperlipidemia and Psoriasis may unveil the discovery of another novel treatment option for psoriasis with most promising outcome.

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Examining and Supporting Frontline Mental Healthcare Professionals (FMHP) during the COVID-19 Pandemic and its Aftermath

By Jennifer Reddin

Introduction- Novel coronavirus-19 (COVID-19) stealthily began its march across the globe at the tail end of 2019 in Wuhan, China and within months had reached all corners of the planet in devastating fashion. (Rajkumar, 2020; Galea, Merchant & Lurie, 2020). The COVID-19 pandemic has impacted people of all nations, races, socioeconomic groups and genders. The ferocity of the virus led governments to take the unprecedented steps of closing schools, courts of law, businesses and entire communities. Shelter-in-place orders and physical distancing mandates have been implemented worldwide (Shanafelt, Ripp & Trockle, 2020; Galea, Merchant & Lurie, 2020). While COVID-19 is a virus which leads to respiratory illness, medical distress and sometimes death, the physical health manifestations of the virus are just the tip of the iceberg of this pandemic. The seeds of a major global mental health crisis are germinating (Hotpof, Bullmore, O'Connor & Holmes, 2020). COVID-19 appears to be easily transmitted via close person-to-person contact, impacts large swaths of the world's population, and there is no known cure or vaccine.

GJMR-K Classification: *NLMC Code: VM 101*

Strictly as per the compliance and regulations of:

Examining and Supporting Frontline Mental Healthcare Professionals (FMHP) during the COVID-19 Pandemic and its Aftermath

Jennifer Reddin

I. Introduction

Novel coronavirus-19 (COVID-19) stealthily began its march across the globe at the tail end of 2019 in Wuhan, China and within months had reached all corners of the planet in devastating fashion. (Rajkumar, 2020; Galea, Merchant & Lurie, 2020). The COVID-19 pandemic has impacted people of all nations, races, socioeconomic groups and genders. The ferocity of the virus led governments to take the unprecedented steps of closing schools, courts of law, businesses and entire communities. Shelter-in-place orders and physical distancing mandates have been implemented worldwide (Shanafelt, Ripp & Trockle, 2020; Galea, Merchant & Lurie, 2020). While COVID-19 is a virus which leads to respiratory illness, medical distress and sometimes death, the physical health manifestations of the virus are just the tip of the iceberg of this pandemic. The seeds of a major global mental health crisis are germinating (Hotpof, Bullmore, O'Connor & Holmes, 2020). COVID-19 appears to be easily transmitted via close person-to-person contact, impacts large swaths of the world's population, and there is no known cure or vaccine. In the early part of 2020 global health care, economic and social welfare systems were essentially brought to a standstill (Horesh & Brown, 2020). Even more alarming, moving forward, COVID-19 looks to be the foundation for an unprecedented large-scale mental health catastrophe.

Frontline Mental Healthcare Professionals (FMHP) (social workers, case managers, therapists, psychologists and psychiatrists) are the first form of defense that society has, to combat the coming psycho-social consequences of COVID-19. As such the mental health of the FMHP needs to be considered. FMHP are presently and for the foreseeable future working under extreme pressure, stressful conditions and hoping to accomplish near impossible tasks. During the course of helping clients navigate the pandemic, they experience stressors from innumerable sources, and are profoundly vulnerable to their own mental health disruptions, as their occupations require them to come in contact with human suffering on an epic scale day in and day out. Throughout COVID-19 FMHP are charged with the tasks of responding to increasing levels of child abuse and domestic violence (Krasniansky, 2020), supporting clients who have lost loved ones and are forced to forego traditional burial rituals, compounding their grief (Miller & Lee, 2020), and assisting families to avoid housing displacement due to financial hardship due as a result of job/income loss (Krasniasky, 2020).

Compounding professional stressors FMHP face stressors from society, their workplace organizations and from their own personal life. Supporting FMHP begins with examining the pressures that they experience. Understanding the stressors can in turn instruct policies and practices which can support FMHP, the organizations they work for and the clients they serve.

Given the unprecedented nature of the COVID-19 pandemic there is a lacuna of data regarding research pursuits. As such, this essay will rely on research from other national disasters and catastrophic events to extrapolate how FMHP have experienced stressors and how they can be supported in the continuing aftermath of COVID-19.

II. Epistemological Considerations, Positionality & Theoretical Lens

a) *Social constructivism and Relativism*

Social constructivism is a paradigm with a subjectivist epistemology, which puts forth the theory that individuals interpret and construct meaning based on their experiences and evolved beliefs. "Meaning is not discovered, but constructed" (Crotty, P. 9). All findings are a report not of what "*IS*" but what is experienced by the creator of the research (Guba 1990). Social constructivists posit that subjects impose all understanding & knowledge upon objects, and that all understandings, scientific or non-scientific are a form of constructed knowledge, created via the understanding of the viewer and knower (Crotty, 1998). Constructivists put forth the notion that it is impossible for a researcher to inquire from an objective or distant position, as the truth is, the researcher and her subject are fused into a reciprocal loop of understanding & information, which is constantly informed by subjective understanding and developed information (Guba, 1990).

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Social constructivism and subjectivism are a good fit with social issues and the complexities of social work practice, given the specific character of social work, as a profession with roots in understanding and helping people, pursuing social justice and recognizing the importance of human relationships. The values and underlying mission of social work, which seeks to recognize the individual worth of each human, the development of support for oppressed populations and the growth and empowerment of society in general are all supported by the inclusive and introspective nature of subjectivist epistemology. In essence being "objective" when dealing with human problems is near impossible, which is the most compelling argument as it relates to the strength of the subjectivist epistemology.

The ontology that informs constructivism is relativism, which espouses the idea that all knowledge, scientific and otherwise is construed through social structures and relationships. Relativists understand that the amount of possible interpretations of reality are limitless and assert that various interpretations can co-exist. (Guba, 1990)

Relativism advances the position that all knowledge exists in relation to culture and that there is not and cannot ever be an independent objective reality. Creating reality, via examining relationships and point of view, rather than seeking to understand the one true reality is an ideal fit for social work, given the complex, ever evolving and truly human nature of the profession, as well as a study about stressors experienced by FMHP in the wake of COVID.

Ontology and epistemology influence methodology, as it depends whether as a researcher you see participants as subjects or active contributors to the project. Given my epistemological location, the most powerful approach to this research is participatory action research (PAR). PAR assumes that clients have the most information to explain the problems they are struggling with. It recognizes the knowledge and the power FMHP have and credits their experiences and points of view as valuable parts of the research journey (D'Cruz and Gillingham, 2017). Participatory research is a self-reflexive inquiry that enlists participants as partners rather than subjects and is action oriented, requiring research, action and further research (Baum, MacDougall and Smith, 2006). My scholarly interest in these issues arises from a very personal place in that many of my family members have been on the frontline of the war against COVID-19 and I seek to understand their experiences and support them in the most meaningful way possible.

b) Compassion Fatigue and Moral Injury

Much of the research in the area of stressors experienced by FMHP view the issue through the theoretical lens of compassion fatigue, also known as secondary traumatic stress, which posits that over time formal caregivers develop a reduced capacity to be empathetic towards clients (Adams, R. Boscarino, J. and Figley, C., 2006). The theory advances the framework that the cumulative and transformative effect of stressors experienced by FMHPs who serve traumatized clients is an expected occupational hazard (Buchanan, M., Anderson, J., Uhlemann, M., and Horwitz, E. 2006), commonly referred to a the "cost of caring" (Killian, K., 2008). While the theory of compassion fatigue/secondary traumatic stress can be effective in measuring long term impact upon mental health professionals, as there are developed scales and long-term research, it does not precisely enough match the examination of the experiences of FMHP during the COVID-19 pandemic. In that COVID-19 is a phenomenon that has had a sudden and generally unexpected onset. The volume of death and suffering due to the virus and its aftershock has been global, intense, and unprecedented in modern times. A theory that does not account for the immensity of the issue will lead to incomplete research.

Another limitation of compassion fatigue/ secondary traumatic stress is that the theory negatively appraises the experiences and reactions of mental health professionals. Use of a theory that normalizes the suffering experienced by FMHP during this crisis is more appropriate, as FMHPs should not be pathologized. COVID-19 is a global mass trauma that brought the world to a standstill. Stress, anxiety and fear in the face of a mass traumatic event is expected and predictable. A more suitable theoretical lens to view research of stressors facing FMHP during the COVID-19 pandemic and its aftermath is moral injury theory.

Moral injury theory is one that can be used to examine the psychological, social and spiritual pieces of individuals. Moral injury is the distress that individuals feel when they witness, fail to prevent or commit acts that transgresses their core ethical belief system (Litz, B., Stein, N., Delaney, E., Lebowitz, L., Nash, W., Silva, c., and Maguen, S. 2009). As a result of moral injury individuals may experiences guilt, shame, anger and self-condemnation (Borges, L., Barnes, S., Farnsworth, J., Bahraini, N. and Brenner, L., 2020). Moral injury additionally includes an aspect of failure of leadership in extreme high stakes situation, and the impact it has on individuals (Shay, 2014). It was first recognized in a military context and is a theory used to explain the cost of processing moral pain and the enduring nature of Post-Traumatic Stress Disorder (PTSD) in soldiers returning from war (Ayyala R., Taylor, G. and Callahan, M., 2020; Shay, 2014).

The basic progression of moral injury begins with a potentially morally injurious event (PMIE), which is a situation occurring in an intense or extreme environment, that is perceived by an individual as a violation of his/her own moral values (Farnsworth J., Drescher, K., Evans, W. and Wasler, R., 2017). The

perpetrator of the violation can be one's own self or another, such as a supervisor or someone who holds authority in the situation (Shay, 2014). Examples of PMIEs in military situations are use of deadly force on civilians and failing to or being unable to provide aid to fellow services members (Farnsworth et al., 2017). Within the context of COVID-19 and FMHP, actions and inactions which could be PMIE could be excluding loved ones from a dying patient's bedside or failure to make a home visit to provide services to a homebound client due to social distancing protocol.

As a result of MIEs individuals can experience moral pain, immediately, later or never. Moral pain is the natural, non-pathological shame, guilt, culpability and self-condemnation that individuals can experience as a result of being exposed to MIEs (Farnsworth et al., 2017).

Examples of experiences FMHP could have during COVID-19 potentially include shame of not providing necessary housing referrals to victims of domestic violence, who were then forced to shelter-in-place with their abusers, guilt over the need to discontinue face-to-face psychotherapy for clients, and self-condemnation for avoiding human touch and physically distancing from clients, due to a fear of infection and subsequent transmission. Moral injury is the suffering of one's psychological, social and spiritual self, as a result of unresolved moral pain (Farnsworth et al., 2017).

A limitation of moral injury theory in this context is that there has not been much application of the theory in non-military personnel (Borges et al., 2020). Although limited research has shown MIE in non-military settings, may expose members of civilian occupations to profound experiences that can be viewed via a moral injury lens (Williamson, V., Greenberg, N., and Murphy, D., 2019).

COVID-19 seems to be an extreme situation, in which FMHP are handcuffed by a lack of resources and are unable to deliver care in the way they have been trained to and expect of themselves (Ayyala, R., Taylor, G. and Callahan, M., 2020). Conflicted allegiances, to self, clients, occupational organizations and national public health may all be at odds for FMHP in COVID-19, who may feel compelled to sacrifice individual client needs for their own, or for the sake of the greater good.

The intersection of a worker's competency, expectations of herself, client's needs, her agency's resources and public health requirements seem precisely the point where moral injury lies.

Given the uniquely personal perspective of moral values, the theory of moral injury seems to fit within a social constructivism paradigm and a subjectivist epistemology. Additionally, the aspect of the theory that considers an individual's spirituality, also aligns with my own positionality as a social worker, researcher and human.

III. Literature Review

Stressors that FMHP face can be organized into four areas of focus, categorized according to the source of the stress. While there seem to be overlapping ideas in each sphere, this area of research can be likened to a compass, with the FMHP in the center experiencing stress from all directions. First, there are individual or personal factors; examples of such work and life balance issues and personal history. Next, there are stressors which originate from clients, including clients requiring higher level of care, due to the pandemic and increased caseloads. Additionally, organizational workplace stressors are impactful, which include agency culture and climate, agency offerings and supervision support. Finally, at a broader level, environmental or societal factors must be considered. Societal stressors include workers perceptions of the way they are regarded by the public, and how those attitudes are internalized, along with issues of race, systematic oppression, marginalization and intersectionality.

a) Individual factors

Personal history and current life experiences of FMHP have been examined and found to be stressors that impact psychological outcomes of FMHP.

Research has shown that FMHP who had a personal history of negative life events experience a higher level of distress, as a result of their work with clients focused on the aftermath of trauma (Adams, Boscarino & Figley, 2006). Similarly, Buchanan and colleagues found that therapists with an acknowledged personal history of trauma experienced elevated levels of compassion fatigue (Buchanan et al, 2006). Compassion fatigue has been explained as the cost of caring, when professionals experience emotional exhaustion as a result of vicarious trauma or secondary trauma, after absorbing the traumatization of clients (Ray, S., Wong, C., White, D. and Heaslip, K., 2013). Compassion fatigue was also found in a study of FMHP, who identified key stressors in the development of their compassion fatigue, including a personal history of trauma, a lack of self-awareness (Killian, K., 2008).

Perceived lack of social support has also been found to be a reliable factor associated with negative psychological outcomes in disaster responders. (Guilaran, de Terte, Kaniasty and Stephens, 2018). Further, research supports that FMHP who had financial problems, poor self-perceived health and outside personal problems are more likely to experience burn out and a lack of job satisfaction (Ray et al, 2013).

During the first weeks of the COVID-19 pandemic, researchers found that sources of anxiety for FMHP were their current life situations, including personal access to food and hydration during extended work shifts, access to childcare during increased work hours, the fear of being exposed to the virus and taking

it home to their family members. This fear led tophysical isolation from family members. Further, the physical strain of the vital protective gear for hours at a time, was physically taxing for personnel (Shanafelt, Ripp & Trockel, 2020).

In support of such, research after the outbreak of severe acute respiratory syndrome (SARS) found that fear of infection and subsequent transmission to family members struck an overwhelming level of fear in frontline health care staff (Chong, Wang, Hsieh, Lee, Chiu, Yeh, Huang, Wen and Chen, 2004).

b) Client stressors

Stressors that emanate from client interaction needs to also be considered. Frontline hospital workers identified client related stressors of failing to meet clients' needs and excessive workload (Hall, D., 2004). Similarly, high caseload demands were found to be the most pressing risk factor in developing work stress and compassion (Killian, K., 2008). Caring for patients who were experiencing extreme and life-threatening medical situations has been shown to be a triggering stressor for hospital staff (Yoder, E., 2010). Additionally, situations involving a demanding patient, or an onerous family were identified as stressors related to compassion fatigue (Yoder, E., 2010). Given the isolation protocols many hospitals implemented during the COVID-19 pandemic, it is expected that the tensions between public health priorities and wishes of patients and their families regarding quarantine will be a source of client related stress for FMHP.

Another factor contributing to mental health care professionals stress is emotional exhaustion, from absorbing the trauma of clients and continually providing unanswered giving and attentiveness (Ray et al., 2013). FMHP often experience exhaustion due to perceived work as a "caregiver" and experienced a sense of hopelessness working with clients (Adams, Boscarino and Figley, 2006).

Further, researchers should be mindful that clients will continue to have social and mental health needs unrelated to the pandemic, during the crisis, which could cause clients to be in need of even more support from FMHP, creating increased stress from clients (Krasnisky, 2020).

c) Workplace stressors

Workplace related stressors encompass organizational factors and social aspects of the workplace.

Regular access to supervision and a reported positive relationship with supervisors were both found to be moderators of occupational stressors for FMHP (Rayet al., 2013). Similarly, professional autonomy, as measured by being able to exercise control over professional decisions, diminished occupational stress in FMHP (Rayet al., 2013). Lack of a supportive work environment, including managers, colleagues and subordinates diminishes the quality of the social context in which FMHP serve, which acts as a significant stressor (Rayet al., 2013).

Similarly lack of role clarity and absence of trust in leadership were deemed among the largest sources of workplace stress (AdibIbrahim, M., Abdul Aziz, A., Suhaili, N., Zahid Daud, A., Naing, L. and Abdul Rahman, H., 2019). Workers with ambiguous roles were less confident and consequently experienced a more negative workplace experience. During the COVID-19 pandemic many agencies experienced a reduction in staff owing to illness. This creates the possibility of workers to be redeployed to new areas and having to undertake new roles (Miller, V. and Lee, H., 2020). It can be anticipated that this will be an occupational stressor experienced by FMHP during and as a result of COIVD-19.

d) Societal Stressors

Societal stressors originate in the global community and can weigh heavy on individuals. The COVID-19 crisis has been reported on within the 24-hour news cycle for months and months. This protracted media coverage has the potential to have an intense impact on those who are working in the thick of it. Research after the SARS outbreak showed that the intense media coverage of the virus heightened the perceptions of personal danger among front line healthcare workers (Bai, Y., Lin C., Lin, Y., Chen, J., Chue, C. and Chou, P., 2004). It also led to a perceived stigma, in that others would fear being in contact with frontline workers who were caring for SARS patients (Bai et al., 2004). The stigma related to COVID-19 and frontline workers, who are more likely to have been exposed, can lead to isolation, depression, anxiety and public embarrassment (CDC-Stigma 2020). Specific racial groups, namely Asians and Asian-Americans are also likely to experience such stigma (CDC-Stigma, 2020).

Larger societal systems have also been found to be impactful upon FMHPs. Social workers, after the 9/11 attacks, were found to have experienced a sense of hopelessness and powerlessness, regarding judicial and social welfare systems, that were failing their clients (Killian, K., 2008).

Systematic and institutional racism and sexism are societal factors that need always be considered in research. Racial and ethnic groups such as, Blacks and Latinx people are historically at a higher risk of illness and death as a result of national public health crises and COVID-19 is no exception (CDC Racial & Ethnic Minority Groups, 2020). During this current pandemic people of color are being hospitalized and dying as a result of COVID-19 in disproportionately high numbers (Wadhera, R.K., Wadhera, P., Gaba, P., Figueroa, J., Joynt Maddox, K., Yeh, R. and Shen, C., 2020). Living conditions, such as institutional racism within public

housing and racially segregated housing; work circumstances, such as having no sick leave or having an employment position which requires face-to-face work, such as grocery store and factory workers; and health circumstances, such as being un or underinsured, all create a greater risk of infection for minority populations(CDC Racial & Ethnic Minority Groups, 2020).Adding to the panoply, unemployment within the United Sates is currently at an unprecedented high, reaching the highest level in the post-World War II era (Kochhar, R., 2020). Examining the disparity via race, highlights racial disadvantages, in that approximately one-in-five Asian, Black and Hispanic workers were unemployed, {Asian, 20.3 %, Black, 19.8% and Hispanic 20.4%} as compared to only 13.5% of white workers (Kochhar, R., 2020). All of this is compounded by the fact that the United States is navigating and absorbing the nation's largest burst of civil unrest since the 1960s as a result of countless police actions against people of color, which came to a head in the midst of COVID-19 (Galea & Abdalla, 2020).

Gender is a significant societal factor as well, being that 70%, a vast majority of frontline health and social care workers are women (Boniol, M., McIsaac, Xu, L., Wuliji, T., Diallo, K., and Campbell, J., 2019). Coupled with the data which reveals that in May 2020 the unemployment rate for women was 17.8 % as compared to 14.5% for men (Kochhar, R., 2020), reinforces the need to conduct this inquiry with consideration of intersectionality.

e) Methodological Critiques

My search strategy began with terms including COVID-19, coronavirus, mental health implications, social workers, compassion fatigue and disaster. After narrowing articles down I then utilized snowball searching by checking references lists of the publications that I found useful and looking at what new work cited articles that I originally found useful. All of the studies had to be reviewed for academic rigor, validity and reliability.

Guilaran, de Tete, Kanisty & Stephens' (2018), publication was a systematic review of twenty-four studies. Initially, it must be noted that no original research was generated, but it did offer a rigorous systematic review. The authors identified a clear objective gave explicit criteria for publications selected to be included in the review, offered a thorough analysis and clear presentation of studies and offered practical conclusions for future research.

Krasniansky, A. (2020); Miller, V. & Lee, H. and Wadhera, et al, (2020) are similarly publications that offer insight, but not original research. These articles are all commentaries and editorial pieces, which seek to address the COVID-19 pandemic, by offering opinions and extrapolating ideas from prior research.

Shanfelt, T., Ripp, J. & Trockel, M. (2020) published an exploratory study, which was based on eight listening sessions, held at Stanford medical school, of 69 clinicians during the first week of COVID-19. The information gained from these sessions was instructive but lacked academic rigor. The authors acknowledge such, but sought to begin discussions and produce knowledge early in the pandemic.

Adams R., Boscarino J. & Figley, C. (2006), conducted a cross-sectional mail survey of 275 NYC social workers, working in clinical practice after the 9/11 terrorist attacks. Given the cross-sectional nature of the study, no causal effect could be determined. The stated purpose of the study was to assess the predictive validity of a compassion fatigue scale and support the validity and reliability of that scale. Only NYC members of NASW were included in the study which impacts the generalizability/external validity of the study.

Bai, Y., Lin C., Lin, Y., Chen, J., Chue, C. and Chou, P. (2004), reported on a study that investigated stress reactions in staff members of a hospital after a SARS outbreak. The questions related to DSM-IV acute stress disorder criteria, so it appeared to have facial validity, although the authors used a self-designed questionnaire, with no reported test/retest reliability, which is a significant limitation.

Buchanan, M., Anderson, J., Uhlemann, M., and Horwitz, E. (2006) published a report of a Canadian study examining compassion fatigue of mental health providers. The self-report questionnaires used included the Impact of Event Scale and the Compassion Fatigue Self-Test for Practitioners, both scales had reported high internal consistency and reliability, as evidence by .89 and .89 test/retest results. Although the survey was sent to 1,200 potential respondents and only 280 completed the surveys, which leaves the possibility of a threat to external validity/generalizability, given the self-selection bias of the respondents.

Chong, M., Wang, W., Hsieh, W., Lee, C., Chiu, N., Yeh, W., Huang, T., Wen, J.& Chen, C. (2004), conducted a mixed method cross-sectional study seeking to assess the immediate psychological impact of SARS in a hospital in Taiwan. Participants included 1310 hospital workers for the quantitative portion of the study and 285 senior level staff members for the qualitative portion. Limits of the study are that the scales used for the quantitative portion of the study (Chinese language Impact of Event Scale, and Chinese Health Questionnaire) had no Cronbach's alpha listed, which limits the ability to analyze internal validity and reliability. The qualitative portion of the study were essentially debriefing sessions which were supportive in nature, where the researchers provided assurances and utilized supportive group psychotherapy techniques. While this may have be purposefully done for ethical reasons to protect the well-being of the participants it could be a

threat to the objectivity and credibility of the study, as these assurances may have had an impact on results.

Hall D. (2004) conducted a qualitative study of 10 nurses in a hospital where she was employed, that focused on work related stressors and coping mechanisms. The author's employment at the hospital creates a threat to the confirmability of the research, given her connection to the institution and the staff. Additionally, there were two raters, which creates a potential for inter-rater inconsistency, although they did employ a peer member check to strengthen interrater reliability.

Adib Ibrahim, M., Abdul Aziz, A., Suhaili, N., Zahid Daud, A., Naing, L. and Abdul Rahman, H. (2019) researched psychosocial work stressors in relation to a healthy workplace. 225 health and allied health professionals from a large hospital in Brunei were included in a cross-sectional study. The Copenhagen Psychosocial Questionnaire and Healthcare Productivity Survey were utilized and were shown to be valid and reliable via .91 and .93 Cronbach's alpha. A limit of this study is its external validity/generalizability, given the cultural and religious components of absolute Islamic monarchy, that may be specific to Brunei.

Killian, K. (2008) conducted mixed method cross-sectional study seeking to assess the therapists' stress and coping in work with trauma survivors. Participants included 104 trauma therapists for the quantitative portion of the study and 20 trauma clinicians for the qualitative portion, no information was provided as to how the 20 subjects were chosen. Within the quantitative portion of the project the Social Support Index, the Maslach Burnout Inventory and the Emotional Self-Awareness Questionnaire were used, and Cronbach's alpha reliabilities were reported as good, ranging from .80 to .91. A limitation of the study can be seen in the administration of the questionnaires which was done at employee's workplaces, which could impact internal validity in that pressure from the location of the survey could be an extraneous source impacting results.

Lai, J., Ma, S., Wang, Y., Cai, Z., Hu, J., Wei, N., Wu, J., Du, H., Chen, T., Li, R., Tan, H., Kang, L., Yao, L., Huang, M., Wang, H., Wang, G., Liu, Z., & Hu, S. (2020) conducted a very recent study at the beginning of the COVID-19 outbreak in Wuhan China. The cross-sectional study included 1257 healthcare workers in 20 hospitals in the epicenter of the COVID-19 outbreak. Measurement instruments utilized were Patient Health Questionnaire, General Anxiety Disorder Scale, Insomnia Severity Scale and Impact of Event Scale. The study doesn't report the validity or reliability of these scale within the report, but to say they are all "validated measurement tools". This is a limitation, but certainly could be owing to seeking to complete the study and publish as immediately as possible. All data was collected within 6 days, and from that time only 6 weeks elapsed until publication, generally this would certainly seem rushed, but given the unprecedented and extreme nature of the outbreak it is not surprising.

Ray, S., Wong, C., White, D. and Heaslip, K. (2013) conducted a study of 169 FMHP in Canada, via a mail survey, which included the compassion satisfaction and compassion fatigue subscales of the Professional Quality of Life Questionnaire, the Areas of Work Life Scale and the Maslach Burnout Inventory General Survey. The reliability of each scale is represented by Cronbach's alpha ranging from .77 to .92. The limits of the study include its cross-sectional nature, making causality and internal reliability difficult to assess. Additionally, the self-report nature of all the scales acts as a common but note-worthy limit.

Yoder, E. (2010) published a mixed method study regarding compassion fatigue, of 106 nurses in a Midwest hospital. Her working definition of compassion fatigue saw the anger and helplessness experienced by respondents as a response to watching experiences that their patients went though. This is limiting as it does not consider how the respondents' own actions or inactions would impact their feelings. The qualitative portion of the study consisted of only two questions, seeking short-answer replies, thereby lacking the depth necessary to insure credibility. Lastly, the researcher was an employee at the hospital, known to many of the respondents, which has the potential of impacting the accuracy of their replies and the objectivity of the study.

IV. Policy Implications

Utilizing the systematic comparisons based on welfare regimes models and social policy developed by Esping-Anderson (1999), the United States operates under a residual welfare model, where individuals and families are responsible for managing social issues and the state interposes in exceptional cases of need. This model is woefully ineffective in addressing social and mental health concerns of individuals and of FMHP. In essence, it requires there to be an identified problem before an intervention can be put in place. The United States must understand that FMHP will be expected to suffer as a result of COVID-19 and need to enact a comprehensive plan immediately, rather than wait until the situation becomes dire.

Moral injury theory is one that normalizes the feelings of pain and suffering during and after traumatic events, rather than pathologizing them. Understanding the issue of stressors experienced by FMHP via a lens of moral injury makes it obvious that mental health support is essential for FMHP and should be viewed as a human right. Doing so requires a much-needed shift to a social democratic model, which begins with an initial understanding that the state should play a larger role of support for the whole population, before it starts to fall apart. This is also consistent with the International

Federation of Social Workers (IFSW) platform which frames health and mental health as a fundamental human right (IFSW, 2008).

Best practices require a whole-of-society approach, which is advanced and supported by the United Nations, which entails the incorporation of mental health care in disaster management plans; the availability of widespread mental health and psychosocial support for and a proactive plan for populations particularly in danger, such as FMHP in an effort to relieve suffering and encourage recovery. (UN Policy brief, 2020).

V. Practice

Practice interventions based in both individual and macro systems theories can be used to impact and improve the experience of FMHPs during the COVID-19 pandemic and its aftermath.

Acceptance & Commitment Therapy (ACT) is trauma related treatment that is related to and born out of cognitive behavior therapy (Farnsworth et al., 2017). ACT is an evidence based behavioral intervention that involves disclosure of and connection to feelings regarding a past traumatic experience and a cognitive restructuring of a clients' understanding of her experiences (Farnsworth et al, 2017). ACT involves a ceaseless process of self-reflection and refraction and encouragement for the individual to make new meaning of their traumatic history, along with self-forgiveness (Farnsworth et al, 2017).

Self-care is another individual practice which is informed by moral injury theory. Practices which can be included in healing from moral injury are mindfulness, meditation and development of a resilient mindset through acceptance and self-compassion (Miller, J., Lianekhammy, J., Pope, N., Lee, J., and Grise-Owens, E.2017).

A larger system theory that can be utilized within the moral injury framework includes weaving care and compassion into the workplace. Understanding the connection that individuals, specifically FMHP have to their workplace and their identity as FMHP is vital from an organizational point of view. Workplaces and agencies need to create policies and protocols that seek to understand and address workers suffering, as a result of working through COVID-19. Examples of such practices are team discussions for decision making, to decrease perceptions of personal culpability for individuals and social support resources to foster team connectedness, boost self-awareness regarding PMIE and encourage workers to support each other. Examples of these would be "Check You, Check Two" and "Code Lavender", which are programs within organizations that encourage workers to seek and offer social support to co-workers during difficult times (Tracy, D., Tarn, M., Eldridge, R., Cooke, J., Calder, J., and Greenberg, N. 2020; Johnson, B., 2014).

Lastly, and bringing the discussion back to the beginning, hearing and acting on the needs of FMHP requires listening to their experiences. Supporting participatory action research allows FMHP to develop, collect and analyze data, so that they can reflexively drive the research, lead the inquires and suggest and implement subsequent actions (Shanafelt, T., Ripp, J., & Trockel, M. 2020).

VI. Conclusion

COVID-19 looks to be the foundation for an unprecedented large-scale mental health catastrophe, greater than we have seen in generations. FMHP will be charged with navigation countless crisis' without being destroyed in the process. Supporting FMHP begins with examining the pressures that they experience. Understanding these stressors can in turn instruct policies and practices which can support FMHP, the clients they serve, the organizations they work for and society as a whole.

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Alteration of Thyroid Hormone Pictures in Absence of Clinically Evident Thyroid Diseases among Type 2 Diabetic Subjects in a Bangladeshi Population

By SA Karim, J Samira, SMR Raihan, MS Emran, O Faroque, L Ali & H Mehtab

Abstract- *Background:* Thyroid diseases and diabetes mellitus are common endocrine disorders and Euthyroid Sick Syndrome is very common in uncontrolled type2 Diabetes Mellitus.

Objective: To evaluate the thyroid hormone pictures in absence of clinical thyroid diseases among type2 diabetic subjects in a Bangladeshi population.

Method: This case and control study was carried out in the Endocrinology department of Sylhet MAG Osmani Medical College (SOMC) and Hospital, Sylhet, Bangladesh in collaboration with the Research Division, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrinology and Metabolism (BIRDEM)), Dhaka, Bangladesh during the period of January 2016 to December 2017.

Keywords: *euthyroid sick syndrome (ESS), diabetes mellitus, body mass index (BMI).*

GJMR-K Classification: *NLMC Code: WK 200*

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SA Karim [1], J Samira [2], SMR Raihan [3], MS Emran [4], O Faroque [5], L Ali [6] & H Mehtab [7]

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Results: During the study, it was found that mean± SD of the thyroid hormone pictures in diabetic and control subjects of TT_3; (ngm/dl) {in controls (88.91±15.88) and in diabetic subjects (84.27±22.29)} was not statistically significant to each other (p=0.209). Mean±SD of TT_4 (µgm/ dl) in control subjects was 8.32±1.64 and in the diabetic subjects was 9.26±1.44, which is almost similar in both the groups (p= 0.589). FT4: (pgm/ml) in control subjects was 2.60±0.54 and in diabetics was 2.53±1.72 (p= 0.830). FT_4. (µgm/ dl) in control subjects was 1.43±0.22 and in diabetics subjects 1.36±0.25. (p 0.179). TSH (µlu/ml) in control subjects was 1.34±1.00 and in diabetic subjects 1.54±1.21 (p: 0.411). FT_3; FT_4 and TSH showed no significant difference between control and diabetic subjects. Thyroid hormones (TT_3, TT_4, FT_3, FT_4) and TSH were reanalyzed according to HbA1c and BMI category and showed no significant differences. But when the FPG and HbA1c goes beyond 12 mmol/l and 10% respectively there was more worsening thyroid hormone pictures in comparison to groups whose FPG and HbA1c were below 12mmol/l and 10%. It was also noticed there was a tendency to develop more lower thyroid hormone pictures and more deteriorating glycemic status in patient with low and normal BMI groups in comparison to higher BMI groups of patients.

Conclusion: Uncontrolled type2 diabetes mellitus is associated with alteration of thyroid hormone pictures particularly affecting TT_3, FT_3 and TSH in absence of clinically evident thyroid diseases. This biochemical feature is more evident if the BMI of the Diabetic subjects is low or within the normal range and also the more worsening the glycemic status as determined by FPG and HbA1c, there was more deteriorating circulating serum thyroid hormone pictures.

Keywords: euthyroid sick syndrome (ESS), diabetes mellitus, body mass index (BMI).

I. Introduction

Diabetes mellitus (DM) is one of the important health problems affecting the major population worldwide. Diabetes mellitus is an endocrine disorder which involves multiple organ systems and leads to significant morbidity and mortality due to accompanying complications.[1.] Diabetes mellitus is characterized by absolute or relative deficiency in insulin secretion or insulin action or both, associated with hyperglycemia and disturbances in carbohydrate, lipid and protein metabolism.[2]

Thyroid diseases and diabetes mellitus are the two most common endocrine disorders[3]. Diabetic patients have increased prevalence of thyroid disorder, with hypothyroidism being the most common.[4] In diabetic patients, thyroid dysfunction varies from 2.2% -17%.

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Diabetic women are more commonly affected than men.[5] Hypothyroidism is a clinical syndrome occurs from a deficiency of thyroid hormones. It is very common thyroid problem in diabetic patients.[6] Thyroid hormones and insulin are the antagonistic, and involved in the metabolism of carbohydrates, proteins, and lipids. Thyroid hormone as well as insulin levels are altered if there is functional impairment of the thyroid gland and endocrine pancreatic beta cells.[7] Thyroid disorders adversely affect diabetes control. Diabetes Mellitus appears to influence thyroid function in two sites; firstly, at the level of hypothalamic control of TSH release and secondly at the conversion of T_4 to T_3 in the peripheral tissue. Increased hyperglycemia causes reversible reduction of the activity and hepatic concentration of T_4-5'-deiodinase, low serum T_3, increase in reverse T_3 and also variation in the level of T_4.[8]

Euthyroid Sick syndrome (ESS) or Non-thyroidal illness Syndrome (NTIS) identifies abnormalities of thyroid function tests observed in patients with systemic non-thyroidal illnesses and in those patients undergoing surgery or fasting.[9-11] Abnormalities of thyroid function tests observed in ESS or NTIS includes 1) Low T_3 Syndrome 2) Low T_3 and T_4 syndrome 3) High TT_4 syndrome 4) other abnormalities like low TT_3 and TSH, high TSH and low TT_3 and TT_4.[12]

Experiments on both animal and human models Diabetes Mellitus was found to be associated with alteration of thyroid hormone picture in absence of clinical thyroid diseases irrespective of the type of diabetes. In both type1 and type2 diabetes significant reduction of both TT_3 and FT_3, increased rT_3 and high rT_3/T_3 ratio was demonstrated.[13-16] Serum FT_4 and FT_4I were normal, TT_4 was normal or suppressed and TSH was found normal or slightly elevated.[17, 18] All the parameters of thyroid function specially TT_3, FT_3 and rT_3 become normal when euglycemia was achived.[14,19,20] More over it was found that reduction of FT_3 and TT_3, rise of rT_3 are significantly correlated with the severity of hyperglycemia and the thyroid secretory response to large dose of TSH is also declined in uncontrolled diabetes mellitus which frequently improves with improved glycemic control.[21]

A previous study among young Bangladeshi diabetic population demonstrated significant alteration of thyroid hormone pictures in absence of clinical thyroid diseases which is consistent with the other study done in abroad earlier.[22] But ESS or NTIS in the setting of type2 diabetes was not investigated extensively earlier and there is no available data regarding the changes in thyroid hormone pictures in patients with uncontrolled type2 Diabetes Mellitus among Bangladeshi population. Although there are few studies in abroad; which revealed that that there are significant alteration of thyroid hormones in uncontrolled type2 diabetic subjects.[19,20,23,24] As most of these studies did not exclude other causes of ESS or NTIS which are responsible for activating inner ring deiodination or inhibition of outer ring deiodination of T_4 to produce T_3 from T_4 instead of rT_3;[25] the result was found to be poorly representative.

In the above context our present study was designed to document the changes of thyroid hormones pictures in absence of clinical thyroid diseases among Bangladeshi population in the setting of uncontrolled type2 diabetes mellitus.

II. Objectives and Methods

To evaluate the circulating thyroid hormone pictures in absence of clinical thyroid diseases among type 2 diabetic subjects in a group of Bangladeshi population.

III. Methodology

a) Types of study

This was a case and control study.

b) Place and duration of study

The study was conducted in the Endocrinology department of Sylhet MAG Osmani Medical College (SOMC) and Hospital, Sylhet, Bangladesh in collaboration with the Research Division, BIRDEM, Dhaka, Bangladesh during the period of January 2016 to December 2017.

c) Study population

A total of 100 type 2 diabetic subjects, 30-50 years of age, irrespective of glycemic status, duration of diabetes, BMI and sex were recruited from the outpatient department (OPD) of SOMC hospital and BIRDEM hospital. Prior to recruitment, diabetes mellitus was confirmed according to current American Diabetic Association (ADA) criteria for the diagnosis and classification of diabetes mellitus.[24] Control subjects (n=100) were selected from friends and family of the patients within 5 years of age band without diabetes or impaired glucose regulation (IFG, IGT) determined according to ADA criteria[24] and having no clinical thyroid diseases or other evident systemic diseases documented on clinical evaluation. Informed written consent was taken from all recruited diabetic and control subjects for the purpose of the study.

d) Exclusion criteria

- Type 2 diabetes with acute metabolic de-compensation.
- Type 2 diabetes with clinically detectable thyroid diseases.
- Type 2 Diabetes with clinically diagnosed other acute or chronic systemic diseases.
- Diabetic subjects with overt nephropathy in which serum creatinine > 2mg/dl
- Pregnancy and postmenopausal woman.

e) Method

Selection criteria as per availability and was given an appointment to come in a particular date. Preparation of the subjects and collection of blood of the Controls and diabetic subjects that were assigned for the purpose of study done according to the recommendation of the "Report of the Expert committee of the Diagnosis and Classification of Diabetes Mellitus.[24] They were requested to fast overnight for at least eight hours and in the subsequent morning 16 ml of venous blood was drawn from the ante-cubital vein by using 25 cc disposable plastic syringe with 18G needle for the estimation of fasting serum insulin, C-peptide, glucose, HbAlc, TT_3, TT_4, FT_3, FT_4 and TSH. One ml of collected venous blood was taken in an anticoagulant containing vial for estimation of HbAlc. Remaining 15ml of blood was kept in 3 separate plain test tubes in equal amounts (5ml in each) to centrifuge immediately. Blood sample contained in the test tube was centrifuged for 15 minutes at a rate of 4000 rpm. A total of 200 μl of serum was collected in appropriately labeled eppendorf in duplicate with the help of micropipette for each of the biochemical parameters. Then the serum sample was preserved immediately at -30° C for analysis.

f) History and clinical examination

Detailed socio-demographic and clinical data were recorded in a pre-designed case record form. These include age, sex, residing area, occupation, socioeconomic status, dietary habit, exercise, alcohol and smoking habit, duration of diabetes, associated diseases like hypertension, obesity, dyslipidemia, coronary artery disease, cerebrovascular diseases, peripheral vascular diseases and crystal deposition diseases. Family history of these diseases were also been noted. Classical and non-classical features of diabetes mellitus and any adverse outcome of diabetes on life style was noted by taking history from the diabetic subjects.

Hight, weight, BMI, waist circumference, hip circumference, waist hip ratio (WHR), waist height ratio (WHtR) of all the controls and diabetic subjects were recorded. Percent body fat and total fat mass was measured by "Body logic Body fat monitor; Omron Corporation, Japan". Systolic and diastolic blood pressure of all patients and control subjects was recorded. Blood pressure was measured by using mercury sphygmomanometer after at least 5 minutes of recumbence in a calm and quiet environment. Systolic blood pressure 130 mm Hg and the diastolic blood pressure 85 mm Hg was taken as the cut-off value for categorizing the normal and the abnormal values among diabetic population.[25] Diabetic neuropathy was tested by appropriate clinical test. Autonomic function test were done by documenting the heart rate variability and blood pressure response on standing. Motor neuropathy was tested by eliciting jerks and reflexes by the percussion hammer. Retinopathy of all diabetic subjects was screened by routine dilated fundoscopy of the BIRDEM opthalmology outpatient department and SOMC hospital outpatient department. For the documentation of nephropathy, urine albumin in mg/l and urine creatinine in g/l was estimated to calculate the albumin creatinine ratio (ACR). FPG was measured by glucose oxidase method and HbA1c wes measured by HPLC based analyzer, Insulin, c-peptide, TT3, TT4, FT3, FT4 and TSH was measured by Chemiluminescence technique in Immulite Auto-analyzer.

g) Statistical analysis

All the data were expressed as mean standard deviation, median (range) and/or number and percentage (%) as appropriate. Statistical analysis was done by using SPSS 7.5 packages for windows. Appropriate statistical test of significance like unpaired t test, one way analysis of variance (ANOVA) and Mann-Whitney test was used as necessary. $P < 0.05$ was taken as minimum level of significance.

h) Data presentation

Tabulation and / or drawing either in the form of graph or in the form of diagram were utilized as necessary for data presentation.

IV. Results

Table-1: Demographic status of the study group

Groups	Age mean ± SD	Annual Income Median (Range)	Family Member	SBP mean ± SD	DBP mean ± SD	Duration of DM, years
Controls (n =30)100	39.53± 5.24	120000 (30000-220000)	6 ±1	120 ±23	80±7	--
DM(n=100)	39.24± 5.79	100000 (20000-200000)	6± 2	124 ±17	80±10	0.02 (0.01- 6)
t/p value		u/p value	t/p value			
Cont vs DM	-.248/0.804	1114/0.32*	1.1770/.241	1.177/0.241	1.101/0.273	--

In table-1 shows demographic status of the study group where mean±SD age of the control and diabetic subjects were 39.5±5.2 and 39.2±5.8 respectively. Duration of diabetes is one month to six years. Systolic and diastolic blood pressure of the control and diabetic subjects were almost similar and it was within normal range. The table given below showed it in detail:

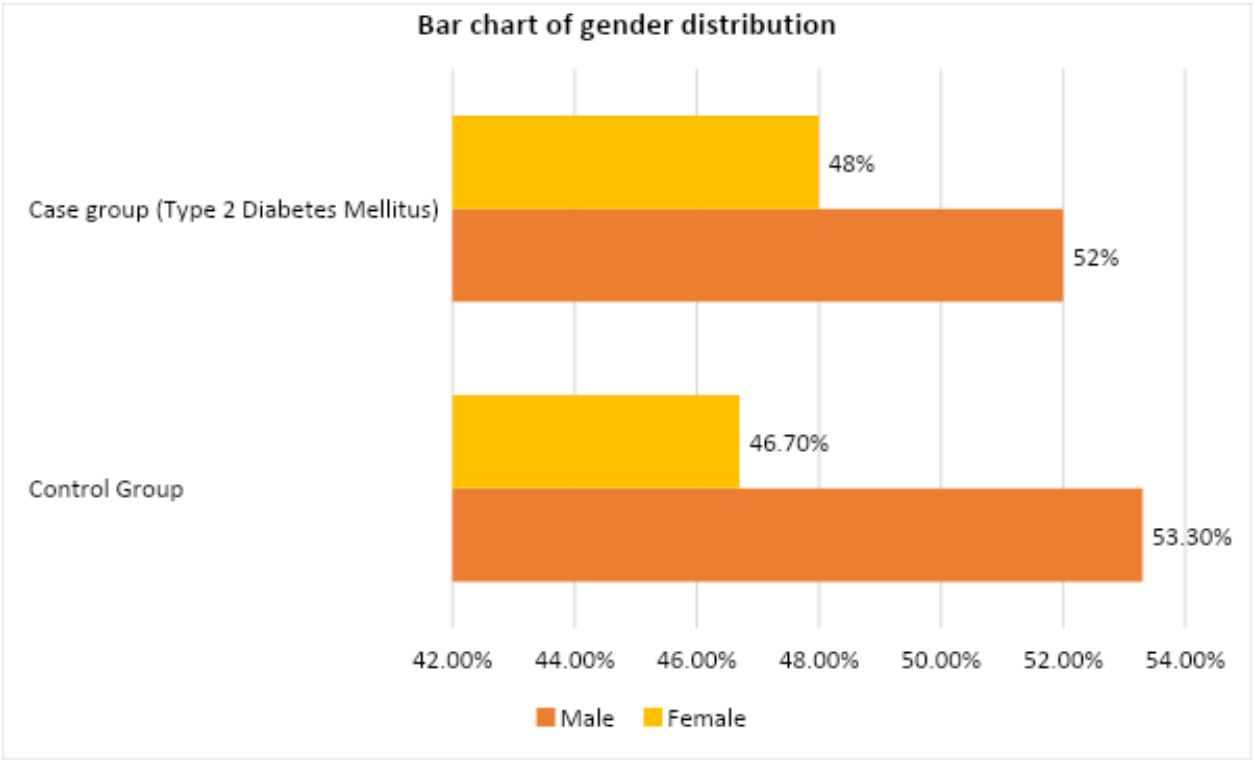

Figure-1: Gender distribution of the study group.

In figure-1 shows gender distribution of the study group where both the groups have shown in the figures in details. Male persons are 53/3% and females are 46.7% in controls and in type Diabetic group male patients are 52% and females are 48%.

Table-2: Clinical status of the study group

Clinical history		Controls		Type-2 Diabetes mellitus	
		Number	Percentage	Number	Percentage
Sex	Male	53	53	52	52
	female	47	47	48	48
Type of work	Sedentary	90	90	84	84
	Physical work	10	10	16	16
Exercise	Regular	37	37	23	23
	Irregular	63	63	57	57
	No Exercise	0	0	20	20
Smoking	Smoker	7	7	20	20
	Non Smoker	90	90	70	70
	Past Smoker	3	3	10	10
FH diabetes	Present	53	53	65	65
	Absent	47	47	23	23
FH HTN	Present	50	50	48	48
	Absent	50	50	36	36
FH obesity	Present	44	44	46	46
	Absent	56	56	54	54
FH CAD	Present	27	27	24	24
	Absent	73	73	52	52
FH CVD	Present	24	24	27	27
	Absent	76	76	50	50
H/O CAD	Present	4	4	38	38

H/O CVD	Present	0	0	06	6
Retinopathy	Present	0	0	35	35
Neuropathy	Present	0	0	35	35
Nephropathy	Present	0	0	25	25
Anti DM drugs	Present	0	0	24	24
Typical Symptoms	Present	0	0	37	37
Atypical Symptoms	Present	0	0	63	63

In table-2 shows clinical status of the study group where 53 out of 100 controls and 65 out of 100 diabetics have family history of diabetes. Family history of hypertension was found in 50 out of 100 and 48 out of 100 controls and diabetic subjects respectively. Family history of obesity was found in 44% controls and 46% diabetic subjects. Around 27% of controls and 24% of diabetic subjects have family history of coronary artery diseases (CAD) and 24% of control and 27% of diabetic patients have family history of cerebrovascular diseases (CVD). Early retinopathy and neuropathy were observed in 45.5% and 36.5% diabetic subjects. Nephropathy was documented in 25 diabetic subjects.

Table-3: Thyroid hormone status in diabetic and control subjects

Parameters	Control			DM			P value			
	MC**	FC**	TC**	MD**	FD**	TD**	MCvs MD	FC vs FD	TC vs TD	MD vs FD
TT_3	93.67± 17.14	83.46 ±12.78	88.91 ±15.88	85.02 ±22.7	83.46 ±22.0	84.27 ±22.3	0.912	1.00	-1.268 /0.209	1.00
TT_4	8.54± 1.9	8.07± 1.31	8.32 ±1.64	8.22 ±1.90	8.63 ±1.69	9.26 ±9.44	1.00	1.00	0.54/ 0.589	1.00
FT_3	2.69 ±0.36	2.56 ±0.55	2.60 ±0.54	2.40 ±0.68	2.37 ±0.74	2.53 ±1.72	0.816	1.00	0.215/ 0.83	1.00
FT_4	1.49 ±0.21	1.37 ±0.23	1.43± 0.22	1.43 ±0.18	1.31 ±0.27	1.36 ±0.25	1.00	1.00	-1.35/ 0.179	0.065
TSH	1.33 ± 0.88	1.35 ±1.16	1.34 ±1.00	1.26 ±0.89	1.84 ±1.42	1.54 ±1.21	1.00	0.961	0.824/ 0.411	0.08

***(MC=Male control, FC= Female Control, TC= Total Control, MD=Male Diabetic, FD= Female Diabetic, TD=Total Diabetic)*

In table-3 shows thyroid hormone status in diabetic and control subjects. Mean±SD of TT3; (ngm/dl) in controls (88.91±15.88) and in diabetic subjects (84.27±22.29) was not statistically significant to each other (p=0.209). Mean±SD of TT4 (μgm/dl) in control subjects was 8.32±1.64 and in the diabetic subjects was 9.26±1.44, which is almost similar in both groups (p= 0.589). FT3 (pgm/ml) in control subjects was 2.60±0.54 and in diabetics was 2.534±1.72 (p= 0.830). FT4 (ngm dl) in control subjects was 1.43±0.22 and in diabetics subjects 1.36±0.25. (p 0.179). TSH (μlu/ml) in control subjects was 1.34±1.00 and in diabetic subjects 1.54±1.21. (p: 0.411). FT3; FT4 and TSH showed no significant difference between control and diabetic subjects.

Table 4: Mean serum level of TT3, FT3 and TSH in patients with low levels of hormones and also in patients with normal values of hormones

Groups	TT3	FT3	TSH
Groups with Low level of Thyroid hormone	58.46±12.32	1.31±0.44	0.50±0.49
Groups with Normal level of Thyroid hormone	93.81±16.91	2.53±0.59	1.50±0.86
T/p value	-11.45/0.0001	-6.83/0.0001	-6.018/0.0001

(Results are expressed as mean±SD, p value was calculated using ANOVA Bonferrony, t/p value was calculated using unpaired 't' test)

Table-4 showed that the mean serum TT3 in patients with low T3 syndrome groups of patients and in patients with normal values of TT3 were 58.46±12.32 and 93.81±16.91 respectively which was statistically significant (p=0.0001) between the two groups. Mean Serum TSH level in low TSH group was 0.47±0.395 and in normal TSH group was 1.50±0.86 which was statistically different significantly (p=0.0001) from each other. Serum FT3 levels in low FT3 groups and normal FT3 groups of patients were 1.31±0.44 and 2.53±0.59 respectively which was statistically different between the two groups.

Table-5: Thyroid hormone pictures in diabetic subjects according to HbA1c

Groups	TT_3	TT_4	FT_3	FT_4	TSH	FPG	HbA1c	S Insulin	Serum C-Peptide
Group A, N=13	78.42± 24.50	8.77± 1.15	2.40 ±0.59	1.37± 0.25	1.37± 0.25	5.97± 1.8	6± 0.56	5.9 (3.5-12.5)	0.88 (0.11-5.1)
Group B, N=15	89.71± 24.33	8.00 ± 1.53	2.35± 0.61	1.22± 0.27	1.90± 1.40	7.7± 1.9	7.37± 0.32	8.0 (3.2-16.3)	0.71 (0.12-2.1)
Group C, n=72	84.19 ±21.51	8.45 ±1.95	2.35± 0.73	1.39± 0.24	1.41± 1.11	12.39± 4.5	10.84± 1.89	7.9 (19-48.9)	0.74 (0.06-3.6)
P value								U/p value	
A vs B	0.554	0.773	0.255	0.774	0.135	0.749	0.09	75/0.30	88.5/0.67
A vs C	1.000	1.000	1.000	1.000	1.000	0.000	0.000	343/0.13	392/0.35
B vs C	1.000	1.000	1.000	0.97	0.862	0.000	0.000	513/0.76	469/0.42

(Group A=HbA1c < 7%, Group B=HbA1c 7%-8%, Group C=HbA1c > 8%)

(P value was calculated using one way analysis of variance, U/p value was calculated using Non-parametric Mann Whitney U test.)

In table-5 shows thyroid hormone pictures in diabetic subjects according to HbA1c. Thyroid hormones (TT_3, TT_4, FT_3, FT_4) and TSH were analyzed according to HbA1c category and showed no significant difference.

Table 6: Thyroid hormone pictures among Diabetic subjects when categorized according to BMI

Groups	Serum Insulin	Serum C peptide	FPG mg/dl	HbA1c%	TT_3	TT_4	FT_3	FT_4	TSH
BMI A N=55	6.0 (1.9-38.0)	0.74(0.06-3.62)	11.77±5.18	10.35±2.63	81.45 ±20.70	8.51± 1.77	2.31± 0.68	1.41± 0.26	1.28 ±0.89
BMI B N=35	8.6 (2.3-48.9)	0.76(0.12-5.11)	9.66±3.79	8.83±2.01	89.54± 24.58	8.46± 1.78	3.02± 2.72	1.32± 0.26	1.78 ±1.51
BMI C N=10	14.9 (4.9-21.5)	0.94(0.20-2.13)	9.96±3.30	9.10±2.01	81.33± 20.62	7.74± 2.15	2.00± 0.56	1.29± 0.18	2.09 ±1.30
u/p value		P Value							
A Vs B	710/0.036	811/0.21	0.108	.013	0.285	1.000	0.164	0.721	0.160
A vs C	140/0.014	219/0.30	0.761	.399	1.000	1.000	1.000	0.651	1.000
B vs C	120/0.13	172/0.93	1.000	1000	0.913	1.000	0.288	1.000	1.000

(BMI A=BMI upto 25). (BMI B=BMI 25.1-30). (BMI C=BMI>30)

(P value was calculated by ANOVA Bonferrony, U/p value was calculated using Mann-Whitney U test)

Table-6 showed that when TT_3 , TT_4, FT_3, FT_4 and TSH were grouped according to BMI category and compared separately among control and Diabetic subjects in three BMI groups; no significant difference was observed.

Table 7: Glycemic Status and indices of obesity in patients with low T_3 syndrome and in patients with having normal T_3 among the Diabetic subjects categorized according to BMI groups

Groups		FPG	HbA1c	BMI	% body Fat	Total Fat Mass	Fasting Serum Insulin	
BMI A	Low T_3	14.24±7.13	10.74±3.15	23.16±1.39	23.46±5.45	7.6 (1.92-18.3)	7.6 (1.92-18.3)	
	Normal T_3	1018±3.61	10.18±2.39	22.74±1.89	25.48±5.84	5.55 (1.9-38.0)	5.55 (1.9-38.0)	
	t/p value	1.96/0.064	0.721/0.474	0.813/0.420	-1.210/0.23	u/p value	u/p value	*281/0.44
BMI B	Low T_3	9.55±4.40	9.08/±2.41	27.19±1.25	33.31±4.83	8.0 (2.3-47)	8.0 (2.3-47)	
	Normal T3	9.70±3.68	8.76±1.93	27.33±1.45	3.72±5.82	8.8 (3.5-48.9)	8.8 (3.5-48.9)	
	t/p value	-.094/0.925	0.380/0.706	-0.233/0.817	1.146/0.26	u/p value	u/p value	*99/0.72
BMI C	Low T_3	10.45±0.64	9.55±1.34	33.41±3.61	36.85±6.29	13.4 (5.3-21.5)	13.4 (5.3-21.5)	
	Normal T_3	9.84±3.73	8.99±2.54	31.63±1.60	37.30±4.10	14.9 (4.9-20.8)	14.9 (4.9-20.8)	
	t/p value	0.222/0.83	0.294/0.776	1.144/0.286	-.182/0.901	0.335/0.726	u/p value	*7.0/0.79

(BMI A=BMI upto 25). (BMI B=BMI 25.1-30). (BMI C=BMI>30)

Table-7 showed that when diabetic patients with low T_3 and Normal T_3 were reanalyzed according to BMI category then it was found that low T_3 subjects having normal BMI showed significantly higher serum fasting glucose (14.24±7.13) levels compared to the patients with normal T_3 (10.66±3.61).

Table 8: Glycemic status, % body fat and total fat mass in different status of thyroid hormones

Groups		FPG	HbA1c	Fasting S. Insulin	BMI	%Body Fat	Total Fat Mass
TT_3	LowTT_3 (n=27)	12.57±6.44	10.16±2.89	7.7(1.9-47.0)	25.11±3.35	27.37±3.74	18.12±5.93
	Normal TT_3 (n=73)	10.21±3.63	9.52±2.32	8(1.9-48.9)	25.41±3.45	28.72±6.83	19.14±6.13
	t/p value	2.30/0.024	1.572/0.119	972/0.92*	-0.384/0.702	-0.856/0.394	-0.753/0.453
FT_3	LowFT_3 (n=12)	11.45±5.09	9.98±2.43	7.4(4.4-38.0)	26.59±3.52	30.42±7.24	21.42±5.94
	Normal FT_3 (n=88)	10.77±4,61	9.66±2.51	7.8(1.9-48.9)	25.16±3.33	28.07±6.91	18.52±6.03
	t/p value	0.475/0.636	0.426/0.671	480.5/0.61*	1.357/0.178	1.096/0.276	1.562/0.122
TSH	Low TSH (n=12)	12.24±4.16	10.86±2.48	8.3(1.9-48.9)	25.27±1.82	30.19±7.65	19.98±5.0
	Normal TSH (n=88)	10.81±4.78	9.65±2.48	7.7(1.96-47.0)	25.21±3.65	27.63±6.66	18.40±6.20
	t/p value	0.986/0.327	1.573/0.137	461.5/0.48*	0.449/0.218	1.524/0.782	0.982/0.624

Table-8 showed the analysis of TT_3, FT_3 and TSH and its relationship to FPG, HbA1c, Fasting serum insulin, BMI and also with %body fat and total fat mass. 27 diabetic subjects have shown T_3 levels below the lower limit of normal range at FPG levels12.57±6.44 and HbA1c 10.16±2.89. FPG value was significantly (p=0.024) higher in low T_3 group compared to normal TT3 group. 12 diabetic subjects were found to have FT_3 below the lower limit of normal range at FPG level 11.45±5.09 and HbA1c level 9.98±2.43. Again 12 diabetic subject were found to have TSH below the lower limit of normal range at FPG level 12.24±4.16 and HbA1c level 10.86±2. Fasting serum insulin levels showed no significant differences among the different groups of TT_3, FT_3 and TSH. Similar observation was also noted in case of %body fat, total fat mass and in BMI.

Figure 2 and Figure 3

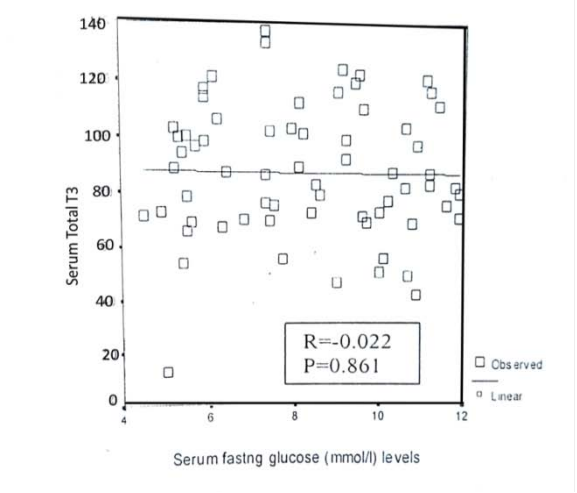

Figure 2: Relationship of FPG with Serum TT3 of the Diabetic Subjects when FPG <12mmol/l

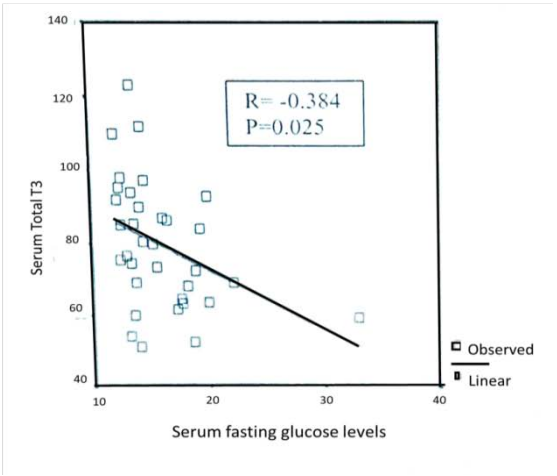

Figure 3: Relationship of FPG with Serum TT3 of the Diabetic subjects when FPG>12mmol/l

V. Discussion

Some studies done earlier in abroad; the age (mean±SD) of type 2 diabetic patients who were participated in study was (47.5±7.4) years coincides with the fact that type 2 diabetes mellitus usually develops after the age 40 years.[9, 26,27]. Where as in our study age (mean±SD) of the control and diabetic subjects were 39.5±5.2 and 39.2±5.8 respectively.

Thyroid hormones among control and Diabetic subjects was evaluated and it was found that the differences observed in serum thyroid hormones and TSH levels between controls and diabetic subjects were not statistically significant. When the thyroid hormones and TSH were reevaluated on the basis of BMI and HbAlc groups among the diabetic subjects, similar observation was noted (table 5 and 6). But when serum TT_3, TT_4, FT_3, FT_4, and TSH values of all the diabetic subjects were divided into two groups by applying the cut off values to each hormone into normal values of thyroid hormones group and TSH with low values of thyroid hormones groups and TSH among the diabetic subjects, 27 diabetic patients were found to have low TT_3 below the lower limit of normal range than that of their normal counterpart which was significant at p=0.0001 level (table-4 and 8), 12 patients were found to have serum

FT_3 levels which was significantly (p=0.0001) lower than their normal counterpart groups. Again 12 diabetic subjects were found to have low TSH level than their normal groups. 4 diabetic subjects were found to have significantly lower serumTT_4 when compare to normal TT_4 groups. When the TT_3. FT_3, TT_4, and TSH groups were reanalyzed in relation to fasting serum glucose and HbA1c, low thyroid hormones and low TSH group have significantly (p=0.0001) higher fasting serum glucose and HbA1c than the groups with hormones within the normal range(table-8). The diabetic patients with Low TT_3 showed strong negative correlation to FPG and HbA1c (Fig: 19, 20). This finding is consistent with the findings of the other studies done in abroad in both type 1 and type 2 diabetic subjects.[11-16,28-31]. Our findings showed that around the level of 12mmoll of fasting serum glucose was associated with marked alteration of thyroid hormone picture in the blood in absence of clinical thyroid diseases. When low TT_3 group was categorized according to BMI, diabetic subjects having BMI within normal range was found to have more deteriorating fasting serum glucose, HbA1c and serum TT_3 levels; compare to other BMI groups (Table-8). This finding suggests that changes in thyroid hormone possibly much more obvious in young diabetic groups who are mostly have low or normal BMI than the type2 diabetic subjects that are mostly associated with obesity and higher degrees of BMI. These findings also supports the findings of the others study done in abroad.[21] and also in the cell and molecular biology department of BIRDEM, Dhaka, Bangladesh.[22] Our findings also conclude that BMI and other indices of obesity possibly have very little or no impact on serum thyroid hormones and TSH levels until and unless they are associated with very high serum fasting glucose levels beyond 12mmol/l.

VI. Conclusion

1. Uncontrolled type 2 diabetes mellitus is associated with alteration of thyroid hormone pictures particularly altering the TT_3, FT_3 and TSH in absence of clinically evident thyroid diseases.
2. This biochemical feature is more evident if the BMI of the subjects is low or normal range and it was also found that the more worsening the glycemic status as determined by FPG and HbA1c, there was more deteriorating circulating serum thyroid hormone pictures and TSH.
3. Interpretation of abnormal thyroid hormone pictures requires a very high index suspicion in patients with uncontrolled type2 diabetes mellitus as it was found to have associated with ESS or NTIS.

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Postoperative Outcome Analyses of Non-Complicated Macula-Off Rhegmatogenous Retinal Detachment: A Retrospective, Long-Term, Multicenter Case Series Report

By Miguel A. Quiroz-Reyes, Erick A. Quiroz-Gonzalez, Jorge Morales-Navarro, Felipe Esparza-Correa, Jorge E. Aceves-Velazquez, Jennifer H Kim-Lee, Alejandra Nieto-Jordan, Margarita Montano, Virgilio Lima-Gomez & Federico Graue-Wiechers

Abstract- *Background:* There is abundant and even confusing information in the available literature concerning the role of internal limiting membrane (ILM) removal in macular conditions secondary to non-complicated macula-off rhegmatogenous retinal detachment (RRD) repair. This retrospective, multicenter, long-term study aimed to analyze the incidence of epiretinal membrane (ERM) proliferation and other surgical complications and to compare the postoperative microstructural and multimodal imaging findings and correlate them with the final postoperative best-corrected visual acuity (BCVA) in selected eyes.

Keywords: *brilliant blue dye; epiretinal membrane; internal limiting membrane; macula-off retinal detachment; non-complicated rhegmatogenous retinal detachment; primary vitrectomy.*

GJMR-K Classification: *NLMC Code: WW 270*

Strictly as per the compliance and regulations of:

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Abstract- Background: There is abundant and even confusing information in the available literature concerning the role of internal limiting membrane (ILM) removal in macular conditions secondary to non-complicated macula-off rhegmatogenous retinal detachment (RRD) repair. This retrospective, multicenter, long-term study aimed to analyze the incidence of epiretinal membrane (ERM) proliferation and other surgical complications and to compare the postoperative microstructural and multimodal imaging findings and correlate them with the final postoperative best-corrected visual acuity (BCVA) in selected eyes.

Methods: This long-term retrospective study included 230 eyes divided into three groups according to the surgical management performed for uncomplicated macula-off RRD: 125 eyes in the buckle group underwent scleral buckle techniques; 55 eyes in the non-peeling group underwent primary vitrectomy with no ILM peeling; 50 eyes in the peeling group with primary preoperative or secondary postoperative presence of significant ERM proliferations underwent the ERM-ILM en-bloc complex removal or double-staining removal techniques.

Results: The postoperative incidence of ERM was 23.2% (29 eyes) in the buckle group, 23.63% (13 eyes) in the non-peeling group, and 2.0% (one eye) in the peeling group ($p<0.05$; Student's t-test). The mean postoperative BCVA difference among the buckle group, peeling group, and non-peeling group was significant (logarithm of the minimum angle of resolution, 0.40±0.33 vs. 0.47±0.16 vs. 0.28±0.19, respectively). Postoperative multimodal imaging tests yielded abnormal retinal thickness in the three groups, with a diffuse optic nerve fiber layer and ellipsoid band disruptions predominantly in the peeling group, and a normal foveal profile in the buckle and non-peeling groups.

Conclusions: Multiple structural alterations in spectral-domain optical coherence tomography biomarkers and a significant reduction in retinal sensitivity were observed in the peeling group. Eyes that developed secondary ERM proliferations in the buckle group and in the non-peeling group showed statistically significant upgrading in BCVA once the ERM proliferation and ILM were removed. Ultimately, our study contributes findings pertaining to severe consequences in macular structure and function. We can conclusively state that ILM removal with the main objective of avoiding macular ERM proliferation is not justified because of the high rate of potential macular complications and poor visual results.

Keywords: brilliant blue dye; epiretinal membrane; internal limiting membrane; macula-off retinal detachment; non-complicated rhegmatogenous retinal detachment; primary vitrectomy.

I. Background

Multiple surgical complications associated with scleral buckle surgery have been reported in the management of primary and non-complicated macula-off rhegmatogenous retinal detachment (RRD). Partial- or full-thickness scleral perforations can give rise to various serious trans-operative vitreoretinal complications, including retinal perforation with vitreoretinal entrapment, choroidal hemorrhage, and subretinal bleeding, that allow access to the sub-macular space with well-known deleterious effects on the photoreceptors. In addition to epiretinal membrane (ERM) proliferation after scleral perforation in buckle and cryotherapy surgery, the most commonly encountered postoperative complications are macular ectopia due to vitreomacular traction and proliferative vitreoretinopathy (PVR) with recurrent and complicated RRD[1-4].

According to the 2005-2019 trending data from the American Society of Retinal Specialists Preferences and Trends Survey [5], primary vitrectomy is the chosen procedure for non-complicated RRD cases not requiring a supplemental scleral buckle in order to reduce the

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aforementioned complications [5]. However, the incidence of macular complications, such as the appearance of epiretinal macular membranes, remains high. Several reports have shown that if the internal limiting membrane (ILM) is removed at the same time as the reapplication of the retina via primary vitrectomy and endolaser treatment, the incidence of significant ERM proliferations is reduced, and thus, additional surgical procedures can be avoided. However, ILM removal still has possible transoperative or postoperative structural and functional complications because the ILM acts as a scaffold for the proliferation of the glial and Muller cells; these cells create ERM proliferations that exert a tangential contraction over the macula [6,7]. Thus, the potential benefits of prophylactic ILM removal remain controversial [8-12].

The main objectives of this study were as follows: (1) to retrospectively determine the postoperative incidence of ERM proliferation over the macula and other postoperative surgical complications; (2) to analyze the long-term final postoperative structural, optical coherence tomography (OCT) findings; (3) to contribute to the analysis of macular microperimetry and multifocal electroretinography (mfERG) findings; and (4) to correlate these results with the final postoperative best-corrected visual acuity (BCVA) in different surgical management methods performed for uncomplicated macula-off RRD.

II. Methods

The Retina Department at the Institute of Ophthalmology. Oftalmologia Integral ABC and Retina Specialists at the American British Cowdray Hospital, and the Retina Service of the Hospital Juarez in Mexico City, Mexico, provided authorization and released the electronic clinical records for the database used in this study. This retrospective, long-term, multicenter, one-surgeon study adhered to the tenets of the Declaration of Helsinki, received full ethical approval from the Research Ethics Committees, and was approved by the Institutional Review Committees and the Teaching Departments of the three participating institutions (no reference number is provided for retrospective studies by these institutions). Written informed consent before the surgical procedure in accordance with the institutional guidelines was obtained from all the patients. Data are available from the Imagenology and Psychophysics Laboratory at the Retina Departments of the three institutions.

The study was designed to comparatively analyze the anatomical and functional outcomes of scleral buckle procedures and vitrectomy techniques with and without ILM removal, to evaluate the postoperative incidence of significant macular ERM proliferations and other main transoperative- and postoperative-related complications in 230 eyes of 164 patients from May 2014 to January 2021. The total sample population was divided into three groups according to the surgical management of non-complicated macula-off RRD: buckle eye group, vitrectomy non-peeling eye group, and vitrectomy peeling eye group. Postoperative eyes that eventually developed significant secondary ERM proliferation over the macula and underwent a second surgical procedure for ERM removal were included in the peeling group. The postoperative redetachment rate was defined in the three surgical groups, and only eyes where the retina was successfully reattached for a minimum of 6 months of follow-up after the first or second surgical procedure were included in the general dataset. Thus, the final sample was composed of 230 eyes of 164 patients that met the inclusion criteria. The scleral buckle group included 125 eyes with no evidence of preoperative ERM proliferation and underwent 360° scleral buckle surgery, rhegmatogenous lesions limited cryotherapy retinopexy, and additional subretinal fluid exo-drainage in selected cases. The non-peeling group included 55 eyes without evidence of preoperative ERM proliferation and underwent primary vitrectomy with no ILM removal. Ten eyes with a significant preoperative presence of primary ERM proliferation over the macula that underwent additional planned macular ERM-ILM complex (en-bloc removal), or double-staining technique removal were assigned to the peeling eye group. Owing to the long-term follow-up of these patients, the methodology of the study made it possible to add 27 eyes from the buckle group and 13 eyes from the non-peeling group that developed significant secondary macular ERM proliferation after the first procedure to the peeling eye group; all cases had at least 6 months of postoperative follow-up after the second surgical approach, consistent with vitrectomy and vitrectomy revision with ERM-ILM complex (en-bloc excision) or two-step (double-staining) removal techniques. To exactly differentiate the complications associated with a scleral buckle from those of vitrectomy with a complimentary buckle, all vitrectomy eyes on which a supplemental scleral buckle was placed were not included in this report.

Only the charts of patients aged 18 years or older who fulfilled the inclusion criteria of a non-complicated macula-off RRD, non-myopia-related RRD (axial length $<$ 26.5 mm), no evidence of complicated RRD, presence of primary ERM proliferation, presence of secondary ERM from the buckle and ILM peeling groups without recurrent RRD, at least 6 months of follow-up, and at least one well-documented structural and functional assessment of the macula at the last follow-up visit evaluation. The exclusion criteria were as follows: prior complicated vitreoretinal surgery or intravitreal injections, trauma-related RRD, occlusive vascular tractional detachment with a rhegmatogenous component, proliferative diabetic retinopathy-related

combined rhegmatogenous and tractional RD or macular diabetic tractional RD, RRD associated with a giant retinal tear, myopic traction maculopathy macular hole associated to RRD, severe PVR recurrent and complicated RRD, presence of intravitreal silicone oil, history of active glaucoma, and placement of a supplemental scleral buckle. The elimination criteria were an impossibility for follow-up, loss of follow-up, surgery in a non-designated institution, presence of severe complications such as endophthalmitis, recurrent, complicated RRD at the last follow-up visit evaluation, and refractory corneal opacity development during follow-up.

The following postoperative assessments were statistically analyzed for the eyes in the three groups (buckle, non-peeling, and peeling groups): Long-term postoperative structural spectral-domain optical coherence tomography (SD-OCT) findings including central subfoveal thickness (CSFT), foveal contour, central subfoveal ellipsoid band status, ELM line appearance, en-face imaging analysis for the presence of dissociated optic nerve fiber layer (DONFL) defects, and the presence of ERM proliferation over the macula. Postoperative multimodal functional evaluations included the final BCVA in logarithm of the minimum angle of resolution (logMAR) units, macular retinal sensitivity (MRS), foveal retinal sensitivity (FRS), and retinal sensitivity analysis mapping assessed by microperimetry with the standard Macular Integrity Assessment (MAIA) examination standard protocol covering a 10^{o} diameter area with 37 measurements points and a light stimulus projected directly over the macula surface, with a size stimuli of Goldman III, background luminance of 4 apostilbs (asb) and maximum luminance of 1000 asb, and 36 decibels (dB) dynamic range. Fixation stability and fixation location patterns parameters are assessed by tracking eye movements 25 times/second and by plotting the resulting distribution over the scanning laser ophthalmoscope image, each movement is represented by a point, and the overall site describes the preferred retina locus (PRL). Computerized mfERG was used to detect focal (regional) outer retinal abnormalities, the amplitude and implicit time of the N1 wave, implicit time of the P1 wave, and elevation electroretinography 3-D maps were assessed in the affected eye and compared to the normal contralateral eye or to the corresponding control normative dataset. 61-hexagon 30^{o} standardized technique to test the macular electrical multifocal outer layers sensitivity point to point at the <2-degree to >15-degree central rings (<2, 2–5, 5–10, 10–15, >15 central rings) was performed at the last follow-up evaluation visit.

a) Examinations

A total of 230 eyes of 164 patients underwent a general ophthalmic evaluation and preoperative examinations, including BCVA assessment, biomicroscopy slit-lamp examination, fundus examination through a panfundoscopic contact lens, and indirect ophthalmoscopy. Cross-sectional images of the macular region were acquired along the horizontal plane through the foveal center using SD-OCT (RTVue-XR platform SD-OCT, Optovue, Inc., Fremont, CA, USA), and the axial lengths were measured using partial coherence laser interferometry (Zeiss IOL Master 700; Carl Zeiss Meditec AG, Oberkochen, Germany). The presence of a simple, non-complicated macula-off RRD or non-complicated, recurrent macula-off RRD in the three groups was confirmed by indirect ophthalmoscopy and B-scan ultrasonography (A and B Ultrasound Unit, Quantel Medical, Du Bois Loli, Auvergne, France). The postoperative microstructural evaluation was performed using SD-OCT (Spectralis OCT Heidelberg Engineering. Heidelberg, Germany) and a swept-source (SS)-OCTdevice (Topcon Medical Systems, Inc., Oakland, NJ, USA) in some cases, while postoperative functional macular evaluation was conducted with microperimetry (MP-3 MAIA Confocal Microperimeter by Metrovision, Pérenchies, France) and mfERG testing (Electrophysiology Vision Monitor Analyzer, Model MonPackONE by Metrovision). All OCT images, mfERG and microperimetry testing were analyzed by three experienced retinal co-authors from the three participating institutions.

b) Surgical procedures

A methodical, standardized, classical scleral buckle surgical procedure was performed (by one of the authors MAQR) in the buckle group consistent with traditional 505, 504, or 503, 360^{o} round Lincoff episcleral sponges (Storz model E-5395-4) and oval foam silicon sponges (506 style S 1981-5 or 501 style S 1981-4) with the newly designed profile (Labtician Ophthalmics, Inc., Ontario, Canada) around the equator of the eye and fixed with polyester 5-0 MERSILENE® Polyester Sutures, double-armed 3/8 circle spatulated needle suture (ETHICON, Johnson & Johnson, Brunswick, NJ, USA). According to the morphological appearance of the RRD, transscleral subretinal fluid (SRF) drainage assisted with a 7-0 vicryl polyglactin suture (needle P-1, 3/8 c, reverse cutting; ETHICON) was performed through the scleral wall on the selected meridian site based on previous visualization and location of large choroidal vessels to avoid potential subretinal or choroidal bleeding, which was prevented or treated by diathermy if necessary, after the SRF drainage. The eye volume and pressure lost were recovered with sterilized air. Only in the buckle group, before or after the retina was reattached, limited transscleral cryotherapy over or around the suspected rhegmatogenous lesions, preferably after retina reattachment to avoid retinal pigment epithelium (RPE) cell dispersion was applied with the assistance of a binocular indirect ophthalmoscope and a 20-diopter

condenser lens. The tenon capsule and conjunctival tissue were repositioned, carefully sutured, and fixed to the episcleral tissue with the same 7-0 vicryl polyglactin to protect the exoplant and prevent infections, conjunctival erosions, and exoplant extrusions. In the vitrectomy groups, a standard 23- or 25-gauge three-port pars plana vitrectomy (Alcon Constellation Vision System. Alcon Labs, Fort Worth, TX, USA) with a total vitreous release of the retina was performed in all eyes under local anesthesia plus sedation by one of the authors (MAQR). The vitrectomy was performed using a contact wide-angle viewing precorneal lens system (ROLS reinverted system Volk Medilex, Miami, FL, USA), the Wide Angle Viewing System with non-contact lens (Insight Instruments, Inc. Stuart, Fl. USA), or recently in the last seven cases, the Zeiss ARTEVO 800 digital ophthalmic 3-D head-up microscope with the Resight non-contact lens system (Carl Zeiss Meditec AG, Jena, Germany); this new digital microscope with a hybrid mode (coaxial and 3-d HD 4K monitor) and integrated transoperative OCT allowed for real-time retinal structural analysis and detection of ERM proliferation, thus enabling a more precise membrane stripping (Figure1D to D-5). In addition to central vitrectomy, our standard technique used a diluted triamcinolone acetonide adjuvant (Kenalog 40 mg/mL; Bristol-Myers Squibb, New York, NY, USA) to identify and better visualize the vitreous and its base and to safely perform integral removal of its cortical face from the surface of the retina using a silicone-tipped cannula with active suction prior to perfluorocarbon liquid (PFCL) infusion and reattachment of the retina, focusing on achieving a free and mobile posterior hyaloid face. The retina was reattached by a PFCL-assisted technique to effectively perform hydropneumatic retinal manipulation and assisted SRF endodrainage in all the vitrectomy eyes (peeling and non-peeling groups).

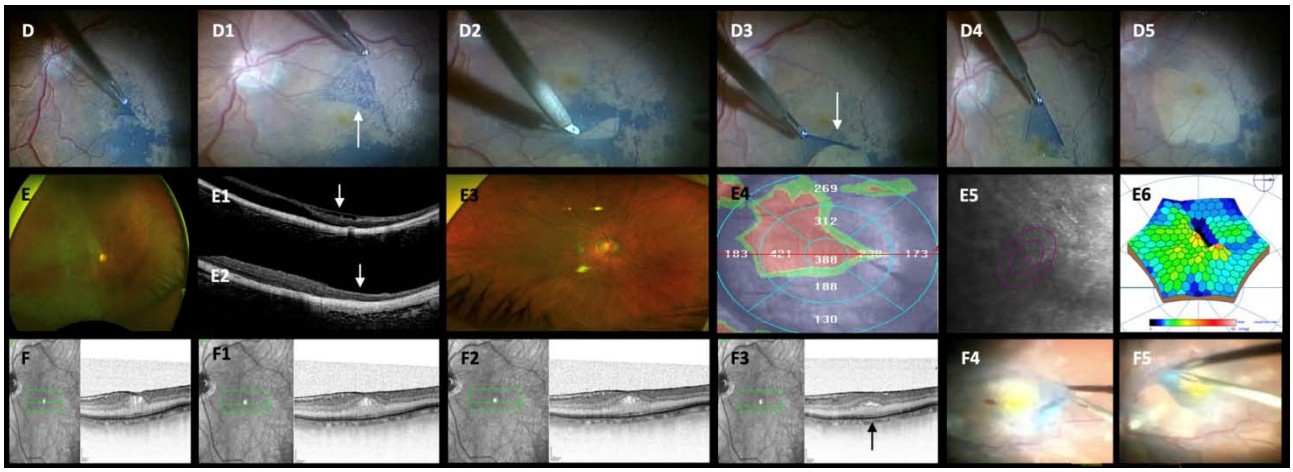

Figure 1: Postoperative structural and functional findings (part 2). (D)–(D-5) Sequence of epiretinal membrane (ERM)-internal limiting membrane (ILM)two-step removal technique events. (D) and (D-1) First-step removal of the dyed trypan blue ERM proliferation (white arrow). (D-2)–(D-5) Uncomplicated second step Brilliant Blue G-dyed ILM removal (white arrow); in this case (case 67), the final best-corrected visual acuity (BCVA) is 0.18 logarithm of the minimum angle of resolution (logMAR). (E) An Optos photo showing rhegmatogenous retinal detachment with macular wrinkling due to postoperative ERM proliferation; the extramacular retina is attached. (E-1) Spectral-domain optical coherence tomography (SD-OCT) of macular thickening with loss of foveal contour due to ERM proliferation and residual subretinal fluid (FRS)(white arrow). (E-2) Corresponding retinal thickening and retinal thinning 6 months after ERM-ILM removal; the ERM, ELM, and ellipsoid band cannot be demonstrated. (E3) AnOptos, color-corresponding photo.(E-4) Abnormal topographic thickness retinal map with irregular and diffuse macular thickening at the end of the follow-up. (E-5) Postoperative microperimetry depicting eccentric and unstable fixation patterns; macular retinal sensitivity and FRS are abnormal, and the retinal sensitivity analysis map shows abnormal macular integrity. (E-6) Abnormal three-dimension topographic map of the macular area showing a very abnormal response due to foveal photoreceptors and bipolar cell sensitivity deep reduction, with very abnormal spatial resolution due to unstable fixation and location (locus) patterns. The final postoperative BCVA after ERM removal, in this case,is0.70 logMAR.(F)–(F-3) Spectralis SD-OCT scans through the center of the macula depicting ERM proliferation associated with diffuse retinal thickening and retinal superficial layer wrinkling with cystic spaces in the Henle nerve fibers; the ELM line shows some attenuation, and the ellipsoid band is disrupted (black arrow). (F-4) and (F-5) Macular surgery sequence of the two-step technique of dyed ERM-ILM complex removal (case 66) 8 weeks after ERM-ILM removal, BVCA is 0.60 logMAR.

The vitreous base was shaved 360°, assisted with scleral depression in all the eyes that underwent vitrectomy; this scleral depression allowed removal of the vitreous traction completely from flap tears and

careful shaving and debulking of the vitreous base using mostly closed port duty cycle and low infusion pressure, even in areas of a detached retina, without producing iatrogenic retinal tears. Our young patients generally showed vitreous that was attached or only partially detached, and removal of the core vitreous was relatively straightforward; however, separation of the posterior hyaloid and other areas of adherent vitreous in the periphery with a very mobile retina was technically intricate, especially when concurrent lattice degeneration was present. Once the retina was reattached and in the absence of a scleral buckle, performing meticulous peripheral vitrectomy and ensuring that all retinal tears were identified and laser treated, were crucial; a benefit of vitrectomy in these groups was that it allowed for the removal of all vitreous opacities, treated the opacified lens capsules, and addressed the cases where macular ERM proliferation was pre- or trans-operatively confirmed. Surgical macular staining was performed using 0.15 mL of a 0.25 mg/mL (0.025%) diluted isomolar solution (pH 7.4) of Brilliant Blue G dye (BBG), to selectively stain and peel off the ILM along with the ERM (en-bloc removal technique). For the ILM-ERM en-bloc removal technique (Figure1 F-4, F-5), a 23- or 25-gauge diamond-dusted membrane scraper and 25-gauge 0.44 ILM forceps (Grieshaber Revolution DSP ILM forceps; Alcon Labs, Fort Worth, TX, USA) and a 23- or 25-gauge Finesse ILM flex loop microinstrument (Grieshaber; Alcon Labs) to facilitate the ERM and ILM removal from arcade to arcade were used. In cases where the removal was performed in two steps (double staining technique), first, trypan blue 0.15% ophthalmic solution (Membrane Blue; Dutch Ophthalmic, USA) was instilled under air to remove the ERM proliferations after washing the dye; afterwards, the MLI was stained with the aforementioned BBG dye, followed by removal (en-bloc or double staining technique removal).

We performed SRF endodrainage by creating a tiny site-selected drainage retinotomy or using preexisting endodiathermy-marked retinal breaks. First, fluid to fluid exchange was done over the retinal break to remove viscous protein aceous SRF, and also to reduce the extent of SRF and minimize the chance of trapped SRF before proceeding to an air-fluid exchange and continuing with SRF drainage. Once the retina was completely free of vitreous traction and completely reattached, argon laser endophotocoagulation around the rhegmatogenous lesions and suspected retina areas was thoroughly performed; to completely dry out the subretinal space, a second air-fluid exchange was performed, and as the last surgical step, a non-expandable bubble containing 15% perfluoropropane (C3F8) gas mixture was used as a long-acting tamponade at the end of the procedure in all the cases.

c) Statistical analyses methodology

A post-hoc power test was used to determine the power of the analyses, and descriptive and analytic statistics were employed to analize our data. Variability of the numerical variables was measured and reported as mean ± standard deviation (SD). The categorical variables are reported as counts (% frequency). For the statistical analyses, all Snellen visual acuities were converted to logMAR visual acuities according to the following formula: logMAR = -log (decimal acuity).

To determine the statistical test required, the Shapiro-Wilk normality test was used to investigate if the variables followed a normal distribution; per the results, the non-parametric Mann-Whitney U-test was used to investigate the associations of the preoperative BCVA, postoperative BCVA, and final BCVA after ERM proliferation removal in terms of the differences in medians with the numerical variables. The Kruskal-Wallis test was used to examine potential differences of the preoperative BCVA, postoperative BCVA, and final BCVA after ERM proliferation removal among the categorical variables. Furthermore, the Wilcoxon rank-sum test was used for the numeric variables, and Fisher's exact test for the categorical variables listed to investigate if the variables presented showed significant differences among the buckle, non-peeling, and peeling eye groups. Spearman's rank correlation coefficient (rho) tests investigated the potential correlations among the numeric variables listed. A generalized linear model (GLM) further investigated potential associations of the preoperative BCVA, postoperative BCVA, and final BCVA after ERM proliferation removal with the other variables listed. To determine the best model for each of these variables, a stepwise algorithm was used to choose the Akaike information criterion (AIC) model from the package step [13]. We set the significance of our tests to be $p < 0.05$. For all statistical analyses, we used R (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/). Additionally, the collected data were statistically analyzed using IBM SPSS for Windows, version 25.0 (IBM Corp. Armonk, NY. USA). The BCVA was evaluated with the Student´s t-test for related samples (statistical hypothesis test in which the test statistic follows Student's t-distribution under the null hypothesis and is used to determine if the means of two sets of data are significantly different from each other); a result of $p < 0.05$ was considered statistically significant.

III. Results

a) Results in the Buckle group

The power of the analysis was very good (Power=99.9%) for the given sample size (n=125) and for a medium effect size (Cohen's d=0.5). The results of the Shapiro-Wilk normality test showed that most of the numerical data followed a normal distribution ($p<0.05$);

hence, we decided to use the non-parametric Mann–Whitney U-test to investigate the associations of the preoperative BCVA, postoperative BCVA, and final BCVA after ERM proliferation removal, in terms of the differences in medians of these variables (Additional Tables S1, S2).

We examined 125 eyes in the buckle group, comprising 59 (47.2%) left eyes and 66 (52.8%) right eyes. From these eyes, 98 (78.4%) were in the phakic group, and 27 (21.6%) were in the pseudophakic group; the state of the lens was not statistically analyzed. The mean age of the study population was 44.3 (±15.9) years, of which 75 (60.0%) were females, and 50 (40.0%) were males. The mean preoperative period with the macula-off before surgery was 3.6 (±2.5) weeks and the mean postoperative follow-up period was 26.1 (±13.4) months with 31 eyes (24.8%) with 20/40 visual acuity or better at the end of follow-up (Table 1 and Additional Table S2).

Table 1: Baseline characteristics of the three groups

Variable	Buckle group (N=125)	Non-peeling group (N=55)	Peeling group (N=50)	P-value significance
Age (mean)	44.3 ± 15.9 sd	50.4 ± 13.5 sd	45.12 ± 15.3 sd	0.054
Sex				
-Female	75 (60%)	19 (34.5%)	18 (36%)	1.00
-Male	50 (40%)	36 (65.5%)	32 (64%)	
Preop lens status				
-Phakic	48 (78.4%)	31 (36.4%)	37 (74%)	
-Pseudophakic	27 (21.6%)	24 (43.6%)	13 (26%)	0.068
Preop macula-off (weeks)	3.6 ± 2.5 sd	4.52 ± 2.4 sd	4.30 ± 2.7 sd	0.425
Postop follow up (months)	26.12 ± 13.4 sd	25.62 ± 12.4 sd	22.66 ± 13.54 sd	0.131
Preop BCVA (mean)	1.03 ± 0.28 sd	1.036 ± 0.258 sd	1.077 ± 0.277 sd	0.386

Preop, preoperative; Postop, postoperative; BCVA, best-corrected visual acuity; sd, standard deviation

Complete descriptive statistics for the numerical and categorical variables are presented in Table 2 and Additional Tables S2 and S3. The Spearman's rank correlation coefficient test showed that there was a moderate to strong positive correlation (rho= 0.57, $p<0.01$) of the postoperative BCVA in logMAR units with the BCVA after ERM surgery. In addition, there was a weak negative correlation (rho = -0.2, $p<0.05$) between postoperative BCVA in logMAR units and follow-up period in months (Additional Table S4; Additional Figure S1).

Table 2: Summary of postoperative outcomes in the three groups

Variable	Buckle group (N=125)	Non-peeling group (N=55)	Peeling group (N=50)	P-value significance
Mean preop BCVA	1.03 ± 0.2 sd	1.036 ± 0.25 sd	1.077 ± 0.27 sd	0.386
Mean postop BCVA	0.40 ± 0.33 sd	0.28 ± 0.19 sd	0.47 ± 0.16 sd	<0.05
ERM detection (weeks)	11.93 ± 4.54 sd	18.00 ± 6.45 sd	12.57 ± 4.38 sd	0.009
RRD recurrence rate	8.8%	1 (1.82%)	12 (24%)	0.001
Mean BCVA before ERM-ILM removal	0.40 ± 0.10 sd	0.297 ± 0.23 sd	0.756 ± 0.32 sd	0.001
Mean final BCVA after ERM-ILM removal	0.43 ± 0.14 sd	0.28 ± 0.19 sd	0.48 ± 0.16 sd	<0.05
Foveal contour abnormalities	19 eyes (15.2%)	Six eyes (11.3%)	18 eyes (37.5%)	<0.05
Mean CSFT (microns)	243.57 ± 41.95	266.71 ± 32.75 sd	253.073 ± 35.66 sd	0.173
DONFL defects present	31 eyes (24.8%)	Five eyes (11.36%)	29 eyes (58%)	<0.05
IS/OS (ellipsoid band) integrity	Disrupted = 25 eyes (20%) Normal = 86 eyes (68.8%)	Disrupted = 16 eyes (29.09%) Normal = 39 eyes (70.40%)	Disrupted = 13 eyes (26%) Normal = 37 eyes (74%)	0.002

ELM line appearance	Abnormal = 24 eyes (19.2%) Normal = 86 eyes (68.8%)	Disrupted = 16 eyes (29.09%)	Disrupted = 35 eyes (76%)	0.654
mfERG alterations	Abnormal = 54 eyes (43.2%) Normal = 45 eyes (36%)	Disrupted = 13 eyes (33.3%)	Disrupted = 30 eyes (88%)	<0.05
Microperimetry alterations	Abnormal = 51 eyes (40.8%) Normal = 56 eyes (44.8%)	Disrupted = 11 eyes (25.6%)	Disrupted = 24 eyes (70.6%)	<0.05
Follow-up period (months)	26.11 ± 13.42 sd	24.80 ± 12.34 sd	21.88 ± 13.32 sd	0.133

Preop, preoperative; BCVA, best-corrected visual acuity; ERM, epiretinal membrane; RRD, rhegmatogenous retinal detachment; ILM, internal limiting membrane; CSFT, central subfoveal thickness; IS/OS, internal segment/ external segment; DONFL, diffuse optic fiber layer; mfERG, multifocal electroretinogram; sd, standard deviation

The Mann–Whitney U test showed that the preoperative BCVA in logMAR units was statistically significantly different ($p<0.05$) for the numeric variables such as age, preoperative period with the macula-off in weeks, postoperative BCVA in logMAR units, postoperative ERM detection in weeks, BCVA in logMAR units after ERM surgery, CSFT alterations (microns), and follow-up period in months (Additional Table S5 A). The postoperative BCVA in logMAR units was statistically significantly different ($p<0.05$) for the numeric variables age, preoperative period with the macula-off in weeks, preoperative BCVA in logMAR units, postoperative ERM detection in weeks, BCVA in logMAR units after ERM surgery, CSFT alterations (microns), and follow-up period in months (Additional Table S5 B). Additionally, the BCVA in logMAR units after ERM surgery was statistically significantly different ($p<0.05$) for the numeric variables age, preoperative period with the macula-off in weeks, preoperative BCVA in logMAR units, postoperative BCVA in logMAR units, postoperative ERM detection in weeks, CSFT alterations (microns), and follow-up period in months (Additional Table S5 C).

The Kruskal-Wallis test results showed that the preoperative BCVA in logMAR units was not statistically significantly different ($p>0.05$) in the buckle group when correlated with any of the categorical variables; in other words, no correlation was found among the preoperative BCVA with any of the categorical variables (Additional Table S6 A). In addition, the postoperative BCVA in logMAR units was statistically significantly different ($p<0.05$) among the following variables: re-detachment, postoperative ERM proliferation, ERM proliferation surgery, BCVA after ERM proliferation surgery, presence of submacular blood, presence of alteration on SD-OCT, mfERG and microperimetry alterations (Additional Table S6 B). Furthermore, the BCVA in logMAR units after ERM surgery was not statistically significantly different ($p>0.05$) among the groups of categorical variables (Additional Table S6 C).

The GLM for the postoperative BCVA in logMAR units showed that the postoperative BCVA in logMAR units was significantly dependent on the postoperative ERM proliferation, increasing the postoperative BCVA by 0.68 in logMAR units, and on retinal entrapment, reducing the postoperative BCVA by 0.21 in logMAR units when adjusting for potential cofounders within the multivariable analyses (Additional Table S7). The GLM also showed that the final postoperative BCVA in logMAR units after ERM surgery was significantly dependent on the postoperative BCVA, preoperative BCVA in logMAR units, and retinal perforation, increasing the postoperative BCVA in logMAR units after ERM surgery by 0.15 logMAR units.

Summarizing the clinically important statistical findings in the buckle group, the Kruskal-Wallis test revealed that the preoperative BCVA, postoperative BCVA, and final BCVA after ERM surgery were compared with all the available variables. For the preoperative BCVA, we did not find any variable that was associated. The postoperative BCVA was statistically significantly associated ($p<0.05$) with the following variables: the presence of a significant postoperative ERM proliferation, retina redetachment, ERM surgery, the presence of submacular blood, and the event of ERM proliferation removal surgery. For the final postoperative BCVA after ERM proliferation removal, we did not find any variables that showed a significant association. The GLM showed that the postoperative BCVA was statistically significant depending on the variables of postoperative ERM proliferation, increasing the postoperative BCVA by 0.68 in logMAR units, and retinal entrapment, reducing the postoperative BCVA by 0.21 in logMAR units. The GLM showed that the final postoperative BCVA after ERM proliferation surgery was statistically significant

depending on the variables of postoperative BCVA logMAR units, preoperative BCVA in log MAR units, and retinal perforation, increasing the postoperative BCVA after ERM surgery by 0.15 in logMAR units.

b) Results in the Vitrectomy groups

The power of analysis for the vitrectomy groups (peeling and non-peeling groups) was very good (Power=95%) for the given sample size (n=105) and for a medium effect size (d=0.5). The Shapiro-Wilk normality test(Additional Table S8)showed that none of the variables followed a normal distribution ($p<0.05$); hence, we used the non-parametric Mann–Whitney U-test to investigate the associations of the preoperative BCVA, postoperative BCVA, and final BCVA after ERM proliferation removal, in terms of the differences in medians of these variables.

We examined 105 eyes in the vitrectomy groups, of which 50 (47.6%) were left eyes and 55 (52.4%) right eyes. The mean age of the study population was 48.9 (±14.6) years, of which 37 (35.2%) were females and 68 (64.8%) were males. The mean preoperative period with the macula-off before surgery was 4.4 (±2.6) weeks and the mean postoperative follow-up period was 23.4 (±12.9) months (Table 1, Additional Table S9).

There were 50 eyes (47.62%) in the peeling group, 27 eyes (23.2% incidence of secondary ERM after buckle procedure) from the buckle group, 13 eyes (23.63% incidence of postoperative secondary ERM proliferation after primary vitrectomy) from the non-peeling group, and 10 eyes (4.34% prevalence of ERM in primary non-complicated macula-off RRD in the whole sample studied in this report) initially diagnosed as having a primary ERM proliferation. The non-peeling group comprised 55 eyes (52.38%).

The Wilcoxon rank-sum tests for the numeric variables (Additional Table S10) and the Fisher's Exact tests for the categorical variables showed that the variables such as first surgery (Additional Table S11), BCVA in log MAR units before ERM-ILM removal, recurrent RRD, additional surgery, postoperative ERM proliferation detection in weeks, final postoperative BCVA, postoperative foveal contour, presence of DONFL defects, mfERG and microperimetry alterations demonstrated statistically significant differences ($p<0.05$) among the peeling and non-peeling groups (Table 2).

The Spearman's rank correlation coefficient test showed a strong positive correlation (rho= 0.78, $p<0.01$) of the BCVA in logMAR units before ERM-ILM removal and the final postoperative BCVA in logMAR units (Additional Table S12).

In addition, the Spearman's rank correlation coefficient test showed a weak positive correlation (rho= 0.32, $p<0.05$) between the preoperative period with the macula-off in weeks and the CSFT findings in microns; it also showed a weak negative correlation (rho= -0.29, $p<0.05$) between the preoperative BCVA in logMAR units and ERM detection in weeks (Additional Figure S2).

The Mann–Whitney U test comparing the peeling versus the non-peeling groups showed that the preoperative BCVA in logMAR units was statistically significantly different ($p<0.05$) for the numeric variables of age, preoperative time period with the macula-off in weeks, BCVA in log MAR units before ERM-ILM removal, ERM detection in weeks, final postoperative BCVA in logMAR units, mean CSFT, and follow-up period in months (Additional Table S13).

The Mann-Whitney U test showed that the postoperative BCVA in logMAR units was statistically significantly better ($p<0.05$) for the numeric variables of age, preoperative period with the macula-off in weeks, BCVA in logMAR units before ERM-ILM removal, ERM detection in weeks, final postoperative BCVA in logMAR units, mean CSFT, and follow-up period in months.

The Mann–Whitney U tests showed that the final BCVA in logMAR units after ERM proliferation removal was statistically significantly different ($p<0.05$) for the numeric variables of age, preoperative time period with the macula-off in weeks, BCVA in log MAR units before ERM-ILM removal, postoperative ERM detection in weeks, final postoperative BCVA in logMAR units, CSFT alterations, and follow-up period in months.

The Kruskal-Wallis test showed that the preoperative BCVA in logMAR units was statistically significantly different (Kruskal x2= 4.17, $p<0.05$) with the ellipsoid band alterations when compared with the other variables (Additional Table S14 A). In addition, the postoperative BCVA in logMAR units was statistically significantly different ($p<0.05$) among preoperative lens status, preoperative ERM, first surgery, recurrent RRD, additional surgery, postoperative ERM proliferation detection in weeks, foveal contour, presence of DONFL defects, mfERG, and microperimetry alterations (Additional Table S14 B). Furthermore, the final BCVA in logMAR units after ERM proliferation removal was statistically significantly different ($p<0.05$) among the preoperative ERM proliferation, first surgery, recurrent RRD, additional surgery, postoperative ERM proliferation detection, foveal contour abnormalities, DONFL defects, mfERG abnormalities, and microperimetry alterations (Additional Table S14 C).

The GLM for the preoperative BCVA in logMAR units showed that no variable was associated with the preoperative BCVA in log MAR units when adjusting for cofounders with multivariable analyses (Additional Table S15 A). It also showed (Additional Table S15 B) that the postoperative BCVA in logMAR units was significantly positively associated with the presence of significant ERM proliferation in the postoperative ERM proliferation analysis (coefficient=0.45, $p<0.01$); significantly negatively associated when only vitrectomy (non-peeling

group) was performed in the first surgery variable (coefficient = -0.23, $p<0.01$); and significantly negatively associated with the variable preoperative period of macula-off in weeks (coefficient= -0.02, $p<0.05$; Additional Figure S3). In addition, the GLM for the final BCVA in log MAR units after ERM proliferation removal showed that it was significantly positively associated ($p>0.01$) with the postoperative BCVA (Additional Figure S4), when only vitrectomy was the first surgery variable, and with the preoperative BCVA (Additional Figure S5) and male variable, when vitrectomy and ERM-ILM removal was the first surgery variable (Additional Table S15 C).

In the three groups in which a total of 230 eyes were analyzed, the general prevalence of preoperative primary ERM proliferation was 4.78% (11 eyes), but only 10 eyes (4.34%) underwent surgery; however, this prevalence should not be statistically considered due to the heterogeneity of criteria used to define a preoperative primary or postoperative secondary ERM proliferation and because the eyes without evidence of preoperative ERM proliferation were intentionally selected, and 10 out of 11 eyes detected with preoperative significant primary ERM proliferation were directly assigned to the peeling group.

The statistical program yielded the following SD-OCT abnormalities in the peeling group: ellipsoid band disruption was observed in 57.9%, CSFT abnormalities in 94.7%, ELM line alterations in 42.1%, mfERG alterations in 89.5%, and an abnormal microperimetry was detected in 78.9% of the eyes. In the non-peeling eye group, ellipsoid band disruption was observed in 21.3%, CSFT abnormalities in 17%, ELM line alterations in 31.9%, abnormal mfERG in 8.5%, and an abnormal microperimetry in 6.3% of the eyes (Table 2).

In the buckle group, the mean postoperative BCVA in logMAR units (0.40±0.33 SD) was statistically significantly associated ($p<0.05$) with the following variables: the presence of a significant postoperative ERM proliferation, the event of a retinal redetachment, ERM surgery, the presence of macular blood, and the event of ERM proliferation removal surgery. The GLM demonstrated that the final postoperative BCVA in logMAR units (0.43±0.14 SD) after secondary ERM proliferation removal was statistically dependent on the following variables: postoperative BCVA after the first surgical procedure (buckle or primary vitrectomy), preoperative BCVA, and retinal perforation as a complication due to the buckling procedure and increased postoperative BCVA after ERM surgery by 0.15 logMAR units.

Analyzing the numeric variables mentioned with Wilcoxon rank-sum test and Fisher's exact test for the categorical variables (first surgery, BCVA before ERM-ILM complex removal, recurrence of RRD, additional surgery, ERM period detection, postoperative foveal contour appearance, DONFL defects, mfERG, and microperimetry alterations), we observed a statistically significant difference ($p<0.05$) with better final BCVA in favor of non-peeling eye group (Additional tables S2 and S11). We used one-factor ANOVA test to compare the postoperative BCVA with the buckle group, the non-peeling group, and the peeling group, and the resultant p-value was 0.001 ($p<0.05$).

In the non-peeling group, we compared postoperative BCVA and abnormal findings on OCT (ellipsoid band, CSFT, ELM line). When comparing the ellipsoid band as a biomarker with the postoperative BCVA in logMAR units, student's t-test was performed, resulting in a $p=0.001$, with a Pearson correlation coefficient of 0.314; hence, a larger value of logMAR was associated with more ellipsoid band disruptions. Further, we compared CSFT with postoperative BCVA in logMAR units, and performed Student's t-test, we obtained the p-value as 0.001 ($p< 0.05$), with a Pearson correlation coefficient of 0.403; hence, a higher BCVA in log MAR units was associated with more CSFT abnormalities. Similarly, on comparing ELM with postoperative BCVA in logMAR units and performing Student's t-test, we obtained the p-value as 0.001 ($p< 0.05$), with a Pearson correlation coefficient of 0.192, showing that a higher logMAR was associated with a greater presence of ELM line abnormalities.

The above analyses also applied to those eyes in the peeling group after ERM proliferation removal complemented with ILM removal. On comparing ellipsoid band disruptions with postoperative BCVA in logMAR units, and subsequently performing the Student's t-test, we obtained the p-value as 0.001 ($p<0.05$) and a Pearson correlation coefficient of 0.061. We observed that a higher value of BCVA in logMAR units was associated with more ellipsoid band disruptions.

On comparing CSFT alterations with postoperative BCVA in logMAR units, the Student's t-test showed p-value of 0.001 ($p<0.05$) and a Pearson correlation coefficient of 0.13. Thus, we observed that a higher value of logMAR was associated with more CSFT alterations.

The relationship of ELM line alterations and postoperative BCVA in logMAR units showed a Student's t-test result of $p=0.001$ ($p<0.05$) and a Pearson correlation coefficient of -0.102. In this case, we observed that a higher BCVA in logMAR units was associated with a lower incidence of ELM line alterations in the SD-OCT.

A correlation was sought between the presence of DONFL defects (dimples) in the peeling group according to the postoperative BCVA in logMAR units. In the non-peeling group, no eyes developed dimples regardless of their BCVA. In the peeling group, the mean postoperative BCVA in logMAR units of eyes that did not have dimples was 0.52±0.14 SD, and the mean postoperative BCVA in logMAR units of eyes that

developed dimples was 0.59±0.16 SD. A necessary comparison of these values was performed to check if the data came from a normal distribution. Hence, the Shapiro-Wilk test was performed, which resulted in 0.89; therefore, coming from a normal distribution, Student's t-test was performed for independent samples, which resulted in $p=0.32$ ($p>0.05$), thereby indicating the absence of a statistical significance.

The postoperative BCVA in logMAR units in the peeling group that did not have ERM proliferation according to the SD-OCT was analyzed and correlated; in this way, no statistical significance was detected in the vision between the eyes with and without SD-OCT abnormalities such as ellipsoid band disruptions ($p=0.848$, $p>0.05$, respectively), CSFT alterations ($p=0.05$), ELM line abnormalities ($p=0.653$, $p>0.05$), mfERG abnormal findings ($p=0.74$, $p>0.05$), and microperimetry alterations ($p=0.20$, $p>0.05$).

The same comparisons were made in the non-peeling eye group who developed ERM proliferations. The BCVA in logMAR units correlated with the presence of ellipsoid band abnormalities, ELM line abnormalities, and mfERG alterations, and microperimetry abnormalities was compared with those of eyes without such defects; we did not find any significant differences ($p>0.05$).

Further, the same groups were compared but without consideration to the presence of an ERM proliferation, a positive statistical significance ($p<0.05$), and BCVA correlation, when CSFT, mfERG abnormalities, and microperimetry alterations were comparatively analyzed between eyes with and without these abnormalities.

Moreover, microperimetry and mfERG revealed abnormal retinal responses with a stable but extrafoveal (eccentric) fixation pattern, a profound reduction in N1- and P1-wave nV amplitudes, and a prolonged P1 implicit time predominantly in the ILM peeling group. The functional responses were predominantly normal in the buckle and non-peeling groups without postoperative ERM proliferation.

Finally, in the peeling group, there was neither statistical significance ($p=0.819$, $p>0.05$) nor visual correlation when the postoperative BCVA in logMAR units was compared between eyes with the presence of DONFL defects and those without it.

In the buckle eye group, more additional surgeries were needed for complications such as recurrent RRD (11 eyes) with an additional surgery rate (ASR) of 8.8%, ERM-ILM complex removal (27 eyes; ASR of 21.6%), buckled revision (4 eyes; ASR of 3.2%), phaco-vitrectomy (3 eyes; ASR of 2.4%), vitrectomy (2 eyes; ASR of 1.6%), phaco-vitrectomy ERM-ILM complex removal (1 eye; ASR of 0.8%), vitrectomy ERM-ILM complex removal (1 eye; ASR of 0.8%), and other serious surgical complications that were treated conservatively and without surgery such as through and through complication drainage phenomenon (8 eyes; 6.4%), retinal perforation (7 eyes; 5.6%), transoperative presence of submacular blood as a complication of SRF drainage or full-thickness scleral perforations (5 eyes; 4.0%) handled with pneumatic displacement, and non-complex vitreoretinal entrapment released with surgical maneuvers in the first surgery (3 eyes; 2.4%), with a general ASR of 37.6% in the buckle group (Additional Table S3). The ASR seen in the vitrectomy group was 9.6% (12 eyes), with vitrectomy revision in 9 eyes (8.6%), only vitrectomy 2 eyes (1.9%), and phako-vitrectomy ERM-ILM peeling 1 eye (1.0%). The comparative incidence of early or short-term postoperative complications between the buckle group and the vitrectomy groups that required additional surgical procedures was statistically significant ($p<0.05$ Student's t-test).

IV. Discussion

Skill and practice are needed to place a scleral buckle in the correct location with the desired indentation to support the vitreous base and retinal tears and to drain transscleral SRF without complications. The use of vitrectomy techniques has expanded greatly nowadays owing to unprecedented advances in vitrectomy platforms, development of more rigid small-gauge cutters with improved fluidics and better instrumentation, and the widespread availability of wide-angle viewing systems with superior endoilluminators. Some studies suggested that vitrectomy techniques alone should be employed in the management of a simple, primary, non-complicated macula-off RRD. While some cases can be managed successfully with vitrectomy, an important subset of non-complicated, macula-off RRD will benefit from buckling techniques. All surgical approaches in this retrospective report were performed to achieve the patient's best interest and to determine the best technique for particular circumstances of RRD. To achieve these, we retrospectively analyzed the charts of scleral buckling techniques in 125 consecutive selected eyes which fulfilled the inclusion criteria and primary vitrectomy or vitrectomy without and with ILM removal in 105 selected eyes which also fulfilled the inclusion criteria that were treated for non-complicated macula-off RRD; we conducted a retrospective, long-term, multicenter, one-surgeon, comparative structural and functional macular evaluation (Figure2 control normal eye; images 1A–1A-6); further, we reported our experience of the real-life postoperative incidence of ERM proliferation over the macula and statistically intercorrelated those findings across the groups. The study aimed to evaluate the main complications of buckling surgery (Figure3 C–P images) and vitrectomy (Figure4 A–H-2 images; Figure5 I–T images) among the groups.

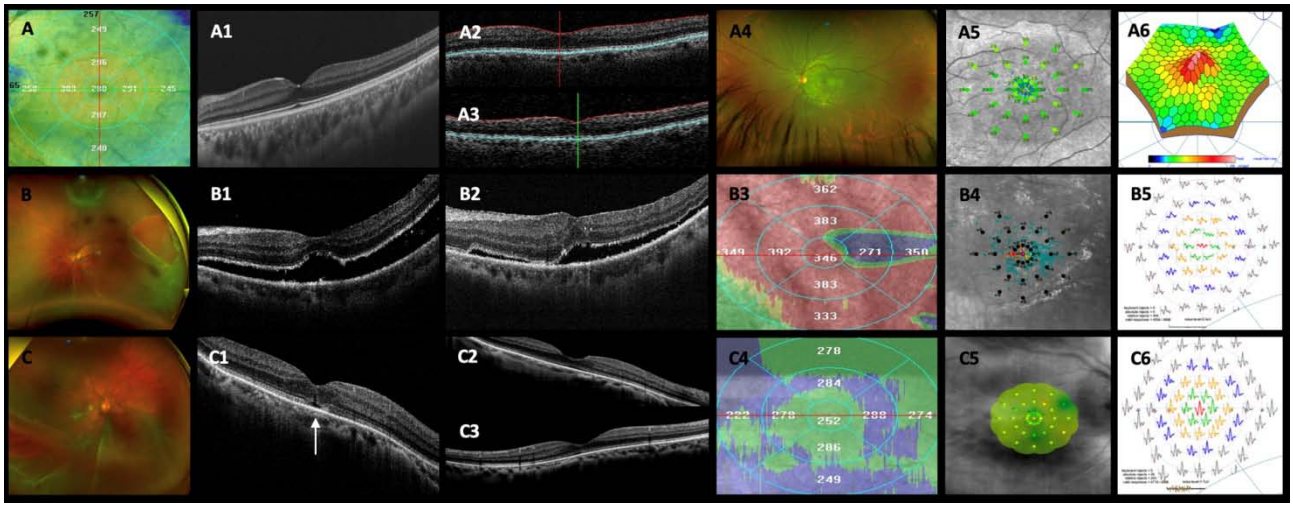

Figure 2: Postoperative structural and functional findings (part 1).

(A)–(A-5) Normal control eye. (B) Primary rhegmatogenous retinal detachment (RRD); large posterior rolled edge retinal tear at 2 o'clock meridian managed with primary vitrectomy (case 2; non-peeling group). (B-1) Spectral-domain optical coherence tomography (SD-OCT) horizontal scan with postoperative subretinal fluid (SRF) 3 weeks after vitrectomy. (B-2) Shallow amount of SRF 8 weeks later. (B-3) Abnormal topographic thickness retinal map on Ret-vue SD-OCT with diffuse retinal thickening. (B-4) Macular microperimetry showing eccentric foveal fixation. (B-5) Corresponding multifocal electroretinogram (mfERG) depicting abnormal electrical response in three central rings with the nV decreased; final best-corrected visual acuity (BCVA) is 0.18 logarithm of the minimum angle of resolution (logMAR) units. (C) An Optos photo showing primary RRD involving the macula; arrow-shaped retinal tears are seen at 7 o'clock, and there is preoperative epiretinal membrane (ERM) proliferation (case 87; peeling group). (C1) SD-OCT image 8 weeks postoperatively depicting defects in the ellipsoid and external limiting membrane (ELM) disruption (white arrow). (C-2) and (C-3) Macula crossline scans, with an ellipsoid and ELM biomarkers recovered. (C-4) Postoperative normal topographic thickness macula map after undergoing a successful, two-step ERM- internal limiting membrane (ILM) removal technique. (C-5) Macular microperimetry with macula retinal sensitivity, foveal retinal sensitivity, and a stable foveocentral fixation pattern; the retinal sensitivity analysis map shows normal macular integrity at the end of the follow-up. (C-6) mfERG of the corresponding macular area. The P1 implicit time is normal in the <2-degree central ring and slightly longer in the remaining central rings. The nV amplitude in the normal range is comparable to the normal age-matched control eye, and the BCVA is 20/40 (0.30 logMAR units). (D)–(D-5) Sequence of macular ERM-ILM two-step removal technique events.

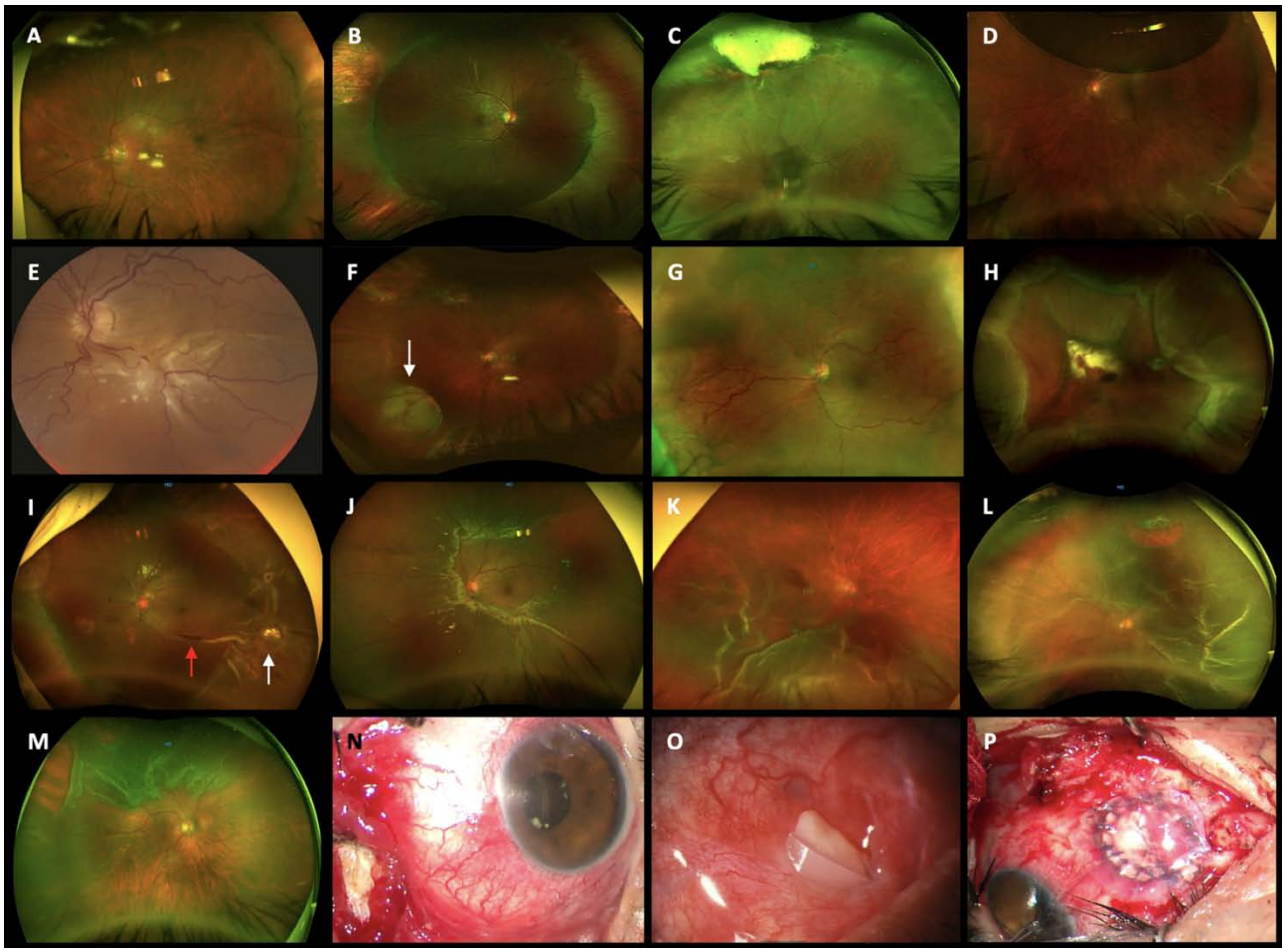

Figure 3: Transoperative and postoperative buckle complications.

(A) and (B) Buckled rhegmatogenous retinal detachment (RRD) (cases 4 and 58); final best-corrected visual acuity (BCVA) is 0.18 logarithm of the minimum angle of resolution (logMAR). (C) Whitish condensed vitreous hemorrhage; a recurrent RRD is detected; the eye underwent vitrectomy. Six weeks after, retina remains attached with epiretinal membrane (ERM); logMAR is 0.60 (D) Buckled eye with 15% sulfur hexafluoridegas; there is a tear at the 5 o'clock with RRD (case 88); after positioning and laser, the vision is 0.30 logMAR. (E) Buckled eye with recurrent RRD (case 15); a retinal fold resolved with ERM-internal limiting membrane (ILM) removal; final BCVA logMAR is 0.40 (F) Case 19. A postoperative granuloma (white arrows) 6 weeks after surgery; a low-grade inflamed course persists; presence of a dome-shaped granuloma (white arrow) at the 7 o'clock meridian, which resolves periocular antibiotics; final logMAR was 0.18is 20/30. (G) Choroidal hemorrhagic detachment after scleral perforation. (H) A 360-degree, non-kissing, hemorrhagic choroidal detachment after a complicated scleral buckle procedure. (I) Vitreoretinal entrapment (white arrow) with retinal fold and preretinal blood (red arrow); BCVA is 20/100 (0.70 logMAR). (J) An Optos photo depicts a recurrent RRD 6 weeks after a buckling procedure (case 112); there is ERM proliferation and PVR over the posterior pole; ERM-ILM removal was performed; final logMAR was 0.60. (K) Scleral perforation; submacular blood displacement is required, and the eye has undergone ERM-ILM removal; BCVA is 0.60 logMAR (case 93). (L) leaking retinal tear; the eye undergoing phaco-vitrectomy; final BCVA is 0.30 logMAR (case 54). (M) A failed buckling (case 43) with a rolled posterior edges retinal tear; ERM proliferation removal; BCVA is 0.40 logMAR. (N) Sponge exposition 32 months after surgery (case 40). (O) Hard silicone extrusion. (P) Scleral patch and amnios graft for buckle-related scleral thinning.

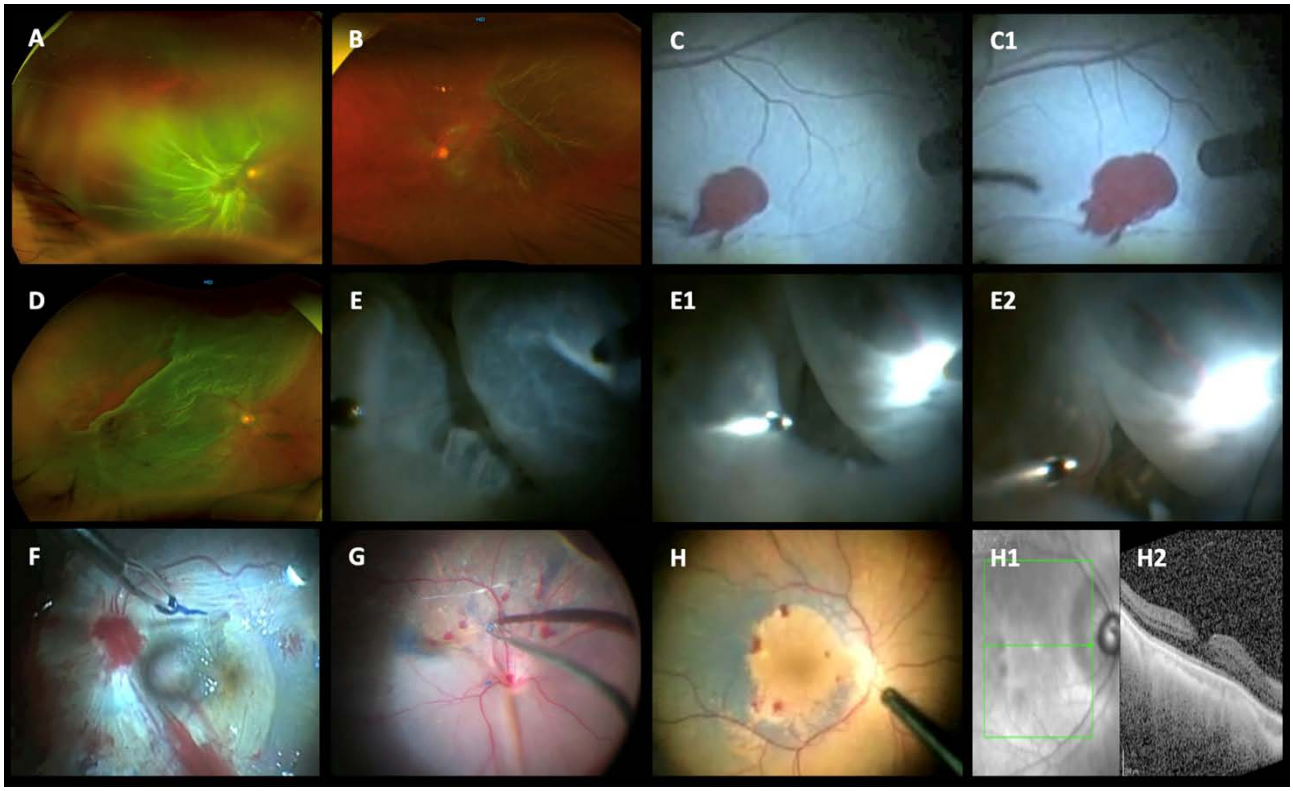

Figure 4: Transoperative and postoperative vitrectomy complications (part 1).

(A) Retinal detachment complicated by posterior proliferative vitreoretinopathy; the retina is totally detached, and the macula appears contracted due to the presence of diffuse epiretinal proliferation. (B) An Optos, color photo of are current rhegmatogenous retinal detachment (RRD) in a failed primary vitrectomy; there is no gas tamponade inside the eye, and the retina is detached mainly over the posterior pole with the macula off; the patient undergoing vitrectomy revision with laser endophotocoagulation. (C) and (C-1) A rather dim brilliant Blue G (BBG) internal limiting membrane (ILM) staining with arterial bleeding at the time of pulling the ILM in a case of a shallow macula-off retinal detachment; this complication is resolved by raising the transoperative intraocular pressure for a few minutes. (D) Complicated RRD 3 weeks after a failed gas-vitrectomy and epiretinal membrane-ILM removal procedure; the retina looks rigid, and there is a large tear with a posterior rolled edge. (E)–(E-2) Sequential hydraulic choroidal and retinal detachment as a transoperative complication due to erroneous positioning of the infusion cannula; the hydraulic complication grows progressively as the cut and suction instrument is working, and by changing the entrance of the infusion cannula, the complication is resolved. (F) Bleeding from the papilla as we peel off the ILM in this macula-off RRD case; in this case, BBG ILM staining-perfluorocarbon heavy liquids are used to reapply the retina-ILM peeling; (G) Tractional bleeding at the moment of the ILM being pulled to release the macula. (H) Multiple spots bleeding due to inner punctate hemorrhagic retinopathy related to ILM peeling. (H-1) and (H-2) En-face superficial imaging of the presence of dark, well-delineated, superficial retinal spots compatible with dissociated optic nerve fiber layer defects; in this case, there is no evidence of superficial dimples on the corresponding Spectralis horizontal spectral-domain optical coherence tomography.

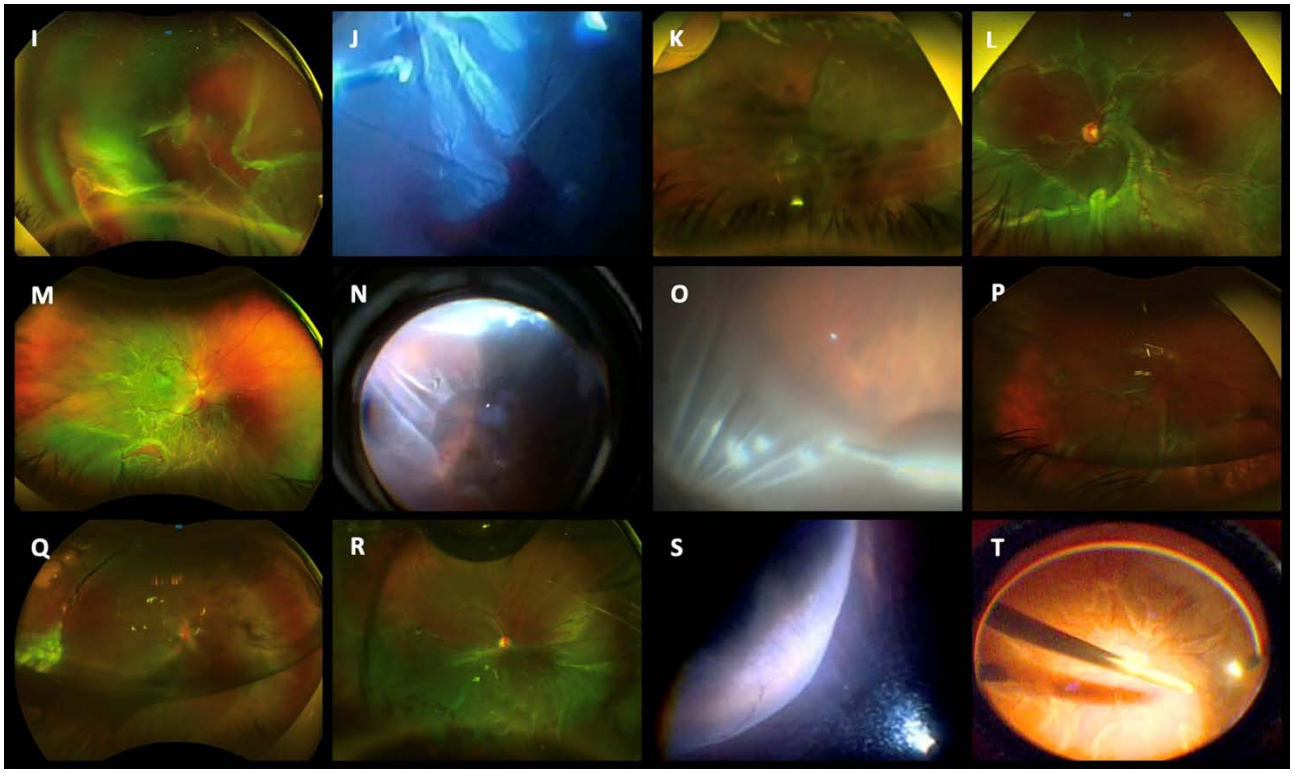

Figure 5: Transoperative and postoperative vitrectomy complications (part 2).

(I) An Optos, wide-angle, color fundus depicts a hemorrhagic choroidal rhegmatogenous retinal detachment (RRD) detected 3 days after primary vitrectomy. (J) Highly complex vitreoretinal entrapment at the level of superior trocar sclerotomy due to undetected transient eye hypotony secondary to transoperative surgical manipulation of the retina. (K) Evidence of vitreous, choroidal, and subretinal bleeding with the persistence of RRD. (L) An Optos, wide-angle, color photo 6 weeks after primary vitrectomy with proliferative vitreoretinopathy (PVR) complicated by RRD as a late vitrectomy complication in the management of primary, non-complicated RRD. (M) Subtotal RRD after primary vitrectomy; an active, leaking retinal tear with rolled-back borders can be seen between the 6 and 7 o'clock meridians; there is evidence of macular rigidity and contraction due to the presence of diffuse epiretinal membrane (ERM) retina proliferation. (N) Transoperative vitreoretinal entrapment at the level of the entry vitrectomy site; an active leaking arrowed-shaped retinal tear is observed at 11 o'clock meridian at the equator zone. (O) shows another transoperative image with a vitreoretinal entrapment at the entry vitrectomy infusion site. (P) shows a low-grade illumination transoperative step of a recurrent complicated PVR case after primary vitrectomy. (Q) A failed buckling of recurrent RRD that has undergone ERM-internal limiting membrane (ILM) complex removal due to significant ERM macular proliferation; there are some recent argon laser spots and a 70% residual sulfur hexafluoride (SF_6) gas bubble with a shallow recurrent retinal detachment. (R) Recurrent inferior RRD after primary vitrectomy with residual SF_6 gas bubble. (S) Hydraulic choroidal and pars plana detachment is caused by mispositioning of the infusion line of the trocar entry sclerotomy site. (T) shows a total recurrent RRD in a pseudophakic eye 30 days after primary vitrectomy with ERM-ILM complex removal due to significant macular ERM proliferation managed with the in-block ERM-ILM technique.

In cases such as those described in the vitrectomy groups in this study, we believe that adding a buckle is unnecessary and adds additional risk and possible undesirable postoperative complications and cost to an already sophisticated procedure; hence, to analyze the complications of scleral buckling (Figure3 D–P) and vitrectomy techniques (Figures4 A–H-2 images; Figure5 I–T images), only eyes without a supplemental scleral buckle were included in the final statistical analyses. The management of non-complicated RRD with scleral buckling was compatible with good anatomic outcomes (Figures3 A and B images); however, this procedure can be associated with transoperative and postoperative complications (Figure3 C–P images), leading to performing additional surgery. Hence, we included only eyes with non-complicated macula-off RRDs, analyzed their management and incidence of complications retrospectively, and compared the incidence of postoperative ERM proliferation and surgical complications as well as structural and functional findings in all three groups (Figure2 B–B5, C–C6; Figure1 E–E6, F–F3; Figure6 A–A5, B–B6). Currently, in the management of macula-off RRD with vitrectomy, we placed a supplemental 360° scleral buckle only in complex or complicated cases involving diffuse tractional membranes such as RDs complicated with significant PVR, failed prior RRD surgery, extensive peripheral vitreoretinal adhesions with multiple retinal tears, RRD associated with penetrating globe-injury

and/or retain intraocular foreign body, and selected RRD associated with giant retinal tears.

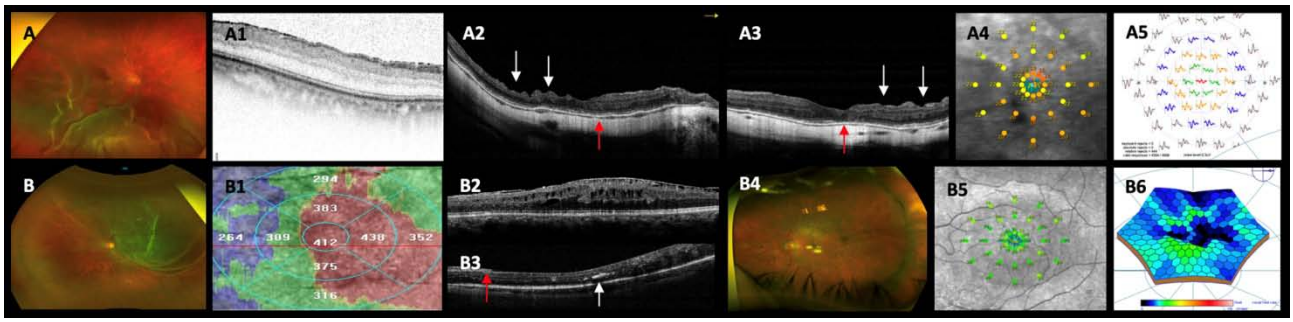

Figure 6: Postoperative structural and functional findings (part 3).

(A) Optos photo of a failed gas-vitrectomy in rhegmatogenous retinal detachment (RRD)(case 78). (A-1) Corresponding macula scan after vitrectomy revision with postoperative epiretinal membrane proliferation with logarithm of the minimum angle of resolution of 1.0 units; the eye underwent a third procedure for ERM-internal limiting membrane removal; final BCVA is 0.70 logMAR. (A-2) and (A3) Foveal crossline depict retinal thinning and dimpling of the superficial nerve fiber layer (white arrows) due to dissociated optic nerve fiber layers defects and loss of foveal contour; the ELM line and ellipsoid show irregular reflectivity (red arrows). (A-4) Macular microperimetry with a reduction in macular retinal sensitivity (MRS); the retinal sensitivity analysis shows abnormal integrity. (A-5) Abnormal multifocal electroretinography (mfERG) response with N1 wave amplitude reductions. (B) An Optos photo of a primary RRD undergoing an uneventful 360-degree 503 round sponge buckling-cryotherapy with subretinal fluid drainage; After 6 weeks, macular thickening associated with epiretinal membrane (ERM) proliferation is depicted in (B-1), with diffuse retinal thickening in the abnormal topographic retinal map.(B-2) Corresponding horizontal scan depicting diffuse macular thickening associated with ERM proliferation and wrinkling of the inner superficial retina layers; multiple deep cystic spaces (sponge-type) and submacular fluid are seen; although the ellipsoid band appears preserved, the ELM line is not; final BCVA is 0.90 logMAR. (B-3) After ERM-ILM removal, the BCVA is 0.70 logMAR. The macula looks atrophic; there are hyper reflective deep lines (white arrow) and ILM remnants (red arrow); no evidence of the ELM and ellipsoid are found. (B-4) An Optos, wide-angle photo of the corresponding cases shows substantial, but non-significant, visual improvement (case 43; peeling dataset). (B-5) The corresponding microperimetry shows subnormal macular integrity with subnormal MRS; stable foveocentral fixation is seen.(B-6) Abnormal three-dimension topographic map of the corresponding abnormal mfERG.

Although the use of a supplemental scleral buckle has evolved throughout the years, its selective use seems to be compatible with good outcomes in non-complicated cases[14]. However, in complex cases with total RD, significant PVR, and posterior insertion of the vitreous base, additional scleral depression to reach the pathological vitreous base to facilitate its dissection must be performed to facilitate vitreous base shaving and release vitreous traction at this level, in addition to the proper placement of a 360° scleral cerclage. Other surgical maneuvers that are considered extreme, such as circumferential retinotomy and retinectomy, are rarely performed[15]; this is because vitrectomy has a better anatomical outcome in such complicated cases when complemented with scleral buckling.

Some surgeons peel the ILM off only if there are pre-existing ERM proliferations in the macula[16], as we reported in the peeling vitrectomy group, while others never perform routine ILM peeling to prevent postoperative ERM proliferation and prefer its removal during a second surgery if there is ERM proliferation occurrence and according to the sight evolution[15-17], this means that they will need an additional vitrectomy procedure only if they are highly symptomatic or show significant structural and functional alterations in the macula due to the secondary postoperative presence of ERM proliferation. The incidence of postoperative ERM proliferation has been reported to range from 27.6% to 38.4% after cryoretinopexy and from 21.5% to 58% after vitrectomy without ILM removal.[12,17,18] Herein, we reported a postoperative ERM proliferation incidence of 23.2% (29 eyes) in the buckle group, 23.63% (13 eyes) in the non-peeling group, and 2.0% (one eye) in the peeling group (Figure1 E1–E2 and F–F3 images).

A previous prospective and comparative study[19] did not identify any functional or structural benefits of ILM peeling during primary vitrectomy for non-complicated RRD; the authors showed a very low incidence (0.003%) of significant ERM in eyes where ILM peeling had been performed and found that these patients had a lower final BCVA than those whose ILM had not been removed (mean logMAR units BCVA 1.0±0.4 vs. 0.4±0.2, $p<0.001$); these functional findings were also found in our study. However, in a retrospective report, Garweg et al.[20] described an unprecedented visual gain over 6 months after successful primary reattachment surgery with peeling of the ILM and sulfur hexafluoride gas tamponade, which did not show the same results as the peeling group in our study. Some authors [19] and the authors of the present study agree that although ILM peeling prevents ERM, it results in a poorer visual outcome in such non-complicated macula-off RRD cases and may therefore be better reserved only for selected complicated cases.

In this study, in the vitrectomy groups, we found that some variables, such as first surgery, BCVA before ERM-ILM removal, recurrence of ERM, additional surgery (Figure1 F-4, F-5), ERM proliferation detection in weeks, final postoperative BCVA, foveal contour abnormalities (Figure 4 D-3 image), DONFL defects (Figure 4 C-2 and C-3 images), mfERG and microperimetry findings alterations (Figure7 C-4 and C-5 images) were more common in the peeling group than in the non-peeling group with significant statistical differences ($p<0.05$) between the peeling and non-peeling groups analysis (Additional Table S5). However, the functional analysis of these structural abnormalities in SD-OCT considered as categorical variables such as ellipsoid band disruptions, CSFT abnormalities, and ELM line discontinuities could not be found a direct correlation with the final BCVA due to a lack of statistical significance.

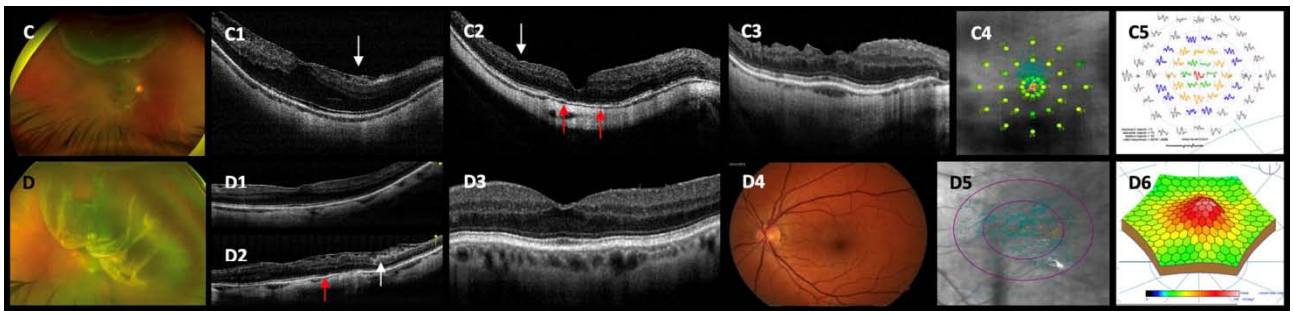

Figure 7: Postoperative structural and functional findings (part 4).

(C) An Optos photo showing rhegmatogenous retinal detachment with epiretinal membrane proliferation (case 23). (C-1) Horizontal foveal scan with macular thickening and subretinal fluid; ERM proliferation is shown (white arrow); foveal thinning and loss of the external limiting membrane (ELM) line with ellipsoid band reflectivity attenuation. (C-2) Horizontal foveal scan of the foveal contour with a thin fovea; dimples are seen (white arrow); ELM looks discontinuous, and the ellipsoid band shows hyporeflectivity (red arrows). (C-3) After 4 months,the vertical foveal scan depicts normal central subfoveal thickness (CSFT); there is loss of the foveal contour and dimpling of the superficial retinal layers. The ELM line and ellipsoid band look normal. (C4) Abnormal macular microperimetry. (C5) Multifocal electroretinography abnormal electrical response. (D) An Optos photo with a bullous pseudophakic RRD (case 6; peeling group) undergoing primary vitrectomy; After 8 weeks, ERM proliferation is depicted on the foveal crossline scan. (D-1) and (D-2) ERM proliferation is clearly seen (white arrows) along with wrinkling of the superficial retina. At this stage, the ELM line looks discontinuous, and the ellipsoid shows disruption (red arrow); the best-corrected visual acuity (BCVA) at this point is 0.48 logarithm of the minimum angle of resolution (logMAR). (D-3) After 12 weeks after, a normal foveal contour is depicted within a normal CSFT; there is a hyper reflective horizontal line below the fovea and a non-cyst hypo reflective space. The reflectivity of the ELM line appears attenuated with discontinuation and a recovered ellipsoid. (D-4) Corresponding color fundus without relevant clinical macular details. (D-5) Abnormal microperimetry, with a central fixation pattern; the retinal sensitivity analysis map shows normal integrity. (D-6) Normal three-dimension topographic macular map compared with the normal matched-age normal control; the final BCVA after ERM-internal limiting membrane complex removal is 0.30 logMAR at the end of follow-up.

Herein, we studied 230 consecutive selected eyes and retrospectively analyzed the cases, and we found functionally unsatisfactory results in the ILM peeling group compared with those in the buckle and non-peeling eye groups. The postoperative BCVA in logMAR units was significantly associated ($P<0.05$) with the following variables: the presence of significant postoperative ERM proliferation, retinal redetachment, the presence of submacular blood, and the event of ERM proliferation surgery, which means that the presence of any of these variables significantly influences the final visual result. The GLM showed that postoperative BCVA was statistically significantly correlated with the following variables: postoperative ERM proliferation, which increased the postoperative BCVA by 0.68 in logMAR units, and retinal entrapment, which decreased the postoperative BCVA by 0.21 in logMAR units. The GLM also showed that the final postoperative BCVA after ERM surgery was statistically significantly dependent on the following variables: postoperative BCVA in logMAR units, preoperative BCVA in logMAR units, and retinal perforation event, which increased the postoperative BCVA after ERM surgery by 0.15 in logMAR units.

The anatomical results regarding successful reattachment of the retina were satisfactory in the three groups; however, we found a significant percentage of postoperative ERM proliferation in the buckle and in the non-peeling groups. Although we found only one eye with postoperative ERM proliferation in the peeling group, we observed multiple structural alterations in the SD-OCT biomarkers, as mentioned earlier, along with multiple functional alterations with a significant reduction in retinal sensitivity. The macular mapping using microperimetry showed excentric with stable fixation patterns in most of the eyes studied; we also observed a significant reduction in the mean MRS and mean FRS at the four central points, starting from the central 2°, as well as an abnormal mean retinal sensitivity analysis map in all the peeling cases studied compared with the

buckle, non-peeling, and normal control eye (Figure2 A-5, B-4, and C-5 images; Figure 6 A-4 and B-5 images; Figure7 C-4 and D-5 images). We found that the three-dimensional mfERG map was abnormal in most of the peeling cases studied, and the electric tracing showed a significant mean reduction in the N1-wave amplitude and prolonged implicit times in P1 waves, indicating low activity of bipolar cells and photoreceptor and inner retinal ganglion cells dysfunction (Figure2 B-5 and C 6 images; Figure1 E-6 image; Figure6 A-5 and B-6 images, Figure7 C-5 image). Notably, the eyes that developed secondary postoperative ERM proliferations in the buckle group and in the non-peeling group showed statistically significant upgrading in BCVA once the macular ERM proliferation was removed, but the abnormalities in the status of the SD-OCT biomarkers, mfERG, and microperimetry did not disappear, as shown in the serial analyses of some of our clinical cases.

Only one study [17] has reported the role of prophylactic ILM removal in reducing the incidence of postoperative ERM proliferations, and few studies have correlated ILM removal with serial or longitudinal findings such as the status of biomarkers from SD-OCT and serial functional results obtained using computerized mfERG and microperimetry [21,22]. Similar to previous studies, we found limited benefits of ILM removal; although there was a significant postoperative reduction in ERM proliferations, this did not justify implementing this technique on a regular basis.

Although this approach avoids new surgical procedures and the patient can be kept free of macular symptomatology, ILM removal is not without potential transoperative complications, such as those related to mechanical trauma, including retinal tears, retinal edema, papilar hemorrhage (Figure 4 F), retinal hemorrhage (Figure 4 F, G), iatrogenic punctuate hemorrhagic retinopathy (Figure4 G and H image), vitreous hemorrhage (Figure 4 C-1 and C-2 images), subretinal hemorrhage; and postoperative late functional findings such as excentric fixation patterns (Figure2 B-4; Figure1 E-5), microperimetric abnormal macular integrity with subnormal macular retinal sensitivity (Figure6 B-5), or central scotomas of different densities described by other[23]; most of them are at the subclinical level but favoring poor quality vision and poor final BCVA recovery. Moreover, possible structural sequelae such as DONFL defects may occur because of a diffuse loss of Muller cell end-feet[22,24,25]. In this study, a DONFL defect appearance in the form of concentric macular dark spots (Figure4 H 1 and H-2 images), known as retinal dimples, was detected in our clinical cases only in the postoperative, long-term SD-OCT evaluations of the peeling eye group (Figure6 A-2 and A-3 images; Figure7 C-1, C-2, and C-3 images), and in some eyes, modified and improved appearance of the external layer of the retina was observed without a total recovery of the normal tomographic pattern (Figure2 C-1–C-3 images). This superficial dimpling finding had been reported as a consequence of ILM removal and was first described by Alkabes et al.[25]as a subclinical finding; its effect on macular function as measured by microperimetry and mfERG [22] is still controversial. Our results revealed that the presence of alterations in the microperimetry and mfERG had no statistically significant correlation with the final BCVA when comparing the eyes with the presence or absence of these DONFL defects (dimples) findings (Figure6 A-2 and A-3 images; Figure7 C-1–C-3 images; Figure4H-1 and H-2 images) nor was statistically significant or statistical correlation was found between the number of dimples and the final visual acuity. We do not know yet how these changes in the retinal nerve fiber layer affect the macular function or how they can impact and correlate with postoperative visual recovery.

The functional analysis correlated with the presence of DONFL defects indicated that in the peeling group, the mean postoperative logMAR units BCVA in the patients who did not have dimples was 0.52±0.14 SD, the mean postoperative logMAR units BCVA in those who developed dimples was 0.59±0.16 SD, and the p-value was 0.89; Student's t-test was $p=0.32$ ($p>0.05$), indicating no statistical significance, meaning that, clinically, the presence of DONFL defects due to the removal of MLI does not appear to have functional repercussions on the final BCVA as previously described by other authors[21]. These defects were not evident when MLI was not removed as we were able to verify this fact in the buckling and in the non-peeling groups; when the ILM is removed, the final BCVA is practically the same in the eyes that develop defects and in the eyes that do not develop them.

In contrast, other studies [26] have shown that the final BCVA correlates better with the time period the photoreceptors remain detached from the RPE. This possible deleterious complication might be correlated with the appearance of the ellipsoid band zone, and this strong SD-OCT biomarker was found to be serially abnormal and disrupted in our study. Schuman et al (27) tried to correlate histopathologically the retinal cleavage plane of the ILM using transmission electron microscopy with the functional results, there was no conclusive remarks if the presence and amount of retinal cell fragments at ILM specimens correlate with functional deficits.

Furthermore, only one (2.0%) out of 50 eyes in the peeling group in this study was found to harvest long-term residual SRF; however, advanced age is considered a significant risk factor for the development of postoperative SRF, especially in patients where the ILM is removed. A gradual decrease in RPE pumping due to aging after reattachment to the neurosensory retina could explain this finding[28,29].The median age

in this study was 51±14 years, and only one eye with chronic residual SRF was reported (Figure2 B-1 and B-2); consequently, this variable was not considered as a cause of poor visual results.

In this retrospective multicenter study using SD-OCT, we documented multiple structural alterations, such as diffuse thinning of the neurosensory macula (Figure1 E-2 image; Figure6 B-3 image), morphological alterations in the foveal contour (Figure6 A-1–A-3 images), a significant decrease in the mean CSFT, and ellipsoid band and ELM line reflectivity discontinuities (Figure2 C-1 image; Figure7 C-2 and C-3) in all three groups; a statistically significant predominance of these alterations was observed in the peeling group (Table 2). However, in this study, in the buckling group, the best functional results were significantly associated ($p<0.05$) with the following variables: the presence or absence of significant postoperative ERM proliferation, RRD recurrence rate (Table 2), eventual ERM surgery, the presence or absence of submacular blood, and the event of ERM proliferation surgery; in the vitrectomy groups, the best functional results were observed in the presence of an intact or untouched ILM and absence of postoperative ERM proliferation at the end of follow-up (Table 2); evidently, prospective and multicenter studies are required to evaluate the SD-OCT findings recovered at serial and longitudinal follow-up in these patients, correlate these findings with visual recovery and final postoperative BCVA, and determine the role of the surgical removal of the ILM in macular and visual function.

Additional statistical analysis of the buckling group for the final postoperative BCVA after ERM surgery did not allow us to find any functional or categorical variables that were significantly associated with it; the GLM showed that postoperative BCVA was statistically significantly dependent on the following variables: postoperative ERM proliferation, which increased the postoperative BCVA by 0.68 in logMAR units, and retinal entrapment, which decreased the postoperative BCVA by 0.21 in logMAR units. The GLM also showed that the final postoperative BCVA after ERM surgery was statistically significantly dependent on the following variables: postoperative BCVA in logMAR units, preoperative BCVA in logMAR units, and retinal perforation, which increased the postoperative BCVA after ERM surgery by 0.15 in logMAR units.

We found a recurrence RRD rate of 1.82% in the non-peeling group, 24.0% in the peeling group, and 8.80% in the buckle group (Table 2). A recently published meta-analysis[30] reported a recurrence RRD rate between 28% and 21% after scleral buckle and primary vitrectomy, respectively, and Deiss et al.[31] reported a recurrence RRD rate of 25.55% after vitrectomy with ILM peeling in the treatment of primary macula involving RRD. Although in our report the recurrences were identified earlier in the buckle group comparatively with the vitrectomy group and consequently resolved timely, in the statistical analysis, this particular variable was not significant but relevant from a clinical point of view; in connection with this, we observed a high rate of recurrence of detachment in the peeling group (24.0%) and speculated that perhaps the ILM removal maneuvers were intimately associated with the risk of producing tiny subclinical iatrogenic rhegmatogenous lesions, therefore significantly raising the incidence of somewhat late recurrences that went unnoticed and became apparent once the gas-tamponade disappeared.

Several reports have indicated poor functional results in eyes with non-complicated macula-off RRD managed with primary vitrectomy and ILM removal; it is well known that the involvement of the macula affects recovery; thus, we investigated what type of damage to the photoreceptors or external layers of the macula could be detected to explain the unfavorable recovery, especially in the peeling group without reaching plausible conclusions. We must recognize, however, that the possible additional mechanisms by which the removal of the ILM could cause a lack of functional recovery are still unclear, and additional prospective and multicenter studies are required[32], as mentioned above. We consider that the only indication for ILM removal in the management of a non-complicated macula-off RRD is to relieve or prevent postoperative macular traction caused by the presence of a well-documented pre- or trans-operative ERM proliferation; therefore, a non-complicated macula-off RRD should be managed with vitrectomy and macular surgery involving the removal of ERM-ILM complex and additional scleral buckling performed at the surgeon's discretion. When the ILM was removed, the incidence of ERM was 0.003% [19] to 2.0% (Table 2) and ranged from 21.5% to 58% when the ILM was not removed. In case a preoperative ERM is concomitant with a non-complicated macula-off RRD, a 3-port plana vitrectomy with concomitant en-bloc removal of the ERM-ILM membranes complex or ERM proliferation and ILM two-step (double-staining technique) peeling surgical removal should be considered as the first surgical approach. A prophylactic approach to prevent the formation of ERM proliferation over the macula is not currently justified in our experience and should be reserved for complicated cases. In our report, only one patient with significant ERM proliferation in the peeling group was detected (2.0% incidence), probably due to an incomplete or failed ILM removal technique (Table 2). Some authors[30] have reported that the duration of RRD prior to primary vitrectomy is not a significant risk factor for postoperative BCVA; this variable was analyzed and compared across groups; however, one of the classic variables that best correlates with the final postoperative BCVA is precisely the shortest time that photoreceptors remain separated from the RPE. Herein,

we found a strong positive correlation between the final BCVA and the mean time period of macular detachment before surgery (3.6±2.5 weeks in the buckle group and 4.4±2.6 weeks in the vitrectomy group), which was considered similar in the groups studied but rather a long period with the macula detached. This factor possibly contributed to the poor functional and structural results, and together with the removal of ILM, may have contributed to the poorer functional results in the peeling group. The same authors reported a greater subfoveal thickness and lower final vision, which should be considered very cautiously since the thickness is also related to the patient's age, and choroidal structure deterioration and photoreceptor loss with aging could explain this finding[33]. We did not find any statistically significant association of the mean CSFT across groups (Table 2). We also did not find any significant association between the hypothetical predictive factors for ERM proliferation, such as age, sex, encircling buckle, transoperative use of perfluorocarbon liquids, and the postoperative presence of ERM, as reported by Schwartz et al.[22] and Schmidt et al.[34] However, the use of cryotherapy, external drainage complications such as retinal perforation, through and through SRF drainage complication phenomenon, vitreoretinal entrapment, subretinal bleeding, and the time of macular involvement before surgery showed consistent statistically significant values ($p < 0.05$) in the logistic regression analysis and were considered good surgical predictors for the final visual acuity (Additional Table S6A, S6B, and S6C).

This study has several strengths, such as the multicenter design and the long-term structural and multimodal functional analyses and complication analyses. However, it also has several limitations, mainly pertaining to its retrospective nature and limited size; accordingly, real-life conclusions cannot be obtained based on a few cases. but this report could be a stimulus for the elaboration of prospective and multicentric studies in relation to this pathology and its consequences and complications.

V. Conclusions

In summary, our findings suggest that non-complicated macula-off RRD should be treated as soon as possible to minimize photoreceptor and RPE damage by involutional changes due to the loss of mechanical, biochemical, and nutritional contact between the photoreceptors and RPE. At present, we cannot determine whether the functional alterations were due to the mean exposure time in weeks of the photoreceptors to the SRF (4.14±2.53 for the general group) or whether they were secondary to possible mechanisms at the cellular level related to the removal of the ILM. Successful early macular anatomical reattachment could only result in subclinical damage, but if the detachment time of the macula is prolonged, significant functional sequelae were observed, as seen in the multimodal functional postoperative eye evaluation in this study.

In conclusion, based on the analyses of our results, as well as those of other authors, we concluded that the peeling of the ILM in non-complicated macula-off RRD cases caused a reduction in glial cell proliferation by inhibiting the scarring process. Consequently, hopefully, our study might contribute with the findings of serious consequences in the structure and especially in the macular function of the eyes, as demonstrated by the analysis of the final vision, where the worst functional results in logMAR units, mfERG, and microperimetry evaluation are seen in the peeling group, although our results are compatible with those of other authors, we can conclusively state that removing the MLI with the main objective of avoiding postoperative or secondary macular ERM proliferation is not justified due to the high rate of potential complications and poor final visual results demonstrated in this study. No ERM proliferations developed in the peeling group; however, significant functional and structural differences among the buckle, peeling, and non-peeling groups were assessed using the mfERG, MRS, FRS, and en-face SD-OCT findings of the peeled area, and the alterations found or the lack of recovery in the postoperative SD-OCT biomarkers should raise deep concerns regarding the use of this technique in non-complicated cases if the only beneficial outcome is to avoid the development of ERM proliferation. If ERM proliferation does occur, it can be managed later, only if they are symptomatic or show significant structural and functional alterations in the macula as mentioned before. Further prospective randomized clinical trials are needed to better establish the role of ILM removal and determine the most appropriate surgical procedures to reduce the incidence of postoperative ERM proliferation. Although the number and complexity of major complications were significantly lower in the vitrectomy group compared to the buckle group, the multidisciplinary postoperative evaluation at long-term follow-up yielded a microstructurally and functionally abnormal macula in the three groups but predominantly in the peeling group ($p< 0.05$). Scleral buckling techniques still have a role in retinal detachment repair, and it remains an important skill for a retinal surgeon, but we need to refine the technique and reduce the risk factors that might raise the incidence of postoperative ERM, mainly the use of cryotherapy and complications related to transscleral drainage of SRF, as we describe in this report.

Sequential and serial postoperative structural and functional multimodal imaging techniques for the diagnosis and follow-up of retinal disorders are continuously being developed not only to offer more precise clinical diagnostic and prognostic insights but also to quantify the visual impact. The anatomical and

functional results of this comparative, retrospective multicentric, long-term, one-surgeon study indicated significant visual damage at the clinical level when a non-complicated macula-off RRD is associated with primary or secondary postoperative ERM proliferation and must be resolved by performing vitrectomy complemented with ERM-ILM complex membranes removal techniques as described in this report.

List of abbreviations

ASR: additional surgery rate; BBG: Brilliant Blue G; BCVA: best-corrected visual acuity; CSFT: central subfoveal thickness; DONFL: dissociated optic nerve fiber layer; ELM, external limiting membrane; ERM: epiretinal membrane; FRS: foveal retinal sensitivity; ILM: internal limiting membrane; logMAR: logarithm of the minimum angle of resolution; mfERG: multifocal electroretinography; MRS: macular retinal sensitivity; OCT: optical coherence tomography; PFCL: perfluorocarbon liquid; PVR: proliferative vitreoretinopathy; RD: retinal detachment; RPE: retinal pigment epithelium; RRD: rhegmatogenous retinal detachment; SD-OCT: spectral-domain optical coherence tomography; SS: swept source

Declarations

Ethics approval and consent to participate: This retrospective study adhered to the tenets of the Declaration of Helsinki, received full ethical approval from the Research Ethics Committees, and was approved by the Institutional Review Committees and the Teaching Departments of the three institutions enrolled (no reference number is provided for retrospective studies by these institutions). Written informed consent in accordance with the institutional guidelines was obtained from all the patients.

Consent for publication: Each patient included in this report has given their written consent to be operated on and also their written consent for the publication of this report.

Availability of data and materials: Photos, composite figures, and laboratory studies supporting the findings of this study may be released upon written application to the Photographic, Psychophysics laboratory and Clinical Archives department at Instituto de Oftalmología Fundacíon Conde de Valenciana (Non-profit Organization), Chimalpopoca 14, Colonia Obrera, Mexico City 06800, Mexico and from the corresponding author upon request.

Competing interests; The authors declare that they have no competing interests.

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Authors' contributions: MAQR, original idea, writing the manuscript, dataset interpretation, statistical analysis interpretation, final revision, and conclusions; EAQG, figures artwork, statistics, tables, and graphics; JGMN, statistics, tables, and graphics; FEC, photographic material compilation; JEAV, photographic material compilation; JHKL, photographic material compilation; ANJ, statistic correlation and final revision; MM, final revision and statistical analysis; VLG, final revision, statistical analysis, artwork and figure formatting, and figure text editing; FGW, final revision.

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Table S1: Shapiro-Wilk normality test results.

	Object	W	p value
1	Age	0.954	0.001
2	Preoperative BCVA (logMAR)	0.930	0.001
3	Postoperative BCVA (logMAR)	0.790	0.001
4	ERM Detection (weeks)	0.961	0.319
5	BCVA after ERM surgery (logMAR)	0.951	0.211
6	CSFT (microns)	0.888	0.001
7	Follow-up period (months)	0.959	0.001

The variables that do not follow a normal distribution are in bold writing. ($p < 0.05$)

W (Shapiro-Wilk normality test): BCVA: best corrected visual acuity; CSFT: central subfoveal thickness

Table S2: Descriptive statistics for the numeric variables.

	Object	Mean	Min	Max	Standard Deviation	Length of Sample (n=125)
1	Age (years)	44.34	18.00	76.00	15.94	125
2	Preoperative macula-off (weeks)	3.60	1.00	12.00	2.47	125
3	Preoperative BCVA (logMAR)	1.03	0.48	1.60	0.28	125
4	Postoperative BCVA (logMAR)	0.40	0.10	1.30	0.33	125
5	ERM detection (weeks)	11.93	5.00	22.00	4.59	125
6	BCVA after ERM surgery(logMAR)	0.43	0.18	0.70	0.14	125
7	CSFT (microns)	243.57	32.00	402.00	41.95	125
8	Follow-up period (months)	26.11	2.00	73.00	13.42	125

Non-parametric Mann-Whitney U-test. min: minimum; max: maximum; BCVA: best corrected visual acuity; ERM: epiretinal membrane; CSFT: central subfoveal thickness

Table S3: Summarized statistics for the categorical variables.

Variable	Value	n	Freq
Sex	Female	75	0.6
	Male	50	0.4
Eye	Left	59	0.472
	Right	66	0.528
Preop Lens Status	Phakic	98	0.784
	Pseudophakic	27	0.216
Preop BCVA	20/100	26	0.208
	20/160	14	0.112
	20/200	35	0.28
	20/300	12	0.096
	20/400	21	0.168
	20/60	1	0.008
	20/70	3	0.024
	20/80	2	0.016
	20/800	11	0.088

Postop BCVA	20/100	11	0.088
	20/120	1	0.008
	20/160	1	0.008
	20/200	4	0.032
	20/25	16	0.128
	20/30	35	0.28
	20/300	3	0.024
	20/40	31	0.248
	20/400	6	0.048
	20/50	4	0.032
	20/60	8	0.064
	20/70	1	0.008
	20/80	4	0.032
Redetachment	No	114	0.912
	Yes	11	0.088
Additional Surgery		114	0.912
	BUCKLE REVISION	4	0.032
	PHAKO-VITRECTOMY	3	0.024
	PHAKO-VITRECTOMY-ERM PEELING	1	0.008
	VITRECTOMY	2	0.016
	VITRECTOMY-ERM PEELING	1	0.008
Postop ERM proliferations	No	96	0.768
	Yes	29	0.232
ERM Surgery	No	98	0.784
	Yes	27	0.216
BCVA after ERM surgery		97	0.776
	20/100	2	0.016
	20/30	2	0.016
	20/40	7	0.056
	20/50	6	0.048
	20/60	4	0.032
	20/70	4	0.032
	20/80	3	0.024
Retinal perforation	No	118	0.944
	Yes	7	0.056

Submacular blood	No	120	0.96
	Yes	5	0.04
Through and through scleral drainage complication phenomenon	No	117	0.936
	Yes	8	0.064
Retinal entrapment	No	122	0.976
	Yes	3	0.024
Foveal contour OCT alterations	Normal	14	0.112
	Abnormal	19	0.152
	Normal	92	0.736
Ellipsoid band OCT alterations		14	0.112
	Disrupted	25	0.2
	Normal	86	0.688
DONFL OCT defects		14	0.112
	Not Present	80	0.64
	Present	31	0.248
ELM line OCT alterations		15	0.12
	Abnormal	24	0.192
	Normal	86	0.688
mfERGregistration		26	0.208
	Abnormal	54	0.432
	Normal	45	0.36
Microperimetry results		18	0.144
	Abnormal	51	0.408
	Normal	56	0.448

Fisher's exact test. freq: frequency; preop: preoperative: postop: postoperative: BCVA: best corrected visual acuity; CSFT: central subfoveal thickness; ERM: epiretinal membrane; DONFL: diffuse optic nerve fiber layer; ELM: external limiting membrane: mfERG: multifocal electroretinography

Table S4: Correlations among the numeric variables in the Buckle group (sample size N=125 eyes)

	Age	Preoperative macula-off (weeks)	Preoperative BCVA (logMAR)	Postoperative BCVA (logMAR)	ERM Detection (weeks)	BCVA After ERM Surgery (logMAR)	CSFT (microns)	Follow-up Period (months)
Age	1 (p=NA)							
Preoperative macula-off (weeks)	0.12 (p=0.17)	1 (p=NA)						
Preoperative BCVA (logMAR)	0.01 (p=0.88)	0.04 (p=0.63)	1 (p=NA)					
Postoperative BCVA (logMAR)	-0.06 (p=0.48)	-0.02 (p=0.78)	0.02 (p=0.85)	1 (p=NA)				
ERM detection (weeks)	-0.27 (p=0.15)	-0.19 (p=0.31)	-0.19 (p=0.31)	0.06 (p=0.74)	1 (p=NA)			
BCVA after ERM surgery (logMAR)	0.05 (p=0.79)	-0.21 (p=0.28)	0.26 (p=0.17)	0.57 (p=0.00)	0.04 (p=0.82)	1 (p=NA)		

CSFT (microns)	0.01 (p=0.9)	0.01 (p=0.93)	0.09 (p=0.37)	0.13 (p=0.17)	0.04 (p=0.85)	-0.06 (p=0.78)	1 (p=NA)	
Follow-up period (months)	-0.17 (p=0.06)	-0.17 (p=0.06)	0.03 (p=0.71)	-0.2 (p=0.03)	0.08 (p=0.68)	-0.05 (p=0.79)	0.12 (p=0.2)	1 (p=NA)

Spearman's rank correlation coefficient test. The significant correlations are in bold text. BCVA: best corrected visual acuity; ERM: epiretinal membrane; CSFT: central subfoveal thickness; NA: not applicable

Table S5: A) PreopBCVA, B) Postop BCVA C) BCVA after ERM surgery in the Buckle group (N=125 eyes)

A. preoperative BCVA (logMAR) Mann-Whitney U tests results		
Object	U	p value
Age	7875	0.001
Preoperative macula-off (weeks)	7140	0.001
Postoperative BCVA (logMAR)	201.5	0.001
ERM detection (weeks)	465	0.001
BCVA after ERM surgery (logMAR)	0	0.001
CSFT (microns)	6105	0.001
Follow-up period (months)	7875	0.001
B. postoperative BCVA (logMAR) Mann-Whitney U tests results		
Object	U	p value
Age	7875	0.001
Preoperative macula-off (weeks)	7866	0.001
Preoperative BCVA (logMAR)	7301.5	0.001
ERM detection (weeks)	465	0.001
BCVA after ERM surgery (logMAR)	0	0.001
CSFT (microns)	6105	0.001
Follow-up period (months)	7875	0.001
C. BCVA after ERM surgery (logMAR) Mann–Whitney U tests results		
Object	U	p value
Age	406	0.001
Preoperative macula-off (weeks)	406	0.001
Preoperative BCVA (logMAR)	378	0.001
Postoperative BCVA (logMAR)	406	0.001
ERM detection (weeks)	406	0.001
CSFT (microns)	406	0.001
Follow-up period (months)	406	0.001

Mann-Whitney U tests. The statistically significant variables ($p < 0.05$) are in bold text.U testBCVA: best corrected visual acuity; ERM: epiretinal membrane; CSFT: central subfoveal thickness

Table S6A: Kruskal-Wallis results of the preoperative BCVA with the categorical variables in the Buckle group

	Object	Kruskal-Wallis x^2.	df	p value	Number of eyes	No. of NAs
1	Male	3.117	1	0.077	125	0
2	Eye	2.132	1	0.144	125	0
3	Preoperative Lens Status	0.130	1	0.718	125	0
4	**Preoperative BCVA**	**124.000**	**8**	**0.000**	**125**	**0**
5	Postoperative BCVA	5.342	12	0.946	125	0
6	Re-Detachment	1.018	1	0.313	125	0
7	Additional surgery	1.238	4	0.872	125	114
8	Postoperative ERM proliferations	0.035	1	0.851	125	0
9	ERM surgery	0.090	1	0.764	125	0
10	BCVA after ERM surgery	4.763	6	0.575	125	97

11	Retinal perforation	0.222	1	0.638	125	0
12	Submacular blood	1.057	1	0.304	125	0
13	Through and through	2.829	1	0.093	125	0
14	Retinal entrapment	0.001	1	0.980	125	0
15	Foveal contour	0.006	1	0.936	125	14
16	Ellipsoid	0.236	1	0.627	125	14
17	DONFL	1.581	1	0.209	125	14
18	ELM	0.384	1	0.535	125	15
19	mfERG	0.242	1	0.623	125	26
20	Microperimetry	0.653	1	0.419	125	18

Table S6B: Kruskal-Wallis results of the postoperative BCVA with the categorical variables

	Object	Kruskal-Wallis x^2.	df	p value	Number of eyes	No. of NAs
1	Male	0.026	1	0.871	125	0
2	Eye	0.047	1	0.828	125	0
3	Preoperative Lens Status	0.234	1	0.629	125	0
4	Preoperative BCVA	3.950	8	0.862	125	0
5	Postoperative BCVA	124.000	12	0.000	125	0
6	Re-Detachment	7.484	1	0.006	125	0
7	Additional surgery	5.331	4	0.255	125	114
8	Postoperative ERMproliferations	68.187	1	0.000	125	0
9	ERM surgery	63.098	1	0.000	125	0
10	BCVA after ERM surgery	13.048	6	0.042	125	97
11	Retinal perforation	1.214	1	0.271	125	0
12	Submacular blood	9.449	1	0.002	125	0
13	Through and Through	0.357	1	0.550	125	0
14	Retinal Entrapment	0.612	1	0.434	125	0
15	Foveal contour	15.821	1	0.000	125	14
16	Ellipsoid	3.479	1	0.062	125	14
17	DONFL	18.677	1	0.000	125	14
18	ELM	0.303	1	0.582	125	15
19	mfERG	20.558	1	0.000	125	26
20	Microperimetry	11.826	1	0.001	125	18

Table S6C: Kruskal-Wallis results of the BCVA after ERM surgery with the categorical variables

	Object	Kruskal-Wallis x^2.	df	p value	Number of eyes	No. of NAs
1	Male	0.499	1	0.480	125	0
2	Eye	0.967	1	0.325	125	0
3	Preoperative Lens Status	1.070	1	0.301	125	0
4	Preoperative BCVA	6.587	7	0.473	125	0
5	Postoperative BCVA	11.572	6	0.072	125	0
6	Re-Detachment	0.428	1	0.513	125	0
7	Additional surgery	1.716	3	0.633	125	114
8	Postoperative ERM proliferations	0.063	1	0.801	125	0
9	ERM surgery	0.063	1	0.801	125	0
10	BCVA after ERM surgery	27.000	6	**0.000**	125	97
11	Retinal perforation	1.847	1	0.174	125	0
12	Submacular blood	2.783	1	0.095	125	0
13	Through and Through	1.144	1	0.285	125	0

14	Retinal entrapment	0.776	1	0.378	125	0
15	Foveal contour	0.178	1	0.673	125	14
16	Ellipsoid	1.235	1	0.266	125	14
17	DONFL	1.230	1	0.267	125	14
18	ELM	0.138	1	0.710	125	15
19	mfERG	0.115	1	0.734	125	26
20	Microperimetry	1.033	1	0.310	125	18

The statistically significant variables ($p < 0.05$) are in bold text.df: difference no: number; NA: not applicable; BCVA: best corrected visual acuity; ERM: epiretinal membrane; DONFL: diffuse optic nerve fiber layer; ELM: external limiting membrane: mfERG: multifocal electroretinography

Table S7: Generalized Linear Model results in the Buckle group (n=125 eyes)

Preoperative BVCA	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.972	0.033	29.046	**<2e-16**	***
Sex Male	0.106	0.051	2.07	**0.040**	*
Through and Throughscleral drainage complication phenomenon	0.164	0.102	1.605	**0.111**	
Generalized					
Postoperative BCVA	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	
(Intercept)	0.218	0.056	3.854	**0.001**	***
Postop ERM proliferations	0.676	0.035	19.055	**< 2e-16**	***
Retinal entrapment	-0.206	0.097	-2.112	**0.036**	*
Preop BCVA logMAR	0.029	0.052	0.567	0.572	
BCVA after ERM surgery	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	
(Intercept)	-0.170	0.130	-1.303	0.206	
Post BCVA logMAR	0.323	0.080	4.003	**0.001**	***
Preop BCVA logMAR	0.194	0.072	2.694	**0.013**	*
Retinal perforation	0.151	0.067	2.251	**0.034**	*
Age	0.002	0.001	1.712	0.100	
Sex Male	-0.021	0.043	-0.498	0.623	

*The statistically significant variables ($p < 0.05$) are in bold text and marked with *.*

Pr: Probabilities using the t distribution, gives the p-value for that t-test; BCVA: best corrected visual acuity; Postop: postoperative; Preop: preoperative: ERM: epiretinal membrane

Table S8: Shapiro-Wilk normality tests results in the Vitrectomy group (n=105 eyes).

Object	W	P value
Age (years)	0.974	0.039
Macula-off (weeks)	0.924	0.001
Preoperative BCVA (logMAR)	0.923	0.001
Follow-up period (days)	0.971	0.023
BCVA before ERM-ILM removal (logMAR)	0.888	0.001
Final postoperative BCVA (logMAR)	0.924	0.001
CSFT (microns)	0.939	0.008
Follow-up period (months)	0.970	0.023

The variables that do not follow a normal distribution are in bold text ($p < 0.05$). BCVA: best corrected visual acuity; ERM: epiretinal membrane; ILM: internal limiting membrane; CSFT: central subfoveal thickness

Table S9: Descriptive statistics for the numeric variables in the Vitrectomy group

Object	Mean	Min	Max	Standard Deviation
Age (years)	47.92	18.00	76.00	14.60
Macula-off (weeks)	4.42	1.00	12.00	2.56
Preoperative BCVA (logMAR)	1.06	0.54	1.60	0.27
Follow-up period (months)	24.2	1.0	58.66	13.02
BCVA before ERM-ILM removal (logMAR)	0.52	0.10	1.30	0.36
ERM detection (weeks)	13.75	5.00	30.00	5.33
Final postoperative BCVA (logMAR)	0.37	0.10	1.00	0.20
CSFT (microns)	256.55	198.00	320.00	35.16
Follow-up period (months)	23.42	1.00	57.00	12.98

Wilcoxon rank sum test. Min: minimum; Max: maximum; BCVA: best corrected visual acuity; ERM: epiretinal membrane; ILM: internal limiting membrane; CSFT: central subfoveal thickness

Table S10: Summarized statistics for the categorical variables in the vitrectomy group (peeling and nonpeeling groups)

Variable	Group	n	freq	% freq
Additional Surgery	BUCKLE REVISION	3	0.03	2.9%
	No	92	0.88	87.6%
	PHAKO VITRECTOMY ERM PEELING	1	0.01	1.0%
	VITRECTOMY	2	0.02	1.9%
	VITRECTOMY REVISION	7	0.07	6.7%
DONFL	Absent	34	0.32	32.4%
	Present	60	0.57	57.1%
	NA	11	0.10	10.5%
Ellipsoid	Disrupted	29	0.28	27.6%
	Normal	76	0.72	72.4%
ELM	Disrupted	27	0.26	25.7%
	Normal	74	0.70	70.5%
	NA	4	0.04	3.8%
ERM 2nd Surgery	VIT and MACULA REVISION	1	0.01	1.0%
	VIT REVISION ERM-ILM REMOVAL	45	0.43	42.9%
	VIT REVISION ERM-ILM REMOVAL	5	0.05	4.8%
	VIT REVISION ERM-ILM REMOVAL	1	0.01	1.0%
	NA	53	0.50	50.5%
Eye	Left	50	0.48	47.6%
	Right	55	0.52	52.4%
First Surgery	BUCKLE	27	0.26	25.7%
	ONLY VITRECTOMY	68	0.65	64.8%

	VIT ERM-ILM REMOVAL	10	0.10	9.5%
Foveal contour	Abnormal	24	0.23	22.9%
	Normal	77	0.73	73.3%
	NA	4	0.04	3.8%
Sex	Female	37	0.35	35.2%
	Male	68	0.65	64.8%
mfERG	Abnormal	43	0.41	41.0%
	Normal	30	0.29	28.6%
	NA	32	0.30	30.5%
Microperimetry	Abnormal	35	0.33	33.3%
	Normal	42	0.40	40.0%
	NA	28	0.27	26.7%
Postoperative ERM proliferations	No	54	0.51	51.4%
	Yes	51	0.49	48.6%
Preoperative ERM proliferations	No	55	0.52	52.4%
	Yes	50	0.48	47.6%
Preop Lens Status	Phakic	68	0.65	64.8%
	Pseudophakic	37	0.35	35.2%
Recurrent RRD	No	92	0.88	87.6%
	Yes	13	0.12	12.4%

Fisher's exact test. freq: frequency; ERM: epiretinal membrane; DONFL: diffuse optic nerve fiber layer; ELM: external limiting membrane; VIT: vitrectomy; ILM: internal limiting membrane; mfERG: multifocal electroretinography; RRD: rhegmatogenous retinal detachment

Table S11: Descriptive statistics with respect to the Vitrectomy (preoperative ERM proliferations) group (nonpeeling and peeling)

Vitrectomy groups	Nonpeeling	peeling	p	
	(N=55)	(N=50)		
Age	50.455 ± 13.52	45.140 ±15.36	0.054	
Sex			1	
- Female	19 (34.545%)	18 (36.0%)		
- Male	36 (65.455%)	32 (64.0%)		
Eye			0.698	
- Left	25 (45.455%)	25 (50.0%)		
- Right	30 (54.545%)	25 (50.0%)		
Preoperative Lens Status			0.068	

- Phakic	31 (56.364%)	37 (74.0%)		
- Pseudophakic	24 (43.636%)	13 (26.0%)		
Macula-off (weeks)	4.527 ±2.403	4.300 ±2.750	0.425	
Preoperative BCVA (logMAR)	1.036 ±0.258	1.077 ±0.277	0.386	
Follow-up period (days)	768.6 ±373.01	679.90 ±407.98	0.131	
First Surgery			0***	
- BUCKLE	0 (0.0%)	27 (54.0%)		
- ONLY VITRECTOMY	55 (100.000%)	13 (26.0%)		
- VIT ERM and ILM REMOVAL	0 (0.0%)	10 (20.0%)		
BCVA Before ERM-ILM removal (logMAR)	0.297 ±0.23	0.756 ±0.319	0.001	***
Recurrent RRD			0.001	***
- No	54 (98.182%)	38 (76.0%)		
- YES	1 (1.818%)	12 (24.0%)		
Additional Surgery			0.004	***
- BUCKLE REVISION	0 (0.0%)	3 (6.0%)		
- No	54 (98.182%)	38 (76.0%)		
- PHAKO VITRECTOMY ERM PEELING	0 (0.0%)	1 (2.0%)		
- VITRECTOMY	0 (0.0%)	2 (4.0%)		
- VITRECTOMY REVISION	1 (1.818%)	6 (12.0%)		
ERM Detection (weeks)	18.00 ±6.45	12.575 ±4.385	0.009	***
ERM 2nd Surgery			0	***
- VIT and MACULA REVISION	0 (0.0%)	1 (2.439%)		
- VIT REVISION ERM and ILM	5 (45.455%)	40 (97.561%)		
- VIT REVISION ERM and ILM REMOVAL	5 (45.455%)	0 (0.0%)		
- VIT REVISION ERM.ILM REMOVAL	1 (9.091%)	0 (0.0%)		
Final Postoperative BCVA (logMAR)	0.280 ±0.192	0.477 ±0.161	0.001	***
CSFT (microns)	266.71 ±32.75	253.073 ±35.66	0.173	

Foveal Contour			**0.002**	***
- Abnormal	6 (11.321%)	18 (37.5%)		
- Normal	47 (88.679%)	30 (62.5%)		
Ellipsoid integrity			0.828	
- Disrupted	16 (29.091%)	13 (26.0%)		
- Normal	39 (70.909%)	37 (74.0%)		
DONFL defects			**0**	***
- Absent	39 (88.63%)	21 (42.0%)		
- Present	5 (11.36%)	29 (58.0%)		
ELM line appearance			0.654	
- Disrupted	16 (29.091%)	11 (23.913%)		
- Normal	39 (70.909%)	35 (76.087%)		
mfERG result			**0**	***
- Abnormal	13 (33.333%)	30 (88.235%)		
- Normal	26 (66.667%)	4 (11.765%)		
Microperimetry evaluation			**0**	***
- Abnormal	11 (25.581%)	24 (70.588%)		
- Normal	32 (74.419%)	10 (29.412%)		
Follow-up period (months)	24.80 ±12.34	21.880 ±13.324	0.133	

*The p-values (p) are the results from the Wilcoxon rank sum test for the numerical variables and Fisher's Exact test for the categorical variables. The variables that showed a statistically significant difference ($p < 0.05$) among the groups of the peeling (preoperative ERM proliferation) are in boldtext and marked with *. sig: significance;BCVA: best corrected visual acuity; VIT: vitrectomy; ERM: epiretinal membrane; ILM: internal limiting membrane; RRD: rhegmatogenous retinal detachment; CSFT: central subfoveal thickness; DONFL: diffuse optic nerve fiber layer; ELM: external limiting membrane: mfERG: multifocal electroretinography.*

Table S12: Correlations among the numeric variables in the Vitrectomy group (peeling and nonpeeling groups)

	Age	Preoperative Macula-Off (weeks)	Preoperative BCVA (logMAR)	BCVA Before ERM and ILM removal (logMAR)	ERM Detection (Weeks)	Final Postoperative BCVA (logMAR)	CSFT (microns)	Follow-up period (months)
Age	1							
Preoperative Macula-off (weeks)	0.03 (p=0.78)	1						
Preoperative BCVA (logMAR)	-0.07 (p=0.47)	0.04 (p=0.68)	1					
BCVA Before ERM and	-0.18	-0.16 (p=0.1)	-0.10	1				

ILM removal (logMAR)	(p=0.07)		(p=0.33)					
ERM Detection (weeks)	-0.18 (p=0.21)	0.03 (p=0.83)	**-0.29 (p=0.04)**	-0.16 (p=0.26)	1			
Final Postoperative BCVA (logMAR)	-0.04 (p=0.72)	-0.05 (p=0.62)	0.10 (p=0.3)	**0.78 (p=0)**	0.04 (p=0.76)	1		
CSFT (microns)	0.15 (p=0.28)	**0.32 (p=0.02)**	0.02 (p=0.89)	-0.14 (p=0.32)	0.02 (p=0.89)	0.02 (p=0.88)	1	
Follow-up period (months)	-0.14 (p=0.18)	-0.08 (p=0.42)	0.09 (p=0.36)	-0.2 (p=0.05)	0.12 (p=0.42)	-0.05 (p=0.61)	-0.08 (p=0.6)	1

Wilcoxon rank sum test. The p-values in parenthesis (p); significant correlations ($p < 0.05$) are in bold text. BCVA: best corrected visual acuity; ERM: epiretinal membrane; ILM: internal limiting membrane; CSFT: central subfoveal thickness. Spearman Rank Test nonpeeling sample=55 eyes. Peeling sample=50 eyes.

Table S13: A) Preoperative, B) postoperative, and C) final BCVA in the Vitrectomy group (peeling and nonpeeling groups)

A. preoperative BCVA (logMAR) Mann-Whitney U tests results		
Object	**U**	**p-value**
Age	5565	0.001
Macula-off (weeks)	5341	0.001
Follow-up period (days)	5565	0.001
BCVA before ERM-ILM removal (logMAR)	238	0.001
ERM detection (weeks)	1326	0.001
Final postoperative BCVA (logMAR)	0	0.001
CSFT (microns)	1540	0.001
Follow-up period (months)	4950	0.001
B. postoperative BCVA (logMAR) Mann-Whitney U tests results		
Object	**U**	**p-value**
Age	5565	0.001
Macula-off (weeks)	5556	0.001
Preoperative BCVA (logMAR)	4712	0.001
Follow-up period (days)	5565	0.001
ERM detection (weeks)	1326	0.001
Final postoperative BCVA (logMAR)	101.5	0.001
CSFT (microns)	1540	0.001
Follow-up period (months)	5049	0.001
C. final BCVA after ERM proliferation removal (logMAR)		
Object	**U**	**p-value**
Age	5565	0.001
Macula-off (weeks)	5565	0.001
Preoperative BCVA (logMAR)	5460	0.001
Follow-up period (days)	5565	0.001
BCVA before ERM-ILM removal (logMAR)	1074.5	0.001
ERM detection (weeks)	1326	0.001
CSFT (microns)	1540	0.001
Follow-up period (months)	5050	0.001

The statistically significant variables ($p < 0.05$) are in bold text. BCVA: best corrected visual acuity; ERM: epiretinal membrane; ILM: internal limiting membrane; CSFT: central subfoveal thickness

Table S14: A) preoperative, B) postoperative, and C) final BCVA in the Vitrectomy group (peeling and nonpeeling groups)

A. preoperative BCVA (logMAR) Kruskal-Wallis tests results			
Object	Kruskal-Wallis x^2.	df	p-value
Male	0.458	1	0.499
Eye	1.878	1	0.171
Preoperative Lens Status	1.640	1	0.200
Preoperative ERM proliferations	0.760	1	0.383
First Surgery	1.055	2	0.590
BCVA Before ERM-ILM removal	9.412	12	0.667
Recurrent RRD	0.208	1	0.649
Additional surgery	1.360	4	0.851
Postoperative ERMs	0.038	1	0.846
ERM 2nd surgery	3.135	3	0.371
Final Postoperative BCVA	11.718	10	0.304
Foveal contour abnormalities	0.385	1	0.535
Ellipsoid disruption	4.175	1	**0.041**
DONFL defects	1.402	1	0.236
ELM line alterations	0.144	1	0.704
mfERG alterations	0.109	1	0.741
Microperimetry alterations	1.623	1	0.203
B. postoperative BCVA (logMAR) Kruskal-Wallis tests results			
Object	Kruskal-Wallis x^2.	df	p-value
Male	0.355	1	0.552
Eye	0.001	1	0.979
Preoperative Lens Status	6.083	1	0.014
Preoperative BCVA	12.845	8	0.117
Preoperative ERM proliferations	50.177	1	0.001
First surgery	47.013	2	0.000
Recurrent RRD	11.364	1	0.001
Additional surgery	12.324	4	0.015
Postoperative ERM proliferations	68.366	1	0.001
ERM 2nd surgery	5.469	3	0.141
Foveal Contour abnormalities	10.021	1	0.002
Ellipsoid disruption	1.091	1	0.296
DONFL defect	19.206	1	0.001
ELM line alterations	0.746	1	0.388
mfERG alterations	31.253	1	0.001
Microperimetry alterations	19.749	1	0.001
C. final BCVA after ERM proliferation removal (logMAR)			
Object	Kruskal-Wallis x^2.	df	p-value
Male	1.561	1	0.211
Eye	0.121	1	0.728
Preoperative Lens Status	1.855	1	0.173
Preoperative ERM proliferations	33.337	1	0.001
First surgery	13.877	2	0.001

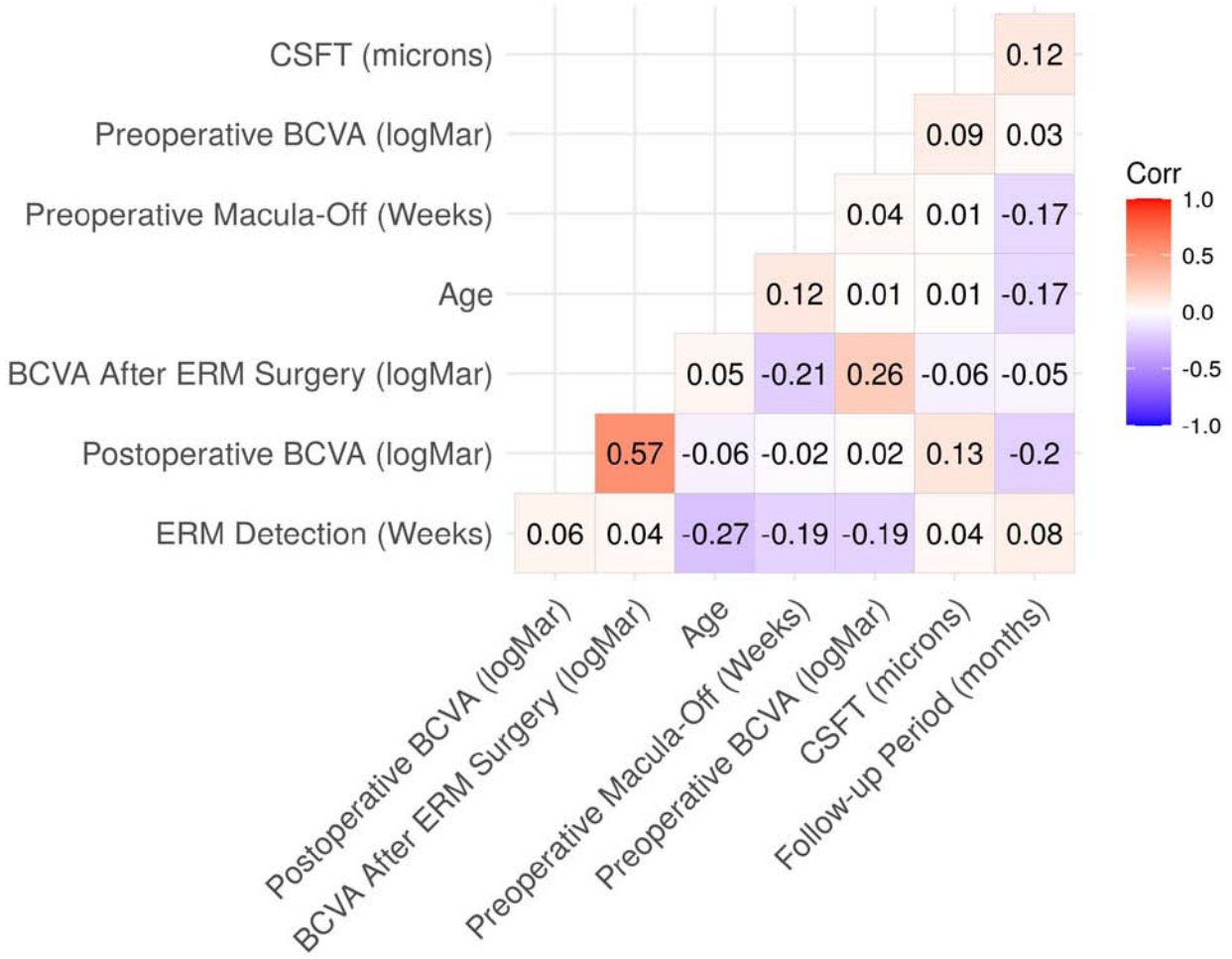
Recurrent RRD	9.223	1	0.002
Additional surgery	10.697	4	0.030
Postoperative ERM proliferations	38.068	1	0.001
ERM 2nd surgery	1.113	3	0.774
Foveal contour abnormalities	6.168	1	0.013
Ellipsoid disruption	0.894	1	0.344
DONFL defect	16.777	1	0.001
ELM line alterations	0.375	1	0.540
mfERG alterations	16.522	1	0.001
Microperimetry alterations	13.150	1	0.001

The statistically significant variables ($p < 0.05$) are in bold text. df: degrees of freedom: BCVA: best corrected visual acuity; ERM: epiretinal membrane; ILM: internal limiting membrane; RRD: rhegmatogenous retinal detachment; DONFL: diffuse optic nerve fiber layer; ELM: external limiting membrane: mfERG: multifocal electroretinography

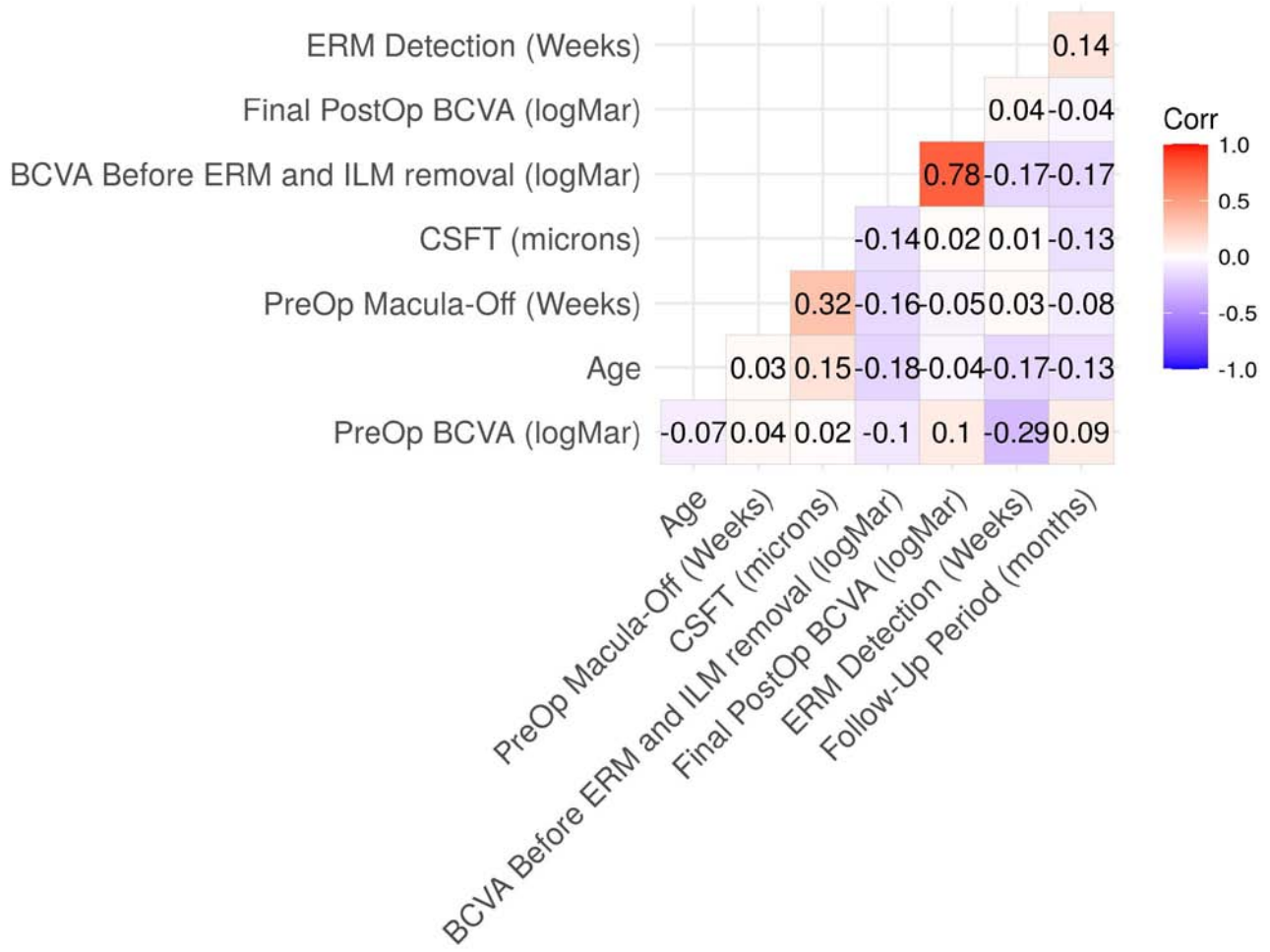
Table S15: Generalized Linear Model results A) "Preoperative, B) Postoperative, and C) final BCVA in the Vitrectomy group (peeling and nonpeeling groups)

A. preoperative BCVA (logMAR) GLM results				
	Estimate	SE	t value	p
(Intercept)	1.055	0.026	40.60	0.001
B. postoperative BCVA (logMAR) GLM results				
	Estimate	SE	t value	p
(Intercept)	0.515	0.067	7.72	0.001
Postoperative ERM proliferations	0.448	0.050	8.97	0.001
First surgery - ONLY VITRECTOMY	-0.235	0.055	-4.30	0.001
First surgery - VIT ERM and ILM REMOVAL	0.034	0.090	0.38	0.704
Macula-off (weeks)	-0.019	0.008	-2.50	0.014
Recurrent RRD	0.118	0.061	1.94	0.055
C. final BCVA after ERM proliferation removal (logMAR) GLM results				
	Estimate	SE	t value	p
(Intercept)	-0.213	0.070	-3.05	0.003
BCVA Before ERM-ILM removal (logMAR)	0.552	0.046	11.98	0.001
First Surgery - ONLY VITRECTOMY	0.201	0.038	5.22	0.001
First Surgery - VIT ERM and ILM REMOVAL	0.275	0.051	5.42	0.001
Preoperative BCVA (logMAR)	0.106	0.046	2.29	0.024
Gender - Male	0.052	0.026	2.03	0.045

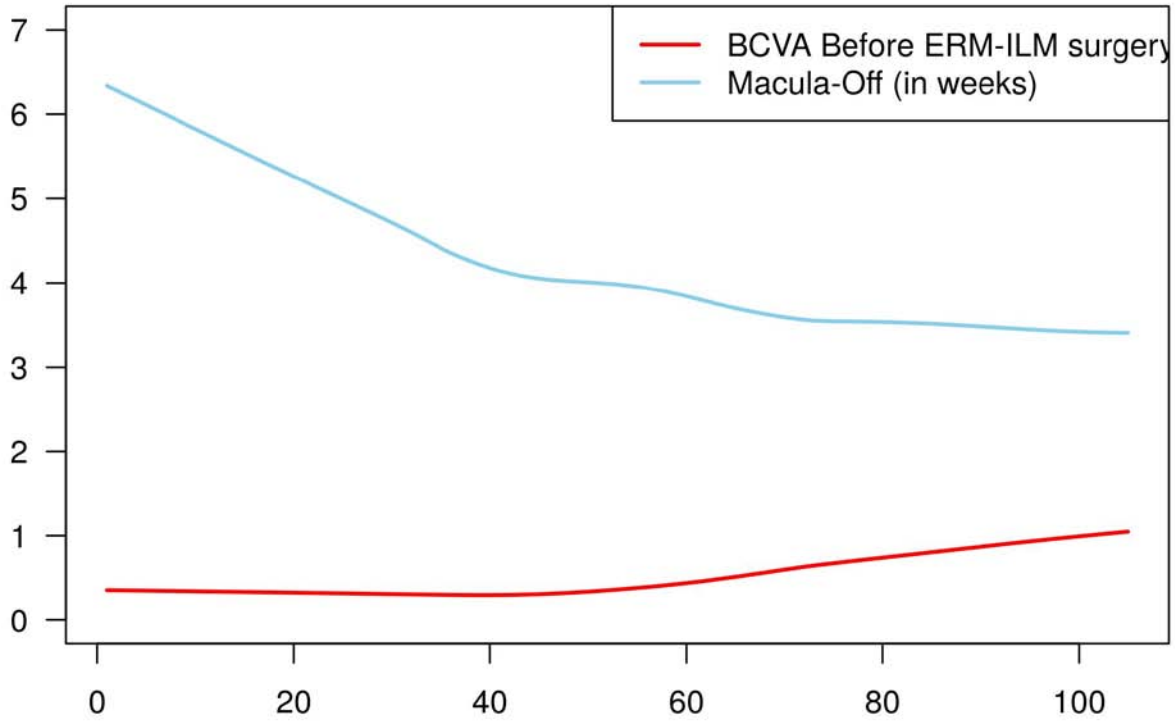
The statistically significant variables ($p < 0.05$) are in bold text. BCVA: best corrected visual acuity; GLM: generalized linear models; SE: standard error; ERM: epiretinal membrane; VIT: vitrectomy; ILM: internal limiting membrane; mfERG: multifocal electroretinography; RRD: rhegmatogenous retinal detachment



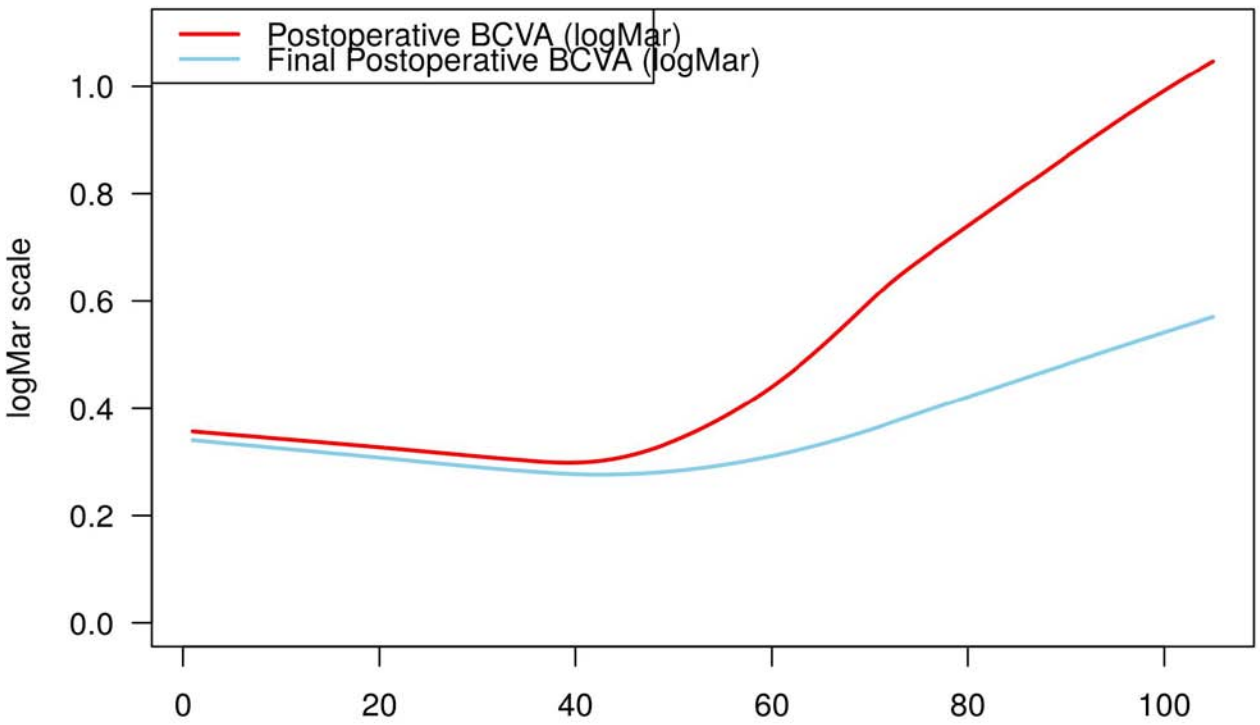
Additional Figure S1: The Spearman's rank correlation coefficient test showed that there was a moderate to strong positive correlation (rho= 0.57, $p<0.01$) of the postoperative BCVA in logMAR units with the BCVA after ERM surgery. In addition, there was a weak negative correlation (rho = -0.2, $p<0.05$) between postoperative BCVA in logMAR units and follow-up period in months.



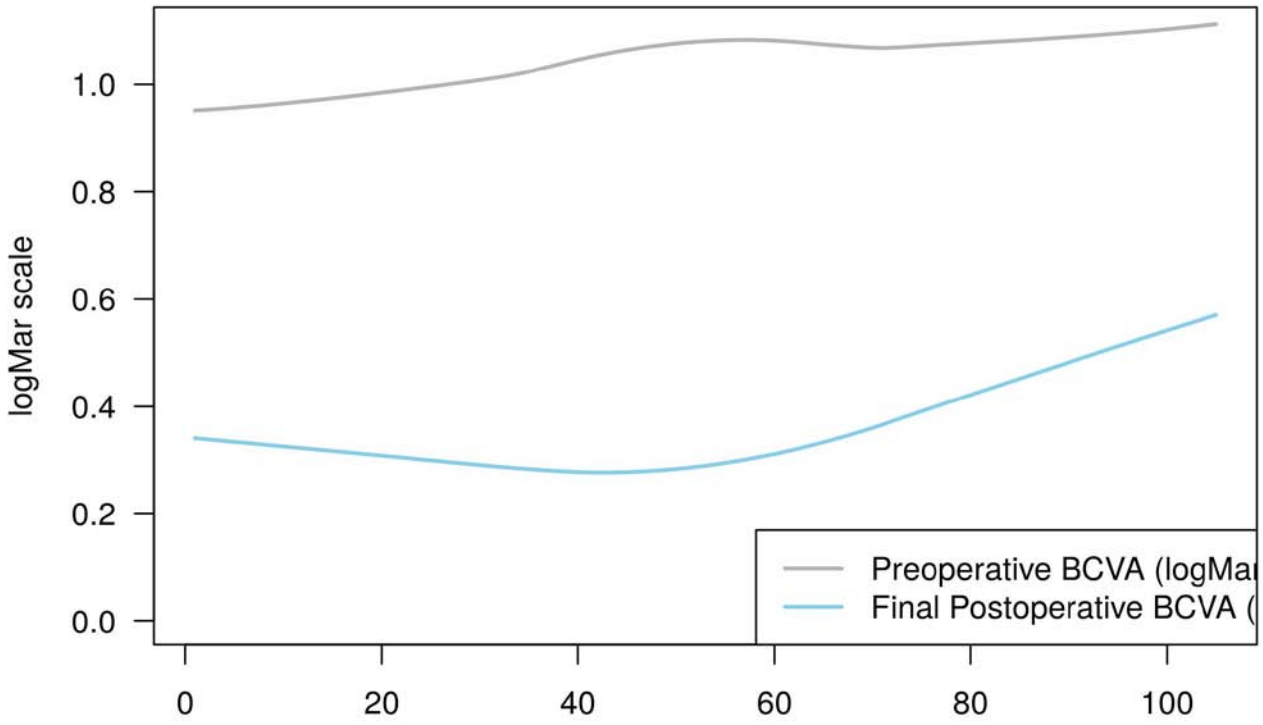
Additional Figure S2: The Spearman's rank correlation coefficient test showed a weak positive correlation (rho= 0.32, $p<0.05$) between the preoperative period with the macula-off in weeks and the CSFT findings in microns; it also showed a weak negative correlation (rho= -0.29, $p<0.05$) between the preoperative BCVA in logMAR units and ERM detection in weeks.



Additional Figure S3: Postoperative BCVA was significantly negatively associated when only vitrectomy (non-peeling group) was performed in the first surgery variable (coefficient = -0.23, $p<0.01$); and significantly negatively associated with the variable preoperative period of macula-off in weeks (coefficient= -0.02, $p<0.05$) is shown.



Additional Figure S4: The GLM for the final BCVA in log MAR units after ERM proliferation removal showed that it was significantly positively associated ($p>0.01$) with the postoperative



Additional Figure S5: Shows when only vitrectomy was the first surgery variable, and with the preoperative BCVA.

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Monitoring and Mapping of Insecticide Resistance in Vector of Cutaneous Leishmaniasis, *Phlebotomus Papatasi* (Diptera: Psychodidae) in Iran

By Maryam Molaeezadeh, Amrollah Azarm, Mohammad Nasrabadi, Fatemeh Shahidi, Faramarz Bozorgomid & Hassan Vatandoost

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Abstract- Sandfly, Phlebotomus papatasi is an important vector of zoonotic cutaneous leishmaniasis (ZCL) that plays the main role in the transmission of leishmaniasis in Iran. ZCL is one of the most common endemic diseases in Iran. The prevalence of resistance to insecticides in vector species around worldwide is a serious threat to the fight against vector-borne diseases. To provide authentic information about this novel, the reliable data on academic resources such as Google Scholar, Scopus, Web of Science, Springer, Pro-Quest, Wiley Online, Science Direct, Research Gate, PubMed, Sage, and SID were used. There are some levels of resistance in some parts of Iran like, Lorestan and Isfahan province. Resistance to DDT 4% was observed in some parts of Iran. In Lorestan province -Pol-e Dokh-tar, Rumeshgan, and Kuhdasht districts- samples collected in the form of hand catch and indoors showed resistance to DDT 4%.Studies in the rural district of Badrood, Natanz County, Esfahan province showed resistance to DDT 4%.

Keywords: *phlebotomus papatasi, insecticide, resistance, leishmaniasis, Iran.*

GJMR-K Classification: *NLMC Code: WR 345*

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Monitoring and Mapping of Insecticide Resistance in Vector of Cutaneous Leishmaniasis, *Phlebotomus Papatasi* (Diptera: Psychodidae) in Iran

Maryam Molaeezadeh [α], Amrollah Azarm [σ], Mohammad Nasrabadi [ρ], Fatemeh Shahidi [ω], Faramarz Bozorgomid [¥] & Hassan Vatandoost [§]

Abstract- Sandfly, *Phlebotomus papatasi* is an important vector of zoonotic cutaneous leishmaniasis (ZCL) that plays the main role in the transmission of leishmaniasis in Iran. ZCL is one of the most common endemic diseases in Iran. The prevalence of resistance to insecticides in vector species around worldwide is a serious threat to the fight against vector-borne diseases. To provide authentic information about this novel, the reliable data on academic resources such as Google Scholar, Scopus, Web of Science, Springer, Pro-Quest, Wiley Online, Science Direct, Research Gate, PubMed, Sage, and SID were used. There are some levels of resistance in some parts of Iran like, Lorestan and Isfahan province. Resistance to DDT 4% was observed in some parts of Iran. In Lorestan province -Pol-e Dokh-tar, Rumeshgan, and Kuhdasht districts- samples collected in the form of hand catch and indoors showed resistance to DDT 4%.Studies in the rural district of Badrood, Natanz County, Esfahan province showed resistance to DDT 4%. The *Phlebotomus papatasi* is susceptible to other insecticides like permethrin 0.75%, deltamethrin 0.1%, cyfluthrin 0.15% and Lambda-cyhalothrin 0.05%. .According to a recent study, *Ph. papatasi* showed resistaceto some kind of pesticides. Constant monitoring, having a map of insecticide resistance can be an alert for the health system and is a good guide for vector disease control. Furthermore, guidelines are needed for monitoring and evaluation of insecticide susceptibility tests against sand flies.

Keywords: phlebotomus papatasi, insecticide, resistance, leishmaniasis, Iran.

I. Introduction

Leishmaniasis is one of the most important communicable diseases between humans and animals transmitted to humans by sand fly species. The prevalence of ZCL in Iran has always been increasing, so that between 2001 and 2005 shows about a 105% increase. The known rural foci of Leishmaniasis have been reported from the villages of East Isfahan, Turkmen Sahara, Natanz, Sarakhs, Lotfabad, Khuzestan, Ilam, Khorasan, Shiraz, and Kashan[1-5] (Fig.1). Due to the widespread prevalence of ZCL in Iran and the world, to break the disease transmission chain, appropriate practical approaches are needed, such as the use of various personal protection methods like long-lasting bed nets and insecticide-impregnated curtains, using insect repellents at work, and outdoors, indoor spraying is limited in scale[6]. As part of control programs, sand flies have been exposed to four major classes of synthetic insecticides: Organochlorine, pyrethroids, Organophosphates, and Carbamates. These exposures have been either intentional in directed vector control efforts or have been inadvertent as part of malaria control efforts against Anopheles[7]. The prevalence of insecticide resistance in vector species worldwide is a continuous threat for any success at mitigating the spread of vector-borne diseases. Most species of phlebotomine sandflies remain susceptible to insecticides. However, around the world, there is increasing evidence of insecticide resistance.

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Figure 1: Map of Iran providing the province outlines, in brown the provinces that are endemic for zoonotic cutaneous leishmaniasis(1)

a) Detecting Insecticide Resistance

Managing insecticide resistance requires timely, accurate data through resistance monitoring and insecticide evaluation to assess a vector species' susceptibility to insecticides. The primary way to assess insecticide resistance in many vectors, including sand flies, is to use insecticide susceptibility bioassays. The two most commonly used bioassays worldwide are the WHO exposure kit bioassay and the Centers for Disease Control (CDC) bottle bioassay[8]. The WHO exposure kit bioassay is a standardized protocol that consists of an exposure kit containing tubes lined with filter papers that are impregnated with a specific concentration of an insecticide. The CDC bottle bioassay protocol consists of exposing insects to concentrations of insecticide that are coated on the interior of glass bottles. Both bioassays have been used to assess insecticide resistance in sand flies, but the WHO bioassay is used more frequently[7].

b) Resistance Mechanisms

Insecticide resistance to synthetic insecticides have been reported in many important insect vectors such as mosquitoes, black flies, Triatomine bugs, lice, fleas, and sand flies. Four mechanisms of resistance are known to exist in insects: reduced penetration, behavior avoidance, target-site insensitivity, and metabolic detoxification. Of the four, target-site insensitivity and metabolic detoxification are the two most geographically and entomologically widespread. Today, there is evidence of target-site insensitivity and metabolic detoxification resistance to the four main classes of synthetic insecticides in all major vector species[7]. The insecticide resistance mechanisms in *Ph. papatasi* have not been identified, unlike the mechanisms of more intensely studied insects such as mosquitoes and house flies. Numerous susceptibility tests have been carried out in the foci of ZCL in Iran in against *Ph. papatasi*. This results are the reviews on the monitoring and mapping of insecticide resistance in *Ph. papatasi* in Iran.

c) Characteristics of Ph.papatasi

Sandflies are tiny insects, 1.5-3.5 mm in length, with a hairy appearance, large black eyes, and long, stilt-like legs. Sandflies can be distinguished from other Diptera, especially members of the Psychodidae family to which these insects belong, by the way, they rest their wings, which look like a V. The Sand-fly *Ph. papatasi*is the well-known vector of zoonotic cutaneous leishmaniasis and sand-fly fever[3](2). This species is endemic to most parts of Iran[3]. The *Ph.papatasi* prefers human habitats rather than other spices even though it found in human habitats in mountainous areas[9](3). Their Resting places are animal and human habitats also that caught from the plains place much more than the mountains. It is found in rodents' nests, rooms, stables, and wall crevices, and in all biotopes. It is interested in heat and humidity as well as this grows well where the groundwater level is high. This species is sensitive to heat but is resistant to rain. In terms of blood-feeding, it is more interested in human, rodent blood and bites several times during feeding to supplement its food[10-12].

d) Distribution of Ph.papatasi in Iran

The sand fly *Ph. Papatasi* is widely distributed in Iran. There are in East Azerbaijan, West Azerbaijan, Ardabil, Isfahan, Ilam, Bushehr, Tehran, Chaharmahal Bakhtiari, Khorasan, Khuzestan, Zanjan, Semnan, Sistan

and Baluchestan, Fars, Qom, Kurdistan, Kerman, Kermanshah, Golestan, Gilan, Lorestan, Mazandaran, Markazi, Hormozgan, Hamedan and Yazd [1,3,14] (Fig.2). Figure 3 shows the symptoms of ZCL.

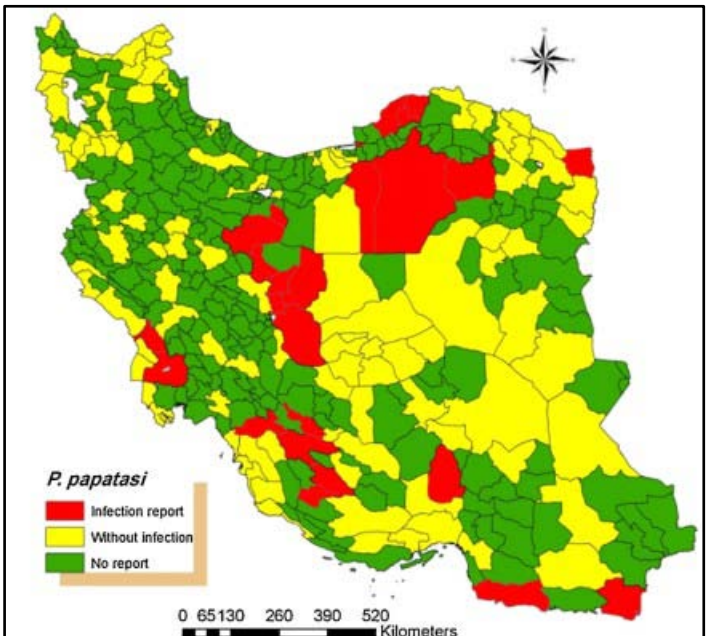

Figure 2: Distribution of *Ph. papatasi* infection to Leishmania parasites in Iran

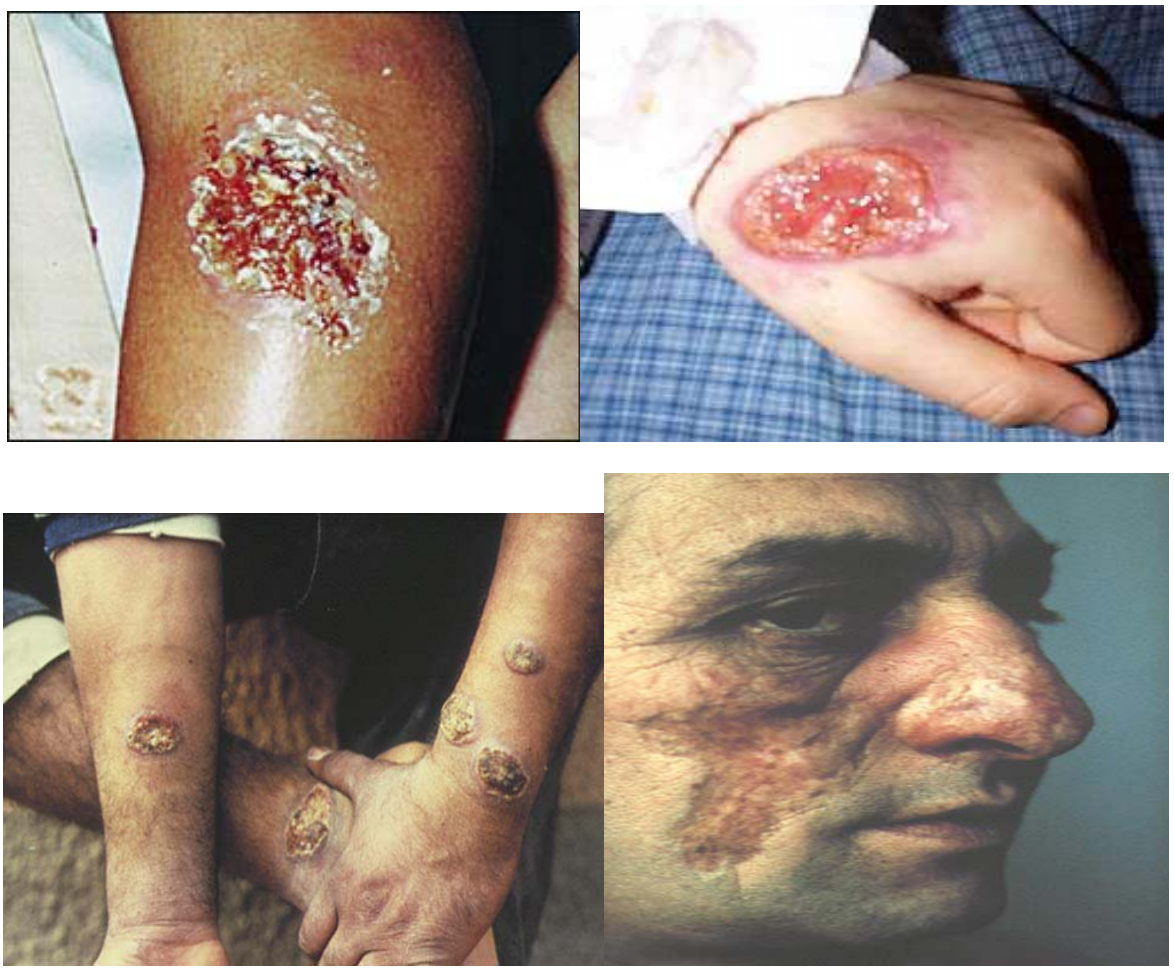

Figure 3: Symptoms of Zoonotic Cutaneous Leishmaniasis (ZCL)

e) The control management Ph.papatasi in Iran

The main vector of the rural type of leishmaniasis is *Ph. papatasi*, which is semi-wild. The most of transmission takes place in an outdoor place, so spraying does not have a significant effect on reducing cases except in the event of epidemics, which may be effective. Although the make use of bed nets, curtains treated by the deltamethrin insecticide with a shelf life of more than five years may lead to the severance of the transmission chain in rural seekers, the covered population must have been properly trained beforehand. Control of sandflies was started using residual insecticides such as DDT, lindane, and aerosols. DDT and lindane were used as emulsions, aqueous suspensions, soapy water suspensions, solutions, and powders. DDT in the form of aerosol was also very effective. DDT and lindane aerosols were mainly used for surface spraying. These compounds were characterized by their lasting effect. Sprayed surfaces retained their insecticidal effect for several weeks and even months after application.

II. Method

To provide authentic information about these novel results, we used reliable data on academic resources such as Google Scholar, Scopus, Web of Science, Springer, Pro-Quest, Wiley Online, Science Direct, Research Gate, PubMed, Sage, and SID.

III. Result and Discussion

A glance at the table number 1 and Fig.4 provided reveals the susceptibility status of *Ph. papatasi* to DDT (4%), permethrin (0.75%), deltamethrin (0.1%), cyfluthrin (0.15%) and lambda-cyhalothrin (0.05%), In four different years 2011, 2013, 2017 and 2020. It has been estimated in the rural district of Badrood, Natanz County, Esfahan province. The results revealed that this species was resistant candidate to DDT but susceptible to other insecticides[13,15-17]. In a similar study in Lorestan Province-Pol-e Dokh-tar, Rumeshgan, and Kuhdasht districts the results showed that this species was resistant to DDT 4% but susceptible to bendiocarb 0.1%, permethrin 0.75%, deltamethrin 0.05%, and cyfluthrin 0.15%[18-23].

Table 1: Status of insecticide resistance in *Ph. papatasi* in different parts of Iran

Area	Method	Insecticide	Susceptibility status	Ref.	year
Dehbakri County, Esfahan	Aspirator	DDT4%	100	(4)	2011
		Deltamethrin 0.05%	100		
Lorestan Province	Hand catch/indoor/baited traps/outdoor	DDT 4%	R(87.7)	(5)	2020
			S(92.0)		
		Bendiocarb 0.1%	S(93.4)		
			S(94)		
		Permethrin 0.75%	S(92.4)		
			S(97.9)		
		Deltamethrin 0.05%	S (96.8)		
			S (97.8)		
		Cyfluthrin 0.15%	100		
			100		
Arsanjan - Fars province	Aspirator	DDT4%	S (96.7)	(6)	2000
Natanz county, Esfahan	Aspirator	Deltamethrin 0.05%	S (97.86)	(7)	2017
		λ-cyhalothrin 0.05%	S (97.78)		
		Cyfluthrin 0.15%	S (100)		
		Permethrin 0.75%	S (98.7)		
		DDT4%	RC (96.)		
Natanz county, Esfahan	Aspirator	DDT 4%	Female, LT50:1312.66	(8)	2013
			Male, LT50:1200.97		
		Permethrin 0.75%	Female, LT50:2 53.66		
			Male, LT50:310.10		
		Deltamethrin 0.1%	Female, LT50:36.47		
			Male, LT50:18.63		
		Cyfluthrin 0.15%	Female, LT50:9.36		
			Male, LT50:6.08		
		λ-cyhalothrin 0.05%	Female, LT50:6		
			Male, LT50 : 0.77		
Natanz county, Esfahan	Aspirator	DDT 4%	Female, LT50:1104.97	(9)	2011

			Male, LT50:973.51		
		Deltamethrin 0.1%	Female, LT50:26.79		
			Male, LT50:4.4,		
		Permethrin 0.75%	Female, LT50:182.35		
			Male, LT50:59.5		
			Female, LT50:1.48		
		λ-cyhalothrin 0.05%	Male, LT50:1.5 s		
		Cyfluthrin 0.15%	Female, LT50:15.42		
			Male, LT50:2.65		
Natanz county, Esfahan	Aspirator	DDT 4%	Female, LT50:641.62	(10)	2020
			Male, LT50:439.28		
		Deltamethrin 0.1%	Female, LT50:146.44		
			Male, LT50:97.75		
		Permethrin 0.75%	Female, LT50:136.15		
			Male, LT50:108.90		
		λ-cyhalothrin 0.05%	Female, LT50:72.69		
			Male, LT50:57.84		
		Cyfluthrin 0.15%	Female, LT50:8.71		
			Male, LT50:5.00		
KoohSangi, Mashhad	Aspirator	DDT 4%	100	(11)	1985
Khajeh Rabi, Mashhad	Aspirator	DDT 4%	100	(11)	1986
Shahporabad, Isfahan	Aspirator	DDT 4%	75	(11)	1985
Komshecheh, Isfahan	Aspirator	DDT 4%	76	(11)	1985
Komshecheh, Isfahan	Aspirator	DDT 4%	100	(11)	1986
Omkolsum, Khuzestan	Aspirator	DDT 4%	100	(11)	1986
Susangerd, Khuzestan	Aspirator	DDT 4%	100	(11)	1986
Natanz county, Esfahan	bed nets	Deltamethrin 0.1%	100	(12)	2006

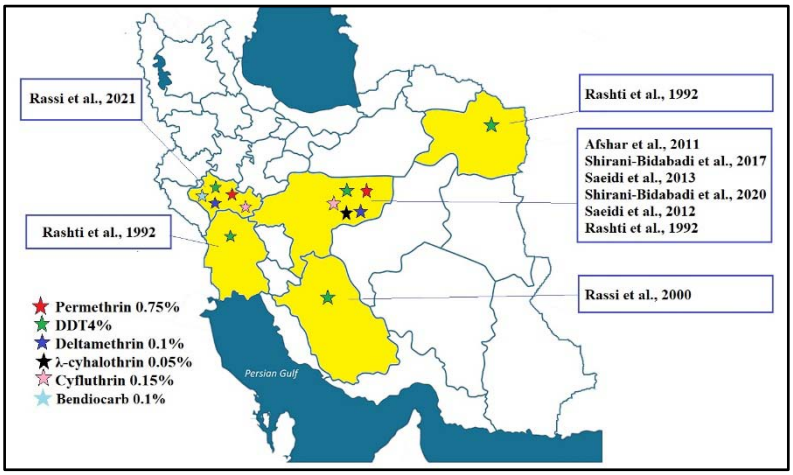

Figure 4: Status of insecticide resistance in *Ph. papatasi* in different parts of Iran

IV. Conclusion

Constant monitoring, having a map of insecticide resistance in Iran can be alert for the health system and is a good guide for vector disease control. Furthermore, guidelines is needed for monitoring and evaluation of insecticide susceptibility tests against sand flies.

Declarations: All the author declare that there is no conflict of interest.

Statements on the authors' contributions: All the authors were involved.

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Conflict of Interest: All authors declare that there is no conflict of interest.

Ethical approval: Not applicable

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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.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

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:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

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.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- 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.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

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.

Introduction:

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.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

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.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

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.

Materials:

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

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- 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.

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.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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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