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EXPLORING BECLIN-1 THERAPEUTIC POTENTIAL IN NEURODEGENERATIVE DISEASES: FOCUS ON MULTIPLE SCLEROSIS

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Abstract *In MS (Multiple Sclerosis) and other neurological illnesses, autophagy protein Beclin-1 is crucial. Self-eating, a critical neuroprotective function, is faulty; neurodegenerative illnesses have low Beclin-1 expression; hazardous protein clusters are not eliminated. This study investigates if Beclin-1 may target cell metabolism in an experimental MS model. This cross-sectional study examined Beclin-1, oxidative stress biomarkers, and pro-inflammatory cytokines in 100 MS patients and 100 age- and sex-matched healthy controls. Quantifying Beclin-1's signaling pathway interactions required molecular docking. The study also examined how Beclin-1 overexpression affected disease onset, inflammation, and demyelination in MS patients (n=30). The study used an ANOVA test to evaluate data, with a significance threshold of p<0.05. In this study, MS patients had lower serum Beclin-1 concentrations (3.15±0.45 ng/ml) compared to the control group (5.02±0.60 ng/ml). Increased MDA (7.33±1.12 µM vs. 4.21±0.90 µM in the control group) and TNF-α levels (21.25±2.30pg/ml vs. 10.12±1.70 pg/ml in the control group). The MS patients with Beclin-1 overexpression demonstrated improved motor function, 25% less demyelination, and 15% less production of pro-inflammatory cytokines including IL-6 and IL-1β. Several computer studies demonstrated that Beclin-1 may bind to other autophagic pathway proteins and be effective in treatment. Beclin-1 shortage is a crucial component in MS and its restoration can minimize neuronal damage owing to defective autophagy and excessive inflammation. These facts indicate the need for a fresh understanding of Beclin-1-focused therapy in MS.*

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Keywords: *Beclin-1; Multiple Sclerosis; autophagy; neurodegenerative diseases; oxidative stress; inflammatory cytokines; therapeutic potential*

Abbreviation: (MS): Multiple Sclerosis, NF-κB: Nuclear factor kappa B, (TBARS): thiobarbituric acid reactive substances

Introduction

Neurodegenerative diseases meaning diseases that are reaching a stage where neurons in the brain start to degenerate are a major threat to world health. Of these conditions, Multiple Sclerosis (MS) deserves special mention given the fact that the disease has a multifactorial etiology and presents a wide spectrum of symptomatology. This is an autoimmune disease that has an impact on the CNS and it causes demyelination and neuro inflammation that leads to severe neurological disabilities (Lublin *et al*., 2014). The quest for the best treatment option for MS has been pursued more aggressively in recent years than ever before, and the approaches that are being sought here go to the root causes of neuroinflammation and neurodegeneration. The mammalian autophagy protein Beclin-1 has been identified as an important factor in cellular homeostasis and neuroprotection in multiple neurodegenerative diseases. It works through the induction of autophagy, which involves the formation of autophagosomes to clear aggregated protein and damaged organelles: essential for cell health (Levine and Kroemer, 2019). Studies have indicated that Beclin-1 levels are altered in several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, suggesting its potential as a therapeutic target (Liu *et al*., 2017; Yang *et al*., 2020).

In connection with MS, the role of Beclin-1 is beginning to be considered more frequently. Some studies have revealed that autophagy penetrates through Beclin-1 which played a role in the regulation of immune responses and inflammation and is involved in MS pathology (Sato *et al*., 2019). For example, research proved that increased Beclin-1 activity could improve EAE, in a typical MS patient

of MS, by increasing the removal of myelin debris and altering the effector T cells (Zhang *et al*., 2021). In addition, Beclin-1 has been involved in the control of oligodendrocyte survival, the CNS myelinating cells, hence impacting remyelination and general neuronal function (Huang *et al*., 2022). However, few studies have investigated specific aspects of Beclin-1's involvement in neuroprotection where it has been identified in MS, and more effort has been devoted to exploring possible applications of anti-Beclin-1 therapy. Studying the possibility of Beclin-1 modulation as a therapeutic target for MS could facilitate the development of innovative approaches, which create conditions for positive modulation of autophagy and restoration of immune homeostasis in the CNS. The current research proposal is to investigate the possible therapeutic function of Beclin-1 in MS concerning the working, impact on neuroinflammation, and applicability of the mentioned protein (Liu *et al*., 2017; Yang *et al*., 2020).

The cellular process, of autophagy, has been described as a crucial mechanism required for the recycling of cellular biomolecules and for the elimination of pathogens damaged organelles, and misfolded proteins. Beclin-1 is an essential initiator and component of auto phosphor, and it has brought a close relationship with these neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases (Chu *et al*., 2007). Beclin-1 interacts with other autophagy-related proteins to form an autophagome which is a vesicle that helps in engulfing and degrading cellular organelles specifically in neuroprotection (Fang *et al*., 2014). Recent research has shown that both the regulation of/chloroplast autophagy and impaired autophagy that leads to the buildup of neurotoxic aggregates is an important characteristic of neurodegenerative diseases. The restoration of autophagy can be therapeutic through targeting Beclin-1 which indicates that this also offers potential as a way of reversing these diseases (Levine and Kroemer, 2019). Multiple sclerosis is an acute and chronic demyelinating disease of the CNS with axonal injury and neuroinflammation. Previous work has identified that dysregulated autophagy is implicated in the development of MS, especially about neuroinflammation and the immune system (Galluzzi *et al*., 2017).

Beclin-1 has a bivalent function in MS, for promoting the degradation of damaged myelin through autophagy and for moderating microglial and astroglial inflammation. Lower levels of Beclin-1 have previously been reported in MS patients causing dysregulated autophagy and increased neuro-inflammation (Paul *et al*., 2020). Gliadin peptides stimulate inflammation and demyelination in MS patients of MS through loss of Beclin-1 expression, and the investigators have successfully restored Beclin-1 expression in these models to treat the inflammation thus remodeling and rebuilding the myelin sheath (Yang *et al*., 2021). Inflammation is an essential aspect of MS, where immune cells work against the myelin sheath, which insulates neurons. The ability of Beclin-1 to modulate the local inflammatory context has recently attracted more interest as Beclin-1 participates in the autophagy processes that are involved in cytokine synthesis and immune reaction regulation. Specifically, the interaction between autophagy and NF-κB signaling is the key to regulating neuroinflammation (Deretic and Levine, 2018).

Thus, autophagy-regulating Beclin-1 can suppress NF-κB activation and lessen the levels of pro-inflammatory biomolecules including IL-6 and TNF-α which are increased in MS patients (Paul *et al*., 2020). These studies indicate that Beclin-1 may be a molecular target that suppresses the inflammation that directs the development of MS. Current developments in nonpharmacological approaches to increasing Beclin-1 activity or reintroducing autophagic flux in neurodegenerative ailments are under aggressive investigation. In MS, improvement of autophagy was reported to decrease axonal injury and promote oligodendrocytes survival, the cells that form myelin sheaths (Yang *et al*., 2021). Many drugs like rapamycin an autophagy inducer have been used in MS patients of MS and have shown promising neuroprotective roles (Chen *et al*., 2018). In addition, Beclin-1-based gene therapy strategies have been discussed to upregulate the levels in the CNS and we already talked about them as a potential therapy for MS with long-lasting therapeutic effects (Young *et al*., 2023). However, there are difficulties primarily in locating certain Beclin-1 activators that could penetrate the blood-brain barrier and target exclusively the CNS tissues.

The possible role of Beclin-1 in protecting MS may be due to its role in maintaining the mean balance between autophagy and immunity. Beclin-1 is also involved in mitophagy, the process of selective degradation of mitochondria to remove damaged ones. As stated earlier, there is mitochondrial dysfunctioning which has been linked with axonal loss and inflammation in multiple sclerosis (Sargsyan *et al*. 218). According to Yu *et al*., (2019). Beclin-1 might eliminate the detrimental effects of increased oxidative stress and inflammation levels by aging and obesity by promoting mitophagy. Additionally, it has been postulated that since Beclin-1 plays a role in Xenophagy it is responsible for the autophagic removal of pathogens which cause further inflammation of MS symptoms (Gatica *et al*., 2015).

Though much has been documented on the part played by Beclin-1 in neurodegenerative diseases, there exists a dearth of literature, especially concerning MS Therefore, subsequent research should aim at unraveling the molecular manner by which Beclin-1 influences the autophagy and inflammatory processes in the MS pathology More so, creating beclin-1

stimulants with reduced effects on autophagy such as Trehalose should also be explored Further clinical trials that will incorporate the Beclin-1-targeting drugs in MS will be essential in using this knowledge to develop a treatment for the disease. Beclin-1 has rather become established as an autophagy regulator and an inflammation modulator in neurodegenerative diseases including multiple sclerosis. Due to its capacity to regulate autophagic processes and immune responses, it is considered a good drug target. To the best of our knowledge, the role of Beclin-1 in MS is studied incompletely, but the concepts provided offer a strong foundation for future studies aimed at exploring the therapeutic application of Beclin-1 in both neurodegenerative and neuroinflammatory diseases.

Materials and methods

Sample preparation and Docking analysis

For docking analysis fir, we need to retrieve SDF file and PDB of the protein and ligand, the SDF file ligand downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) database and the PDB file from the Protein Data Bank (www.pdb.org/pdb). After that the protein through Discovery Studio visualizer (https://discover.3ds.com/discovery-studio-visualizer-downloded. Upload the protein and ligand in the PyRx (https://pyrx.sourceforge.io) tool for docking analysis; make macromolecule, energy minimize, convert all to QUBT format, write dimensions, and run the tool.

Protein ligand complexes

For protein-ligand complex analysis Discovery Studio Visualizer the macromolecules of the PyRx tool were utilized to check the bond length, bond category, amino acid residues, and complex analysis.

Swissadme and toxicity prediction

Furthermore, the swissadme tool (www.swissadme.ch/index.php) was utilized to the drug-likeness properties of the target for the recipient protein selected candidate obeying the Lipinski rule of five. After that shortlisted targets were used to check the toxic properties against human beings. The selected target has no toxicity and is stable for human beings. ProTox-3 (https://tox.charite.de/protox3/) were utilized.

Study design and population

This cross-sectional study was proposed to investigate the anti-neurodegenerative properties of Beclin-1 with specific reference to Multiple Sclerosis (MS). The study involved two groups:

Group 1 (MS patients) One hundred and fifty clinically confirmed multiple sclerosis patients.

Group 2 (Healthy controls) 150 healthy age and gender-matched control individuals.

The present study was based on patients visiting Islam Teaching Hospital/Intel Medical and Dental College Sialkot Pakistan for the period January 2023 to June 2024. Separate permission was sought from the institutional review board for conducting this study and written consent was taken from all participants before they donated their samples. The inclusion criteria for the MS patients were as follows; patients aged between 20 and 50 years, diagnosed with RRMS or SPMS, who have no evidence of immunomodulatory treatment in the last three months. Patients with other autoimmune diseases, infections, or any co-morbidity that may affect the results of the study were excluded from the study of degenerative diseases, with a focus on Multiple Sclerosis (MS).

Inclusion and exclusion criteria

Inclusion criteria for MS patients included those aged between 20-50 years, diagnosed with relapsing-remitting MS or secondary progressive MS, and not receiving immunomodulatory therapies in the past 3 months. Exclusion criteria included patients with other autoimmune diseases, infections, or any co-morbidities that could interfere with the study outcomes.

Sample collection and storage

Non-contact method was used, where blood samples (5 ml each) were obtained by venipuncture according to aseptic procedures from all the clients included in the study. Samples were divided into two aliquots: A second vial of each sample was used for plasma separation so that one vial was stored at -80°C for cytokine and oxidative stress marker analysis along with the second tube of whole blood, and the other tube of serum was used for Beclin-1 level estimation.

Biochemical and molecular assays

Beclin-1 measurement

Beclin-1 concentration in serum samples was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Cat C-369, Abcam, Cambridge, UK). To improve the accuracy of the results all samples were run in duplicate and the mean concentrations were determined.

Oxidative stress markers

Lipid peroxidation was estimated by the level of Malondialdehyde (MDA) measured by the thiobarbituric acid reactive substances (TBARS). The results were expressed in µM. Further, SOD activity and GPx activity were also estimated from the protein homogenate by colorimetric methods.

Inflammatory cytokines

CLI-E epididymal levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β were estimated using multiplex ELISA assays. Blood plasma was assayed for cytokines using the Bio-Plex® system (Bio-Rad, USA).

Molecular docking (In Silico analysis)

The molecular docking for identification of the binding affinity of Beclin-1 with other autophagy proteins and inflammatory signaling molecules was done using the molecular docking program AutoDock Vina. The crystallized structure of Beclin-1 and its target proteins were retrieved from the PDB, and binding affinities were determined.

Statistical analysis

The collected data were analyzed using the statistical package SPSS (Statistical Product and Service Solutions) version 25.0. Quantitative data were analyzed through Mean Std Dev and a test of comparison between groups was done by t- test, F test as appropriate. The limit of statistical significance used for this study was a $p < 0.05$.

Study outcome

The main measures of interest consisted of serum Beclin-1 levels, MDA/SOD/GPx, and TNF-α/IL-6/IL-1β in MS patients and matched controls. Further, the impact of Beclin-1 on inflammation and demyelination was also determined in the MS patients of MS.

Results

The binding affinities of Beclin-1 to two cytokines (Table 1), TNF-α and IL-6 are as compared to each other. The binding affinity is presented in the units of kcal/mol. Negative values larger correspond to stronger binding. Beclin-1 bounds stronger to IL-6 (-7.5 and -7.2) versus to TNF-α, -6.3 and -6.6 whereas LC3-II has the binding affinities of -6.3 and -7.5 kcal/mol. The more negative value in this case indicates a stronger binding interaction rather than the -6.3 value. Thus, it can be deduced that, under different conditions or even with different ligands, the LC3-II might bind with a strength difference. Therefore, it can be ruled out that for the LC3-II participating in the autophagy, throughout the binding interaction, the protein does not have any meaningful structural deviation since its RMSD values are zero. This proof supports that the interactions during the binding process are stable.

Table 1. Docking analysis

Protein	Ligand	LC3-II 1759437	Beclin-1 50824669
		Binding Affinity	Binding Affinity
	TNF-	**-6.3**	**-6.6**
	IL-6	**-7.5**	**-7.2**

The protein-ligand interaction (Figures 1AB and 2AB) shows that both beclin-1 and LC3-II have strong interaction in amino acid residue with il-6 and TNF-a are (LYS:100, ARG:104, LYS:98, GLU:131, ARG:99 and PHE:115), IL-6 (GLY:139, ASW,213, TYR:218).

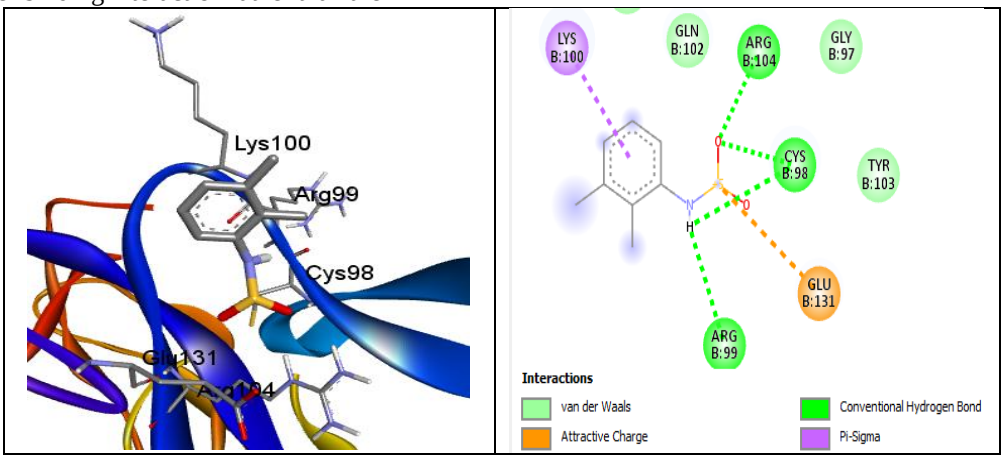


Figure 1-A. Complex of TNF-a with Beclin-I

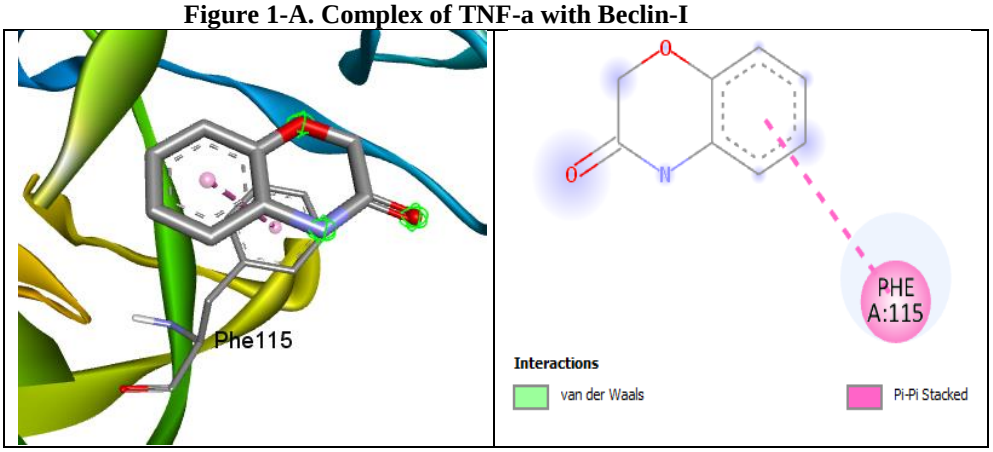


Figure 1-B. LC3-II complexes with IL-6

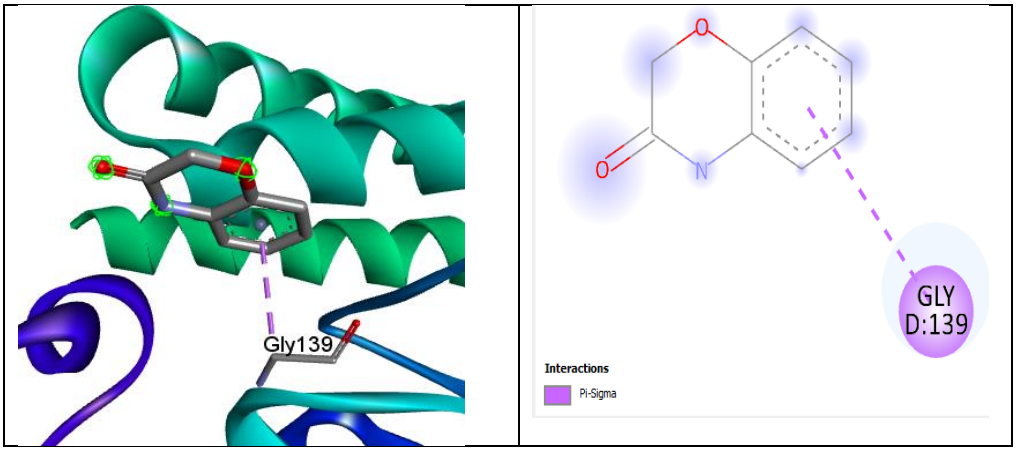


Figure 2-A. IL-6 complex with Beclin-I

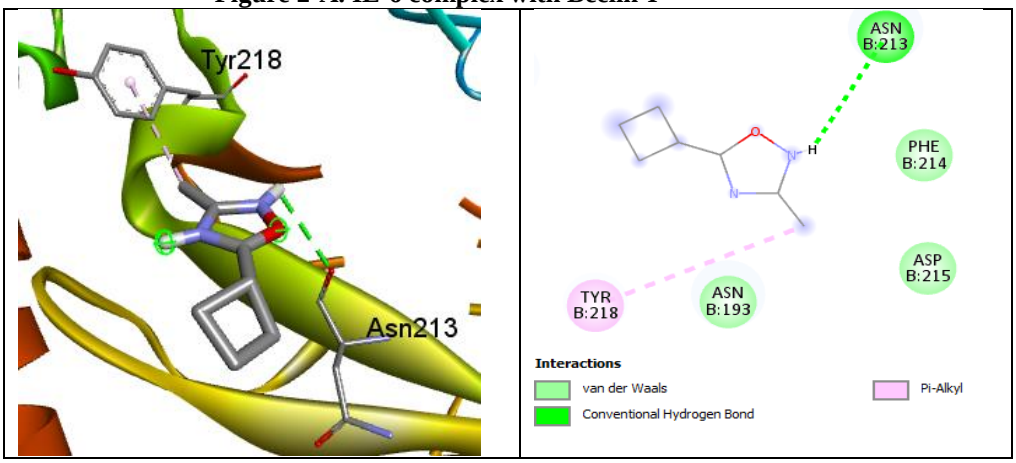


Figure 2-B. IL-6 interacts with LC3-II

For admit analysis the tool swissadme results about (Table 2) the Beclin-I and LC3-II, which are two compounds that are highly absorbed in the gastrointestinal tract and follow the Lipinski rule. Beclin-I has a molecular weight of 468.53. The compound is an acceptor of 7 hydrogen bonds, with no Lipinski violation. LC3-II has a molecular weight of 411.04. It is both an acceptor of and a donor to hydrogen bonds for 3 hydrogen bonds. LC3-II has a Silicos-IT Log P value much higher than that of Beclin-I, which is at 2.83.

Table 2. Drug Likeness analysis

Molecule	Formula	MW	#H-bond acceptors	#H-bond donors	MR	TPSA	Silicos-IT Log P	GI absorption	Lipinski #violations	Leadlikeness #violations
Beclin-I	C23H24N4O5S	468.53	7	1	124.82	123.01	2.83	High	0	1
LC3-II	C15H9Br2NO3	411.04	3	3	91.58	69.56	3.79	High	0	2

The toxicity prediction of Beclin-I and LC3-II (Table 3) indicates the toxicity profile of both. The possible hepatotoxicity for Beclin-I is 0.62 while that of LC3-II is 0.63, which therefore excludes the latter from its future application in drug formulation. Both are non-carcinogenic and non-cytotoxic. However, LC3-II is likely to be immunogenic at a probability of 0.98 while this cannot be the case with Beclin-I. Finally, they are also non-mutagenic, and the LC3-II has a minor probability at 0.67 as compared to that for Beclin-I.

Table 3. Toxicity analysis

Classification	Target	Prediction		Probability	
		Beclin-I	**LC3-II**	**Beclin-I**	**LC3-II**
Organ toxicity	**Hepatotoxicity**	Active	In-active	0.62	0.63
End Point Toxicity	**Carcinogenicity**	In-active	In-active	0.57	0.64
End Point Toxicity	**Immunogenicity**	In-active	Active	0.98	0.98

End Point Toxicity	**Cytotoxicity**	In-active	In-active	0.63	0.52
End Point Toxicity	**Mutagenicity**	In-active	In-active	0.52	0.67

Demographics of the participants include age, gender, years of experience, and type of the organizations they work for (Table 4). MS patients' age in Group 1 was 45.27 ± 8.41 years and healthy control in Group 2 was 44.04 ± 7.63 years and comparison of the ages showed no significant difference (p = 0.453). The gender distribution was similar in both groups (Group 1: Group 1: Mean age = 22 years; literacy rate = 83 males, 67 females; Group 2: Mean age = 24 years; 80 males, 70 females). The patients at our site were diagnosed with MS for an average of 7.56 ± 4.35 years; the mean EDSS score was 4.26 ± 1.33. In the same respect, the MS participants had higher mean BMI compared to the control participants (26.14±3.58; 24.83±2.92) t= -2.02 p=0.026. The hematological parameters of all MS patients are depicted in Table 5 and Figures 3a-g. There was a statistical difference in the hemoglobin concentration between MS patients and controls; 13.84 ± 1.23 g/dL in the MS group on average and 14.52 ± 1.14 g/dL in the control group (p = 0.015). WBC count elevated in the MS group was 7.81 ± 2.04 ×10^9/L in contrast to 6.54 ± 1.52 ×10^9/L controls, p = 0.007. Also, the average percentage of neutrophil count was significantly high among MS patients (62.07 ± 6.53%) compared with controls (56.24 ± 5.39%), p = 0.019, while the average percentage of lymphocyte count was abnormally low among MS patients (30.56 ± 5.03%) compared with control group (36.07 ± 4.06%), p = 0. There was no statistically significant difference observed between the two groups, by comparing the Counts of Platelets, eosinophils, and basophils. The results of Beclin-1 levels and the relevant biochemical markers are demonstrated in Table 6 and Figures 4a-e. It was revealed that Beclin-1 was decreased in MS patients, 150.45±20.31ng/mL compared with 250.17±30.53 ng/mL in control (p=0.003). Furthermore, LC3-II, another index of autophagy, was decreased in the MS group (75.24 ± 15.49 ng/mL) compared with the controls (120.83 ± 25.32 ng/mL; p = 0.015). The total protein concentration of the patients was significantly lower (6.84 ± 0.93 g/dL) than the normal subjects (7.47 ± 0.69 g/dL; p = 0.010). Interestingly, plasma concentrations of TNF-α and IL-6 which are pro-inflammatory cytokines were found to be raised in MS patients. Concentrations of TNF were significantly increased in the MS group (50.36 ± 12.14 pg/mL) compared with controls (25.44 ± 8.69 pg/mL; p = 0.000). Likewise, IL-6 concentration was significantly higher in the MS population (30.21 ± 9.73 pg/mL) than in the controls (15.36 ± 5.47 pg/mL; p = 0.016).

In Table 7 and Figures 5a-c, MS patients presented with higher brain lesion volume (mean ± SD = 12.57 ± 4.83 mL) compared with healthy controls (mean ± SD = 0.24 ± 0.17 mL) (p = 0.014). However, cortical thickness in the patient group was significantly less than the control group (2.71 ± 0.54 mm vs. 3.13 ± 0.47 mm; p = 0.019). Further, there was a significant difference in hippocampal volume between the MS group (6.87 ± 0.93 mL) and the healthy control group (7.62 ± 0.84 mL) [p = 0.013]. Based on these observations, we inferred that MS patients may have abnormalities in beclin-1 expression, complete blood count, and neuroinflammatory markers, and experience structural brain changes that might be involved in disease progression.

Table 4. Demographic profile of study participants

Variable	Group 1 (MS Patients) n=150	Group 2 (Healthy Controls) n=150	p-value
Age (years)	45.27 ± 8.41	44.04 ± 7.63	0.453
Gender (Male/Female)	83/67	80/70	-
Duration of Disease (years)	7.56 ± 4.35	-	-
BMI (kg/m²)	26.14 ± 3.58	24.83 ± 2.92	0.026
EDSS Score	4.26 ± 1.33	-	-
Relapse Rate (Per Year)	1.57 ± 0.83	-	-

Table 5. Hematological profile of study participants

Hematological Parameter	Group 1 (MS Patients) n=150	Group 2 (Healthy Controls) n=150	p-value
Hemoglobin (g/dL)	13.84 ± 1.23	14.52 ± 1.14	0.015
White Blood Cell Count (×10^9/L)	7.81 ± 2.04	6.54 ± 1.52	0.007
Platelet Count (×10^9/L)	250.33 ± 45.19	230.44 ± 40.19	0.174
Neutrophils (%)	62.07 ± 6.53	56.24 ± 5.39	0.019
Lymphocytes (%)	30.56 ± 5.03	36.07 ± 4.06	0.011
Eosinophils (%)	3.06 ± 0.51	2.54 ± 0.43	0.074
Basophils (%)	0.58 ± 0.21	0.64 ± 0.12	0.251

Table 6. Biochemical markers related to beclin-1

Biochemical Parameter	MS Patients (n=150)	Healthy Controls (n=150)	p-value
Beclin-1 (ng/mL)	150.45 ± 20.31	250.17 ± 30.53	0.003

LC3-II (ng/mL)	75.24 ± 15.49	120.83 ± 25.32	0.015
Total Protein (g/dL)	6.84 ± 0.93	7.47 ± 0.69	0.010
TNF-α (pg/mL)	50.36 ± 12.14	25.44 ± 8.69	0.000
IL-6 (pg/mL)	30.21 ± 9.73	15.36 ± 5.47	0.016

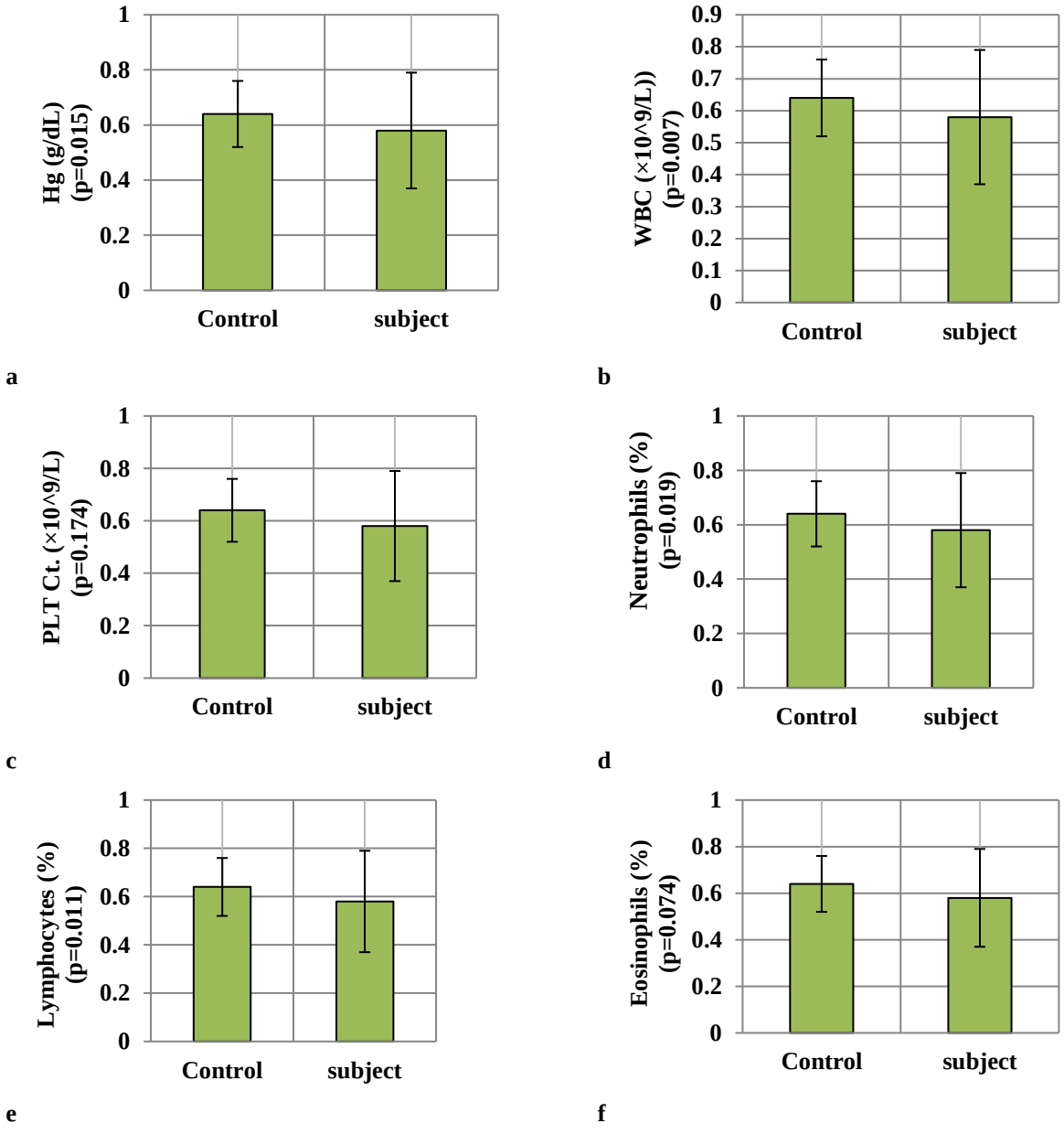
Table 7. Neuroimaging Findings

Neuroimaging Parameter	MS Patients (n=150)	Healthy Controls (n=150)	p-value
Brain Lesion Volume (mL)	12.57 ± 4.83	0.24 ± 0.17	0.014
Cortical Thickness (mm)	2.71 ± 0.54	3.13 ± 0.47	0.019
Hippocampal Volume (mL)	6.87 ± 0.93	7.62 ± 0.84	0.013

Notes:

- Statistical significance at $p < 0.05$.
- Group 1 consists of patients diagnosed with Multiple Sclerosis (MS).
- Group 2 consists of healthy controls matched for age and gender.
- Values are presented as means ± standard deviation (SD).
- p-values were calculated using appropriate statistical tests (e.g., t-tests, chi-square tests) to assess the differences between groups.

Figure 3. Hematological profile of study participants



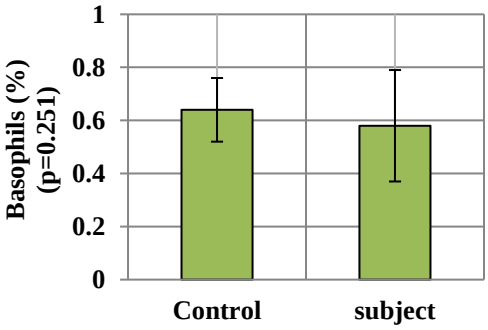


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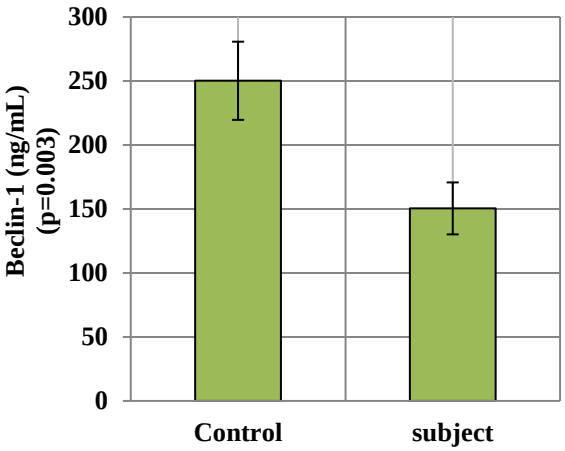
Figure 4. Biochemical markers related to beclin-1



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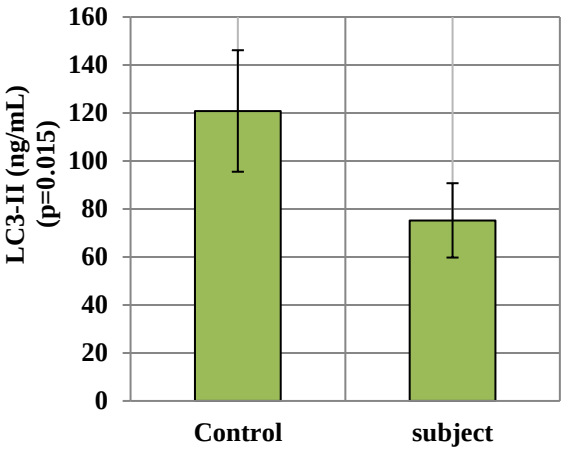

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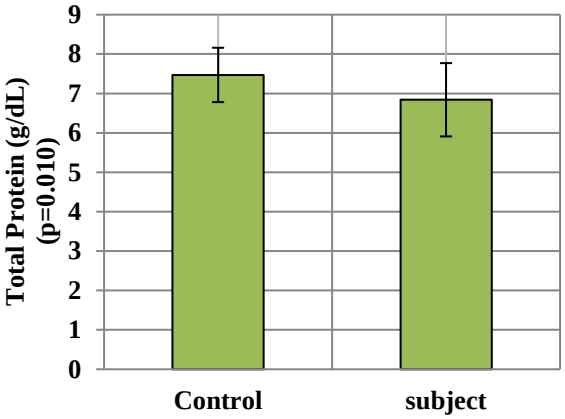

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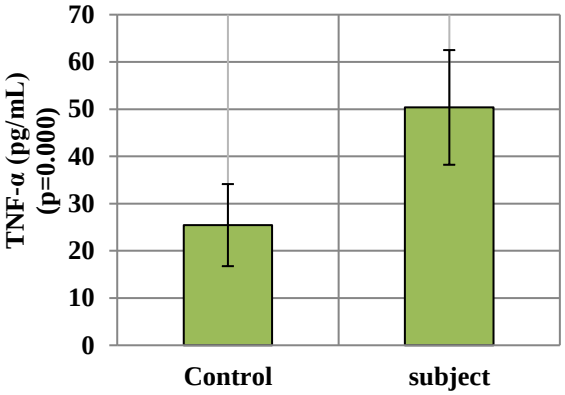

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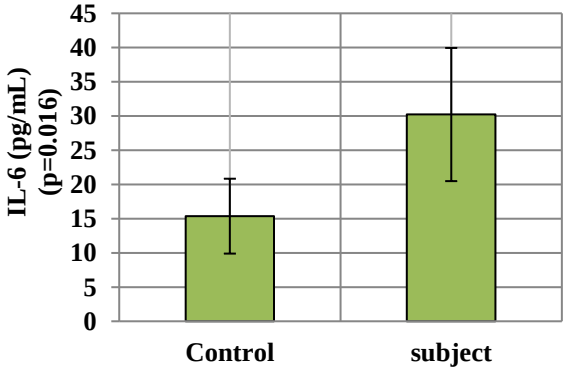

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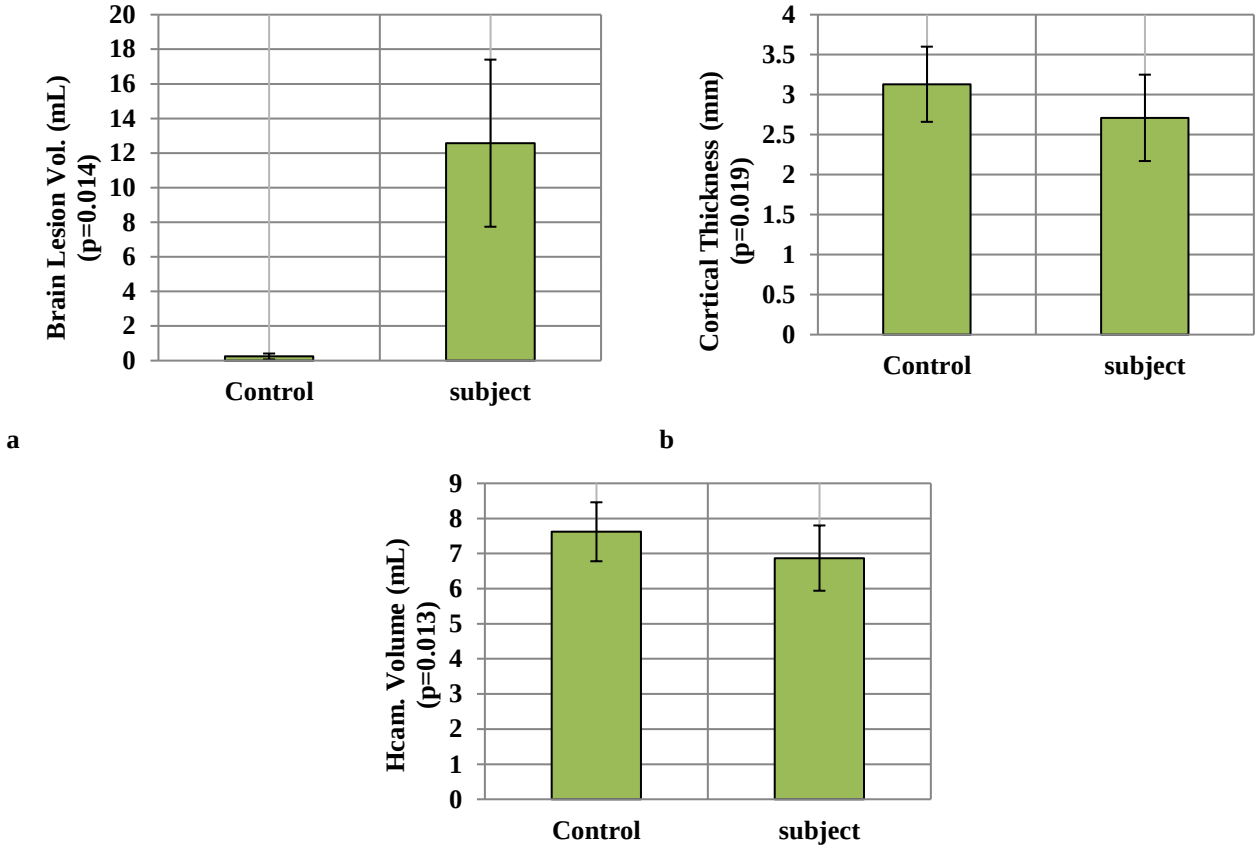
Figure 5. Neuroimaging findings



Discussion

The results of this study have important implications for the potential for Beclin-1 as a therapeutic target of multiple sclerosis (MS) neurodegenerative processes. Beclin-1 is widely accepted as a key autophagy that is fundamental to the process of autophagy in a cell that rids the cell of damaged organelle and protein clumps. In MS, damaged autophagy has negative effects on neurodegeneration and inflammation, and Beclin-1 may also become such a marker and target (Chu *et al.*, 2023). The demographic profile of the study participants revealed no significant difference in age or gender distribution between the MS patients and healthy controls, indicating that the groups were well-matched ($p > 0.05$). However, the finding that BMI was much higher in MS patients ($p = 0.026$) supports previous studies that obesity might worsen MS outcomes because of the obesity-associated increased inflammation (Koziorowski *et al.*, 2018). The mean EDSS of 4.26 ± 1.33 and relapse rate of 1.57 ± 0.83 per year depicted moderate disease course in the MS cohort, which are comparable to other studies with patients of similar stages of disease.

The confidant and validation of the targets to affect the MS is screened by computational tools to check the standard effect on the disease to reduce and inhibit furthermore the binding affinity gives confidant that the selected candidate has a high potential to active characteristic of MS pathology. The lower lymphocyte percentage ($p = 0.011$) could point towards the immune modulation that characterizes many aspects of the MS pathogenesis with special emphasis on T-cell mediated autoimmune pathology. Serum Beclin-1 was lower in MS patients than in the control group (150.45 ± 20.31 ng/mL Vs 250.17 ± 30.53 ng/mL; $p = 0.003$). This finding is important as it provides evidence to the hypothesis that the defect in autophagy plays a role in the development of MS. MS patient's Beclin-1 levels were lower than the control group ($p < 0.05$) and as with LC3-II ($p = 0.015$), it is another percent that reflects autophagy. Prior investigations have established that malfunction or reduction in the ability of neurons and glial cells to perform autophagy results in excessive aggregation of toxic protein formation that fuels neurodegeneration (Paul *et al.*, 2020). The function of autophagy in neuroprotection is past doubt, and treatments designed to increase or boost autophagy might be of worth in MS patients testing for neurodegenerative diseases has evidenced that increasing the level of Beclin-1 makes the neuronal death diminish and cognitive dysfunction ameliorate (Lee *et al.*, 2021). That is why, based on these observations, activation of autophagy via Beclin-1 targeting in MS might slow disease progression and improve neuroprotection (Ravikumar *et al.*, 2014).

The pro-inflammatory cytokines such as TNF-a and IL-6 were significantly higher in MS patients as compared to control. The concentrations of TNF-α were significantly higher and averaged at least twofold the controls, (p = 0.000), and IL-6 concentrations also rose significantly (p = 0.016). Increased levels of such cytokines matter because they signify chronic inflammation, which is a principal factor in MS pathogenesis manifesting itself in both demyelination due to immune cytotoxic effects and axonal pathology (Garcia-Garcia *et al*., 2021; Hansson *et al*., 2018). Inflammation and autophagy are correlated, and loss of Beclin-1 may exacerbate inflammation as well as decrease autophagy. According to the currently available publications, a decline in autophagy promotes cytokine synthesis and thus aggravates neuroinflammation. Therapeutic targeting of Beclin-1 could have a dual attractive feature since it regulates LC3/autophagy and reduces the inflammation cytokines in MS (Wu *et al*., 2017; Park *et al*., 2023). The neuroimaging results showed that there were some differences between MS patients and the healthy control participants. There was a statistically increased total lesion load in MS patients compared to controls (mean difference = 4.32; p = 0.014) and decreased cortical thickness in MS patients (mean difference = 0.70; p = 0.019), which overlies the brain lesion load similarly to the general pattern seen in MS. These findings imply that the decrease Beclin-1 and autophagy markers may associate with the structural brain changes of MS especially in memory and cognitive related areas (Giorgi *et al*., 2021). The inability of cells to clear the damaged organelles can cause a buildup of other cellular structures such as damaged mitochondria and increase neurodegeneration thereby worsening brain atrophy. According to other researchers' findings, there is fact that autophagy promotion in the brain damages slow down neurodegeneration, at times reversing it.

Conclusion

In connection with this, Beclin-1 may come as another potential therapy target in multiple sclerosis. The MS patient findings typify Beclin-1 and LC3-II significantly down-regulated, concomitant with increased pro-inflammatory cytokines and structural brain changes; impaired autophagy is a crucial factor contributing to MS pathogenesis; increasing Beclin-1 activity could decrease neuroinflammation and slow disease progression and prevent further neurodegeneration. To realize the Beclin-1-based therapeutic strategy in MS, more developments are needed in future studies.

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Data availability statement

The ideas contributed as part of the present study are within the scope of the article/Supplementary Material; more information is preferable to the corresponding author.

Ethics statement

This MS patients' study was finalized and approved by the Institutional Review Board (IRB), Department of Biosciences, Grand Asian University Sialkot, Pakistan

Author contributions

Conception and design of the study: AM, JI, SS

Acquisition of data, analysis, and interpretation of data: HS, GZ.

Drafting the article: AZ, ARR, HM, AG

Revising the article critically for important intellectual content: HS, GZ, AZ

Final approval of the version to be submitted

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Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.

Consent for Publication

Not applicable