Hotair (Homeobox Antisense Intergenic Rna) And Gas-5 (Growth Arrest–Specific 5) Expressions As Biomarkers For Early Diagnosis Of Multiple Sclerosis (Ms)

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Abstract: Background: Multiple sclerosis is a central nervous system autoimmune illness that is characterized by chronic inflammation, gliosis, demyelination, and neuronal loss. Long noncoding RNAs (LncRNAs) play a significant role in regulating immune response, as well as the development of immune cells.
Objective: This study aims investigate the role of lncRNAs; HOTAIR (HOX transcript antisense intergenic RNA) and (HOX transcript antisense intergenic RNA) in MS pathophysiology and their impact on clinical course of the disease.
Patients and methods: The present study was conducted on 134 subjects; 74 patients with MS (relapsing remitting and secondary progressive types) and 60 age and sex matched controls. Expression of both lncRNAs; HOTAIR and GAS-5 was assessed in serum using Real-time quantitative PCR (qPCR). The clinical disability in the patients was evaluated using the Expanded Disability Status Scale (EDSS) at the time of patient enrollment.
Results: The relative expression levels of HOTAIR were significantly down-regulated in serum samples of MS patients relative to the control group; (p value < 0.001) while relative expression levels of GAS-5 were significantly up-regulated in serum samples of MS patients relative to the control group; (p value = 0.002). There is significant positive association between GAS-5 expression level and age of onset in MS patients (P value= 0.020).
Conclusion: This differential expression of both lncRNAs may have an important role in MS pathophysiology. This study clarified the molecular pathways through which those lncRNAs contribute to MS clinical presentation.

Key words: Multiple sclerosis, LncRNAs, HOTAIR, GAS-5.

1. INTRODUCTION: Multiple sclerosis (MS) is a chronic demyelinating disorder that affects the central nervous system due to axon degeneration and gliosis, contributing to progressive neurological disability **(Azimi et al., 2018)**.

Multiple reports have stated that the human genome encodes long coding RNAs (lncRNAs), which in turn can participate in different biological processes involving regulation of gene expression. These molecules have tissue specific expression pattern that it accurately regulated in the CNS **(Gharesouran et al., 2018; Eftekharian et al., 2017)**.

Dysregulation of these molecules have been linked to multiple degenerative neurological disorders including multiple sclerosis, Parkinsonism, Alzheimer, and Huntington's diseases. Nevertheless, the exact mechanism linking that dysregulation with these disorders remains unclear **(Gharesouran et al., 2018)**.

HOX Transcript Antisense Intergenic RNA (HOTAIR) is one of these transcripts that was confirmed to contribute in the pathophysiology of MS in both animal and human studies **(Taheri et al., 2020)**. It is a lncRNA molecule encoded within the HoxC gene cluster located on chromosome 12 **(Pahlevan et al., 2018)**. Pahlevan and his associates have reported the significant high expression in MS cases with vitamin D deficiency. However, the exact mechanism behind that upregulation in such cases remains doubtful **(Pahlevan et al., 2018)**.

The growth arrest-specific 5 (GAS5) is long non-coding RNA whose gene is located on chromosome 1q25. It accumulates inside the human cells in response to lack of nutrition **(Senousy et al., 2020)**. It was initially detected during searching for tumor suppressor genes, and it was found to be highly expressed in during growth arrest **(Schneider et al., 1988)**. It has multiple functions including cell arrest control, protein synthesis alternation with apoptosis modulation **(Pickard and Williams, 2015).**

Furthermore, GAS5 has a crucial role in the modulation of glucocorticoid receptors, as it can bind to the receptor (DNA binding domain) and interfere with glucocorticoid response element (GRE) **(Mourtada-Maarabouni and Williams 2013;, Kino et al., 2010).** As steroids are known to modulate gene transcription in different autoimmune disorders, GAS5 blocks the activation of target gene transcription by the prevention of receptor binding to glucocorticoid response elements (GRE) in target genes. GAS5 lncRNA is known to serve as a decoy molecule or a steroid hormone receptor action riborepressor by competing with GREs for GR binding **(Pickard and Williams , 2015)**.

This study was aiming to assess the role of HOTAIR and GAS-5 in genetic susceptibility to MS and to study their potential role in clinical presentation, disease severity, course and progression.

2. PATIENTS AND METHODS

This is a prospective case control study that was performed at Al-Kasr Al-Ainy Multiple Sclerosis Unit-Cairo University Hospital. We included a total of 134 subjects; 74 patients who were diagnosed with MS (aged > 18 years and fulfilling the 2010 revised McDonald's

criteria for diagnosis of MS **(Polman et al., 2011)**, in addition to 60 age-and sex-matched healthy controls without clinical evidence, or family history of MS or any other autoimmune disorders.

On the other hand, pregnant females, patients with chronic infectious disease or recent infection within month, patients on steroid therapy, and patients with history of other autoimmune diseases or cancer were excluded from the current study.

An informed written consent has been obtained from all participants (cases and controls). Besides, this research has been accepted by Ethical Committee at Faculty of Medicine, Cairo University **"Ethical Code I-211016"**.

All cases were subjected to detailed history taking, thorough neurological examination, along with routine laboratory and radiological investigations. Also, the Expanded Disability Status Scale (EDSS) **(Zurawski et al., 2019)** was calculated for the included cases for evaluation of the clinical disability. Whole blood samples (5ml) were taken and serum was separated for quantification of serum expression levels of the two target lncRNAs, HOTAIR and GAS-5, using real-time quantitative PCR (qPCR).

Technique for assessment of serum expression levels of the two target markers:

A. RNA extraction:

This step was performed using miRNeasy mini kit and protocol for purification of serum total RNA, including noncoding RNAs (Qiagen, Valencia, CA, USA).

B. RNA quantitation and purity assessment:

RNA samples were subjected to RNA quantitation and purity assessment using the NanoDrop®-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA).

C. Reverse transcription of RNA into complementary DNAs (cDNAs):

Reverse transcription (RT) was carried out on total RNA in a final volume of 20 uL RT reactions using the RT^2 first strand kit (Qiagen, Valencia, CA, USA).

D. Real-time quantitative PCR (qPCR) for detection of lncRNAs, lncRNA HOTAIR and lincRNA GAS5:

This step was carried out using RT^2 qPCR Sybr Green/Master Mix kit and RT^2 lncRNA qPCR primer assays for HOTAIR and GAS-5 and GAPDH (used as reference gene). All these kits were purchased from (Qiagen, Valencia, CA, USA) with catalog no. of **HOTAIR; LPH07360A-200**, catalog no. of **GAS-5; LPH11340A-200** and catalog no. of **GAPDH; QT00079247.**

3. *Statistical analysis*

Microsoft Excel software is used to enter and analyze data. Data was then imported for review into the Statistical Package for Social Sciences (SPSS 22.0, IBM/SPSS Inc., Chicago, IL). For categorical data, the basic characteristics of the study population were represented as frequencies and percentages (%), while the quantitative data were presented as mean values and standard deviations (SD) or median (Range) according to the normality of the data.

For data comparison, two independent groups of qualitative data were compared using the Chi-Square test (or Fisher's exact test). To compare two groups of parametric and non-

parametric quantitative data respectively, the independent-Samples t-test and the Mann-Whitney U test were used for quantitative data.

In order to determine the best cutoff value of markers for case detection, the ROC (receiver operating characteristic) curve was constructed with area under curve (AUC) analysis. P values <0.05 are deemed to be significant for all the tests used.

4. RESULTS

Our study shows that the mean age in MS group was 32.5±8.1 years and in the control group was 30.2±8.9 years. This current study included a total of 134 subjects; 74 patients who were diagnosed with MS in addition to 60 age-and sex-matched healthy controls without clinical evidence, or family history of MS or any other autoimmune disorders.

In the current study, the average age in MS group was 32.5±8.1 years and there were 12 (16.2%) males and 62 (83.8%) females. In the MS group there were 12 (16.2%) males and 62 (83.8%) females while in the control group there were 12 (20%) males and 48 (80%) females. There was no statistically significant difference in the mean age and sex distribution between the two groups.

In cases with MS, only 4 cases had positive family history of MS, 54 cases (73%) were diagnosed with PRMS and 20 cases (27%) were diagnosed with SPMS. Regarding the symptoms of initial presentation, 24 cases (32.4%) presented mainly with optic neuritis, 30 cases (40.5%) presented mainly with weakness, 10 cases (13.5%) presented mainly with sensory manifestations and 10 cases (13.5%) presented mainly with ataxia. According to the disease severity, 40 cases (54.1%) had mild activity, 14 cases (18.9%) had moderate activity and 20 cases (27%) had severe activity. There were 18 drug naiive cases and 56 cases who received drug for treatment (Interferon, azathioprine or cyclophosphamide)

The relative expression levels of HOTAIR were significantly down-regulated in serum samples of MS patients compared to the control group; (p value < 0.001) while relative expression levels of GAS-5 were significantly up-regulated in serum samples of MS patients compared to the control group; (p value = 0.002).

The expression levels of HOTAIR didn't show a statistically significant difference between MS patients according to gender, family history, MS type, initial presentation, disease activity as detected by EDSS and treatment.

As regard to gender of patients, male patients had significantly higher expression levels as compared to female patients (p= 0.049). As regard to Initial presentation, patients with weakness had significantly higher expression levels as compared to patients with ataxia (p=0.008). The expression levels of HOTAIR didn't show a statistically significant difference between MS patients according to family history, MS type, disease activity as detected by EDSS and treatment

The distribution of HOTAIR and GAS-5 relative serum expression levels were shown in box plot diagrams (Figures (2), (3) respectively). Each box plot diagram displays the relative serum expression values of each gene according to the $2^{-\Delta\Delta CT}$ method (y-axis) by MS patients and control groups (x-axis). The data are presented as the median and inter-quartile range (IQR). The bold black line in the box represents the median value of our data. The

entire box represents the IQR. The bars across the boxes show the minimum and maximum values. Asterisks and circles indicate values (outliers) falling beyond the statistical distribution of the related variables (serum HOTAIR and GAS-5 respectively). (Figure 2 and 3)

There was significant positive correlation between GAS-5 expression level and age of onset in MS patients **(P value= 0.020)**. No statistically significant correlation was found between these two long non coding RNAs expressions, no significant correlation between EDSS and those two LncRNAs expressions in MS patients and no significant correlation between ages of the MS patients and those 2 lncRNAs expressions. There is no significant correlation between HOTAIR expression and age of onset in MS patients.

ROC (Receiver Operating Characteristic) curve of HOTAIR and GAS-5 relative expression levels for MS diagnosis:
ROC curve analysis of HOTAIR relative expression levels; **HOTAIR** with an area under the curve **(AUC) of 0.730, (95% confidence interval (CI) = 0.629–0.831, p < 0.001**). The sensitivity and specificity of HOTAIR were calculated using a **cut-off value of 0.8455** for serum HOTAIR expression.

ROC curve analysis of GAS-5: GAS-5 with an area under the curve **(AUC) of 0.649 (95% confidence interval (CI) = 0.540–0.757, p=0.003)**. The sensitivity and specificity of GAS-5 were calculated using a **cutoff value of 1.0435 for serum GAS-5 expression.**

Table (1): The demographic and clinical data of all subjects

Variables		**MS patients (n=74)**		**Control (n=60)**		**P-value**
Age [years] Mean ± standard deviation (SD)		32.5±8.1		30.2±8.9		0.119
		Count	**%**	**Count**	**%**	
Gender	**Female**	62	83.8%	48	80.0%	0.570
	Male	12	16.2%	12	20.0%	
Family history	**Yes**	4	5.4%			---
	NO	70	94.6%			
MS Type	**RRMS**	54	73.0%			---
	SPMS	20	27.0%			
Initial presentation	**Optic neuritis**	24	32.4%			---
	Weakness	30	40.5%			
	Sensory symptoms	10	13.5%			
	Ataxia	10	13.5%			
EDSS	**EDSS=1-3**	40	54.1%			---
	EDSS=3.5-5	14	18.9%			
	EDSS≥5.5	20	27.0%			
Treatment	**Interferon**	40	54.1%			---
	Azathioprine	12	16.2%			
	Cyclophosphamide (Endoxane)	4	5.4%			
	No treatment (Drug naiive)	18	24.3%			

Table (1): shows the demographic and clinical data of all subjects.

Table (2): Relative expression levels of HOTAIR and GAS5 the two study groups

	MS patients			Control			P value
	Median	Minimum	Maximum	Median	Min.	Max.	
HOTAIR	0.11	0.00	7.90	1.00	0.8	1.3	< **0.001**
GAS-5	1.48	0.13	8.82	1.00	0.9	1.2	**0.002**

Table (2) shows that relative expression levels of **HOTAIR** were significantly down-regulated in serum samples of MS patients compared to the control group; **(p value < 0.001)** while relative expression levels of **GAS-5** were significantly up-regulated in serum samples of MS patients compared to the control group; **(p value = 0.002)**.

Table (3): HOTAIR levels based on different variables among MS patients

		HOTAIR			P value
		Median	Minimum	Maximum	
GENDER	**FEMALE**	0.11	0	7.9	0.86
	MALE	0.1	0	7.04	
family hisrory	**Yes**	0.94	0.32	1.57	0.192
	NO	0.1	0	7.9	
MS TYPE	**RRMS**	0.13	0	7.9	0.884
	SPMS	0.11	0	6.24	
Initial presentation	**optic neuritis**	0.12	0	7.68	0.475
	Weakness	0.08	0	7.9	
	Sensory	0.44	0	1.17	
	Ataxia	0.32	0.01	1.82	
EDSS	**EDSS=1-3**	0.14	0	7.9	0.585
	EDSS=3.5-5	0.32	0	6.24	
	EDSS≥5.5	0.09	0	2.38	
TREATMENT	**INTERFERON**	0.13	0	7.9	0.521
	AZATHIOPRINE	0.14	0.01	6.97	
	Cyclophosphamide (ENDOXANE)	0.05	0.01	0.08	
	No TREATMENT	0.11	0	2.38	

Table (3) shows that the expression levels of **HOTAIR** didn't show a statistically significant difference between MS patients according to gender, family history, MS type, initial presentation, disease activity as detected by EDSS and treatment.

Table (4): GAS-5 levels based on different variables among MS patients

		GAS-5			P value
		Median	Minimum	Maximum	
GENDER	FEMALE	1	0.13	8.82	**0.049**
	MALE	1	0.83	7.79	
family hisrory	Yes	1.74	0.66	2.82	0.525
	NO	1.48	0.13	8.82	
MS TYPE	RRMS	1.56	0.13	8.76	0.679
	SPMS	1.26	0.48	8.82	
`Initial presentation	optic neuritis	1.35	0.13	8.82	**0.008 a**
	Weakness	2.82	0.68	8.76	
	Sensory	1.09	0.61	3.41	
	Ataxia	0.66	0.18	1.55	
EDSS	EDSS=1-3	1.35	0.13	7.79	0.56
	EDSS=3.5-5	2.82	0.18	8.76	
	EDSS≥5.5	1.42	0.48	8.82	
TREATMENT	INTERFERON	1.22	0.18	8.76	0.2
	AZATHIOPRINE	1.32	0.13	3.81	
	Cyclophosphamide (ENDOXANE)	1.11	0.68	1.55	
	NO TREATMENT	3.24	0.48	8.82	

(a) a ataxia vs weakness

Table (4) shows that as regard to gender of patients, male patients had significantly higher expression levels of **GAS-5** as relative to female patients **(p= 0.049).** As regard to Initial presentation, patients with weakness had significantly higher expression levels as compared to patients with ataxia **(p=0.008)**. The expression levels of **GAS-5** didn't show a statistically significant difference between MS patients according to family history, MS type, disease activity as detected by EDSS and treatment.

Table (5):Correlation between HOTAIR and GAS-5 with other variables among MS patients

	HOTAIR (N=74)		GAS-5 (N=74)	
	Correlation Coefficient	P value	Correlation Coefficient	P value
GAS-5	-0.083-	0.485	1	--
AGE	0.005	0.968	0.22	0.06
EDSS	-0.119-	0.313	0.113	0.34
Age of onset	-0.001-	0.99	0.27	**0.020**

As shown in table (5), there is significant positive correlation between **GAS-5** expression level and age of onset in MS patients **(P value= 0.020)**. No statistically significant correlation was found between these two long non coding RNAs expressions, no significant correlation between EDSS and those two LncRNAs expressions in MS patients and no

significant correlation between ages of the MS patients and those 2 lncRNAs expressions. There is no significant correlation between **HOTAIR** expression and age of onset in MS patients.

Table (6): ROC (Receiver Operating Characteristic) curve of HOTAIR relative expression levels for MS diagnosis:

Area Under the Curve	P value	95% Confidence Interval				
		Lower Bound	Upper Bound	Cut off	Sensitivity %	Specificity %
0.730	< **0.001**	0.629	0.830	0.8455	73%	93.3%

Table (6) shows that shows ROC curve analysis of **HOTAIR** relative expression levels; **HOTAIR** with an area under the curve **(AUC) of 0.730, (95% confidence interval (CI) = 0.629–0.831, p < 0.001**). The sensitivity and specificity of **HOTAIR** were calculated using a **cutoff value of 0.8455** for serum **HOTAIR** expression.

Table (7): ROC curve analysis of GAS-5.

Area Under the Curve	P value	95% Confidence Interval		Cut off	Sensitivity %	Specificity %
		Lower Bound	Upper Bound			
0.649	**0.003**	**0.540**	**0.757**	**1.0435**	**64.9**	**100**

Table (7): shows ROC curve analysis of **GAS-5: GAS-5** with an area under the curve **(AUC) of 0.649 (95% confidence interval (CI) = 0.540–0.757, p=0.003)**. The sensitivity and specificity of **GAS-5** were calculated using a **cutoff value of 1.0435 for serum GAS-5 expression.**

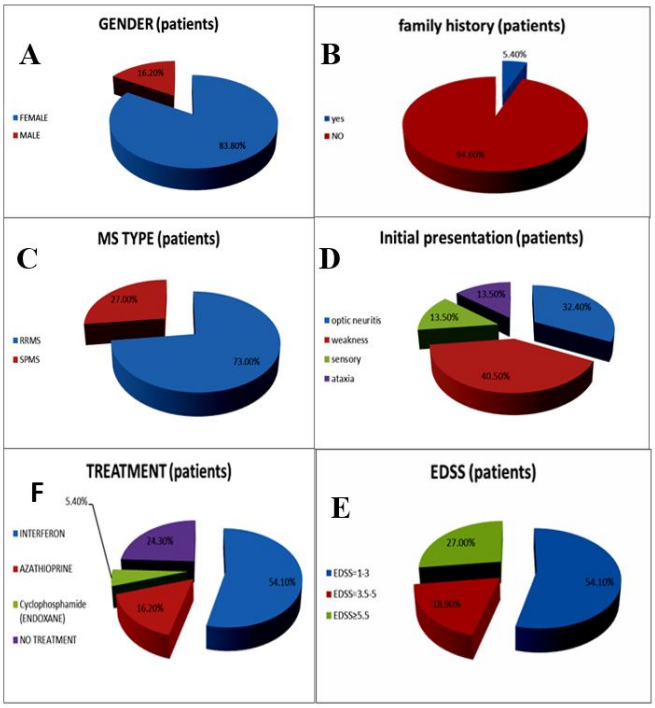

Figure (1): A: Gender distribution among MS patients. B: Family history distribution among MS patients. C: MS types distribution among MS patients. D: Initial presentation distribution among MS patients. E: EDSS distribution among MS patients. F: Type of treatments distribution among MS patients.

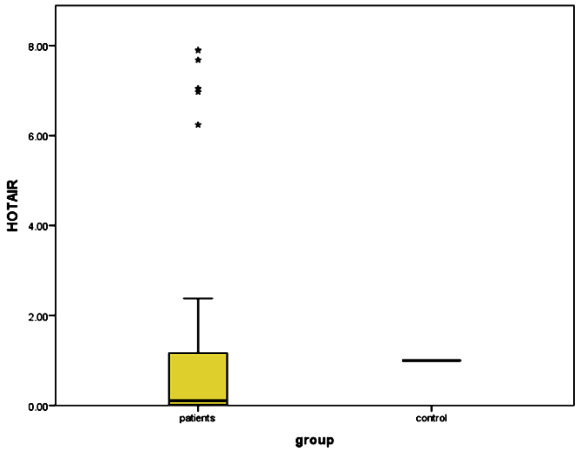

Figure (2): Box plot diagram of relative expression levels of serum HOTAIR in MS patients compared to control; asterisks indicate outliers.

FIGURE (3)

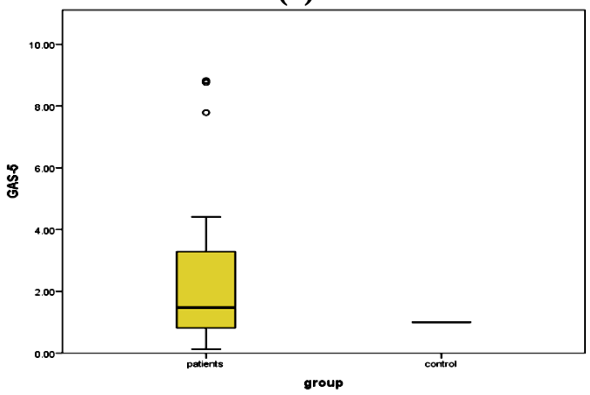

Figure (3): Box plot diagram of relative expression levels of serum GAS-5 in MS patients compared to control; circles indicate outliers.

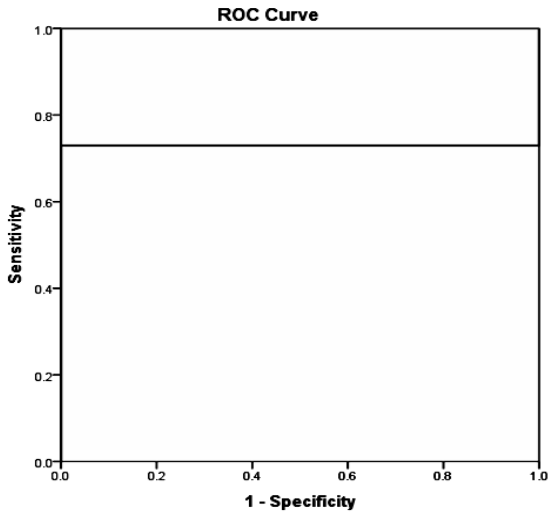

Figure (4): The ROC curve of HOTAIR relative expression levels in serum for MS diagnosis.

ROC curve of HOTAIR expression levels is plotted between sensitivity on y-axis and (1– specificity) on x-axis. Every point on the ROC curve represents a chosen cut-off (Figure 4).

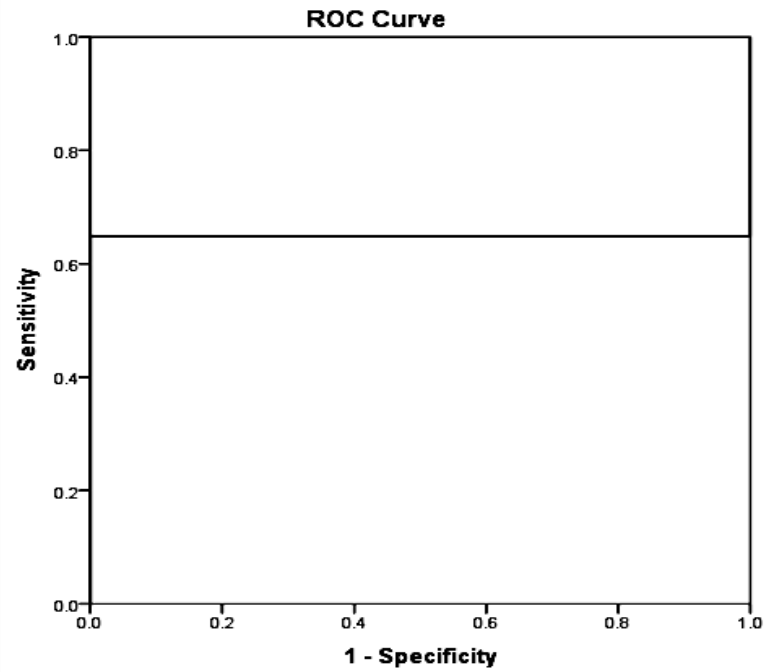

Figure (5): The ROC curve of GAS-5 relative expression levels in serum for MS diagnosis.

ROC curve of GAS5 expression levels is plotted between sensitivity on y-axis and (1– specificity) on x-axis. Every point on the ROC curve represents a chosen cut- off (Figure 5).

5. DISCUSSION:

Multiple sclerosis (MS) is a chronic idiopathic autoimmune demyelinating disease of the CNS and is the main trigger of disabilities in younger people **(Habek, 2013).**

In the mammalian genome, several lncRNAs have been transcribed, and only a small portion of lncRNAs have been functionally distinguished. LncRNA dysregulation is associated with the pathogenesis of several neurological disorders and immunological disorders. Few studies have shown that dysregulated expression of profiles of lncRNAs within CNS lesions plays a major role in MS pathogenesis. In order to forecast their role in disease activity, progression and treatment response, there is an interest in studying biomarkers of lncRNAs **(Aune et al., 2017).**

Certain circulating lncRNAs, such as inflammation regulators, and immune response, have been identified as useful biomarkers for neurodegenerative disorders as well as pathway prediction and determination **(Gharesouran et al., 2018).**

This study was conducted to assess the role of **HOTAIR and GAS-5** in genetic susceptibility to MS and study their potential role in clinical presentation, disease severity, course and progression.

It was previously reported that MS usually starts among 20 and 40 years of age and is the main cause of young adult non-traumatic disability **(Tullman, 2013)**. Also, female predominance was present in that study and that confirmed our findings regarding gender.

Our results showed that relative expression levels of HOTAIR were reported to be significantly down-regulated in serum samples from MS patients compared with the control group; (p value < 0.001).

In accordance with our study **Duan et al. (2018)** demonstrated that microglia plays a main role in brain inflammatory progression. In the CNS, activated microglia leads to demyelination. Microglia is distinguished: M1-like phenotype and M2-like phenotype. The M1-like microglia is distinguished by the production of pro-inflammatory factors whereas the M2-like microglia is distinguished by the production of anti-inflammatory factors. The transition from the phenotype of M1 to M2 leads to the resolution of inflammatory responses and during CNS remyelination, drives oligo differentiation.

Duan et al. (2018) LncRNA HOTAIR upregulation has been shown to inactivate the AKT2-NF-κB axis by targeting miR-136-5p.

They demonstrated that downregulation of lncRNA HOTAIR alters microglial phenotype through miR-136-5p. Upregulation of lncRNA HOTAIR blocks microglia was concluded by switching to a pro-inflammatory M1-like phenotype via ceRNA (competitive endogenous RNA) effect of lncRNA HOTAIR and miR-136-5p by downregulating AKT2-NF-κB axis. MiR-136-5p stimulated the production of inflammatory factors in astrocytes via NF-κB/A20 signaling. This disparity may be attributed to the heterogeneity in various neuronal types and species (microglia versus astrocytes; mice versus rats) of miR-136-5p expression).

In accordance of our study, **Pahlevan et al. (2018)** demonstrated the role of PRC-like complexes in the responses of the mouse immune system and haematopoietic lineage development**.** T helper type 2 cell differentiations appears to regulate the PRC-1-like complex. HOTAIR knockdown can up-regulate miR-326 expression (a T helper type 17-associated miRNA) involved in MS disease, that agrees with our findings that HOTAIR is down-regulated in patients with MS.

In contrast to our study, **Duan et al. (2018)** also reported that overexpression of lncRNA HOTAIR contributed to rising Ago2 (The core component of the RNA-induced silencing complex (RISC)) enrichment, however reduced enrichment on AKT2 transcripts. By growing Tumor necrosis factor (TNF-α) and Interferon gamma (INF-γ) expression, AKT2/NF-βB mediates demyelination. The knockdown of lncRNA HOTAIR had the opposite results.

They concluded that switching to a pro-inflammatory M1-like phenotype via downregulating AKT2-NF-κB axis via competing endogenous RNA (ceRNA) impact of upregulated lncRNA HOTAIR**.**

In contrast to our study, Other studies by **Obaid et al. (2018)** who demonstrated that, in response to LPS therapy, HOTAIR expression is stimulated in macrophage cells. Various cytokines, chemokines and inflammatory genes, such as ILs, TNFs, interferons (IFNs), iNOS, are triggered by LPS stimulation.

Interestingly, HOTAIR is needed for the expression of LPS-induced cytokines and inflammatory genes. The expression of IL-6 and iNOS, both at mRNA and protein levels, was regulated by HOTAIR-knockdown down.

Obaid et al. (2018) proved that LPS-induces TLRs activation that activates downstream signaling cascades and ultimately leads to IκBα degradation and NF-κB activation; NF-κB activation causes the expression of the target gene and triggers immune and inflammatory response.

They also showed that HOTAIR controls the activation of NF-κB and its expression of target genes (IL-6 and iNOS) by promoting the degradation of IκB-α. HOTAIR knockdown decreases the expression of NF-κB target gene expression.

In contrast to our study, **Pahlevan et al. (2018)** proved that in PBMCs from vitamine D (VD) deficient patients with MS prior to supplementation, the level of HOTAIR expression became substantially higher than in healthy controls. They proved that the c-Myc factor, that has been found to mediate the effects of VD and activate HOTAIR expression, may be a potential explanation.

Pahlevan et al. (2018) observed dysregulation (up or down-regulation) of HOTAIR in both the immune and nervous compartments.

This discrepancy in the expression of HOTAIR in MS patients was explained by the differential expression of HOTAIR in the central nervous system of EAE mice. Raised HOTAIR in the cerebellum and decreased HOTAIR in the spinal cord and cortex, suggesting context- or tissue-specific control of HOTAIR lncRNA inside the central nervous system and probably among peripheral pathogenic cells like PBMCs and the target organ.

Our results demonstrated that relative expression levels of GAS-5 were considered to be significantly up-regulated in serum samples from MS patients compared to the control group; (p-value = 0.002). As regard to Initial presentation, patients with weakness had significantly higher expression levels as compared to patients with ataxia (p=0.008). Also, our study showed a significant positive correlation between GAS-5 expression level and age of onset in MS patients (P value= 0.020).

In accordance with our results, functional studies by **Gharesouran et al. (2018)** showed that bad respondents to glucocorticoids have higher levels of GAS5 and NR3C1 compared to better respondents among MS patients.

They proposed the upregulated expression of GAS5 as a pathologic event in MS GAS-5 might lead to alteration of the NR3C1 gene function or expression. GAS5 considered as a candidate indicator of glucocorticoid tolerance in such disorders

Abnormal GAS5 expression levels can modify glucocorticoid efficacy by interfering with the mechanism of GR autoregulation resulting in the suppression GR-target genes across the genome. These genes are involved in inflammatory pathways in pathogenesis of MS.

GAS5 and its downstream target, (NR3C1), may be involved in a complex multigene interaction network that regulates the expression of several targets in the pathways that may be deregulated in MS.

Also **Duan et al. (2018)** proved that lncRNA GAS5 inhibits microglial M2 polarization and exacerbates demyelination as an epigenetic regulator. This supports our results that GAS5 is upregulated in MS.

Also in accordance to our study, **Eftekharian et al. (2019)** proved that, By binding to its DNA binding domain and serving as a decoy, GAS5 changes the transcriptional activity of the glucocorticoid receptor (GR).

Also in accordance to our results, **Lucafo et al. (2015)** approved that GAS5 may change GC efficacy, which is likely to interfere with the mechanism of autoregulation of GR.
They hypothesized that GAS5 upregulation prohibits the activated GR from attaching to the NR3C1 gene to intragenic control elements, thereby avoiding transcriptional gene repression. Their findings strongly indicate that in the regulation of the response to GCs, GAS5 may be significant.

In agreement with our results**,** a microarray screen was conducted by **Sun et al. (2017)** noticed that lncRNA GAS5 was substantially upregulated in MS patients' amoeboid-shaped microglia and that this characteristic was substantially correlated with MS.

Furthermore, they approved that intervention in transplanted microglia with lncRNA GAS5 was discovered to attenuate EAE progression and encourage remyelination, indicating that lncRNA is a promising target for the treatment of MS.

In our study, GAS-5 expression levels based on different variables among MS patients we found that; male patients had significantly higher expression levels as compared to female patients ($p= 0.049$). Such data should be viewed with precaution, depending on the limited number of participants in this subgroup. These results need more researches to be proved more and to determine the exact relationship between gender and expression of GAS5 in MS patients.

In agreement with our results, the results of **Gharesouran et al. (2018)** study showed higher expression level of GAS5 in male MS patients in the same age range compared with male controls, although the difference was not significant. Such finding might be related to higher threshold for initiation of MS in males.

The sex-based differences observed in the expression of GAS5 lncRNAs in the present research can reflect the existence in the GAS5 lncRNAs of hormone-response elements.
More research studies are required to explain this difference. This could result from interference with sexual hormones and further needs thorough investigation.

Additionally, men comprised only less than third of the participants in our trial (females= 83.80%, males=16.20%), and this comparatively limited sample size can have been

underpowered to identify substantial variations among men and women. Second, our outcomes rely on carefully defined parameters for patient selection could be confused by selection bias which may restrict the generalizability of their findings in real-world medical practice to the typical MS population.

Li et al. (2017) explained this potential gender difference for patients with MS patients due to many factors. (1) The immune response both women and men indicates variations in the regulation of T helper (Th) cell network homeostasis. (2) Lifestyles may be different between men and women, for instance, smoking. Smoking prevalence in men is much higher than that in women**.**

Some of the limitations of our research are as follows:
There was a comparatively small population of enrolled patients and controls that required a wider sample for further study to validate our findings.

6. CONCLUSION:

From this study we concluded that the significant down regulation of HOTAIR and the up-regulation of GAS-5 in serum of MS patients compared to control group. GAS-5 expression levels based on different variables among MS patients, male patients and patients with weakness had significantly higher expression levels of GAS-5. lncRNA HOTAIR and GAS-5 may have a role in genetic susceptibility to MS. It might be new biomarkers for prognosis of MS and as therapeutic targets in treatment of MS patients.

RECOMMENDATION

- Further studies are required for various ethnic groups in terms of race.
- Further research is needed to establish the precise molecular mechanisms in MS pathophysiology through which HOTAIR and GAS5 participate.

It is significantly recommended that well-design studies, which incorporate different ethnicities together with non-genetic factors, should be carried out. We must study these biomarkers on larger sample size. It is recommended that large association studies are needed to assess the effects of GAS5 and HOTAIR functional polymorphisms in conferring risk to MS.

Conflict of Interest
Authors declare no conflicts of interest.
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REFERENCES

1. **Azimi T, Ghafouri-Fard S, Omrani MD, Mazdeh M, Arsang-Jang S, Sayad A, et al.** Vaccinia Related Kinase 2 (VRK2) expression in neurological disorders: schizophrenia, epilepsy and multiple sclerosis. Multiple Sclerosis and Related Disorders. 2018;19:15-9.
2. **Gharesouran J, Taheri M, Sayad A, Ghafouri-Fard S, Mazdeh M, Omrani MD.** The growth arrest-specific transcript 5 (GAS5) and nuclear receptor subfamily 3 group C member 1 (NR3C1): novel markers involved in multiple sclerosis. International Journal of Molecular and Cellular Medicine. 2018;7(2):102.

3. **Eftekharian MM, Ghafouri-Fard S, Soudyab M, Omrani MD, Rahimi M, Sayad A, et al.** Expression analysis of long non-coding RNAs in the blood of multiple sclerosis patients. Journal of Molecular Neuroscience. 2017;63(3-4):333-41.
4. **Taheri M, Noroozi R, Sadeghpour S, Omrani MD, Ghafouri-Fard S.** The rs4759314 SNP within Hotair lncRNA is associated with risk of multiple sclerosis. Multiple Sclerosis and Related Disorders. 2020;40:101986.
5. **Pahlevan Kakhki M, Nikravesh A, Shirvani Farsani Z, Sahraian MA, Behmanesh M.** HOTAIR but not ANRIL long non- coding RNA contributes to the pathogenesis of multiple sclerosis. Immunology. 2018;153(4):479-87.
6. **Senousy MA, Shaker OG, Sayed NH, Fathy N, Kortam MA.** LncRNA GAS5 and miR-137 polymorphisms and expression are associated with multiple sclerosis risk: mechanistic insights and potential clinical impact. ACS Chemical Neuroscience. 2020;11(11):1651-60.
7. **Schneider C, King RM, Philipson L.** Genes specifically expressed at growth arrest of mammalian cells. Cell. 1988;54(6):787-93.
8. **Pickard MR, Williams GT.** Molecular and cellular mechanisms of action of tumour suppressor GAS5 LncRNA. Genes. 2015;6(3):484-99.
9. **Mourtada-Maarabouni M, Williams GT.** Growth arrest on inhibition of nonsense-mediated decay is mediated by noncoding RNA GAS5. BioMed research international. 2013;2013.
10. **Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP.** Noncoding RNA gas5 is a growth arrest–and starvation-associated repressor of the glucocorticoid receptor. Science signaling. 2010;3(107):ra8-ra.
11. **Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al.** Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Annals of neurology. 2011;69(2):292-302.
12. **Zurawski J, Glanz BI, Chua A, Lokhande H, Rotstein D, Weiner H, et al.** Time between expanded disability status scale (EDSS) scores. Multiple sclerosis and related disorders. 2019;30:98-103.
13. **Habek M.** Evaluation of brainstem involvement in multiple sclerosis. Expert review of neurotherapeutics. 2013;13(3):299-311.
14. **Goldenberg MM.** Multiple sclerosis review. Pharmacy and Therapeutics. 2012;37(3):175.
15. **Aune T, Crooke III P, Patrick A, Tossberg J, Olsen N, Spurlock III C.** Expression of long non-coding RNAs in autoimmunity and linkage to enhancer function and autoimmune disease risk genetic variants. Journal of autoimmunity. 2017;81:99-109.
16. **Tullman MJ.** Overview of the epidemiology, diagnosis, and disease progression associated with multiple sclerosis. The American journal of managed care. 2013;19(2 Suppl):S15-20.
17. **Duan C, Liu Y, Li Y, Chen H, Liu X, Chen X, et al.** Sulfasalazine alters microglia phenotype by competing endogenous RNA effect of miR-136-5p and long non-coding RNA HOTAIR in cuprizone-induced demyelination. Biochemical pharmacology. 2018;155:110-23.
18. **Obaid M, Udden SN, Deb P, Shihabeddin N, Zaki MH, Mandal SS.** LncRNA HOTAIR regulates lipopolysaccharide-induced cytokine expression and inflammatory response in macrophages. Scientific reports. 2018;8(1):1-18.
19. **Eftekharian MM, Noroozi R, Komaki A, Mazdeh M, Taheri M, Ghafouri-Fard S.** GAS5 genomic variants and risk of multiple sclerosis. Neuroscience letters. 2019;701:54-7.

20. **Lucafo M, De Iudicibus S, Di Silvestre A, Pelin M, Candussio L, Martelossi S, et al.** Long noncoding RNA GAS5: a novel marker involved in glucocorticoid response. Current molecular medicine. 2015;15(1):94-9.
21. **Sun D, Yu Z, Fang X, Liu M, Pu Y, Shao Q, et al.** Lnc RNA GAS 5 inhibits microglial M2 polarization and exacerbates demyelination. EMBO reports. 2017;18(10):1801-16.
22. **Li R, Sun X, Shu Y, Mao Z, Xiao L, Qiu W, et al.** Sex differences in outcomes of disease-modifying treatments for multiple sclerosis: A systematic review. Multiple sclerosis and related disorders. 2017;12:23-8.