INTRODUCTION

Multiple sclerosis (MS) is an autoimmune demyelinating disorder. ***(Katz Sand I et al.,2015).***

In the majority of patients (85%), the clinical course of the disease is characterized by a bout-onset (relapses) while a progressive onset is seen in approximately 15% of patients. ***(Milo R et al.,2014).***

Over time, most of patients with relapsing-remitting MS (RRMS) acquire a secondary progressive MS (SPMS) clinical phenotype. ***(Katz Sand I.,2015).***

Many immunomodulatory or immunosuppressive treatments are currently available only for (RRMS), while these are uneffective for primary progressive MS or SPMS. ***(Ontaneda D et al.,2015*).**

The etiology of MS remains unknown. Several environmental factors, including microbial agents, have been considered potential inducers of the disease. ***(Grigoriadis N et al., 2006).***

Amongst the microbial agents, Helicobacter pylori (Hp) has been considered a possible infectious trigger of the disease. ***(Smyk DS et al.,2014).***

At the antigen level, several Hp antigens have been considered important for the loss of immunological tolerance to myelin antigens, particularly heat shock proteins (hsp). ***(Scotti C et al., 2010).***

Several approaches have been used to assess whether hsps are implicated in the pathogenesis of MS, including studies on immune responses to hsp in MS and the extent of cross reactivity between hsp and CNS myelin, as well as the expression of hsps in the brain of patients with MS. ***(Salvetti et al., 1992; Tishler andShoenfeld, 1996; Horvath et al., 2001; Gruber et al., 1996; Chiba et al.,2006).***

Because of the conserved nature of hsp there is a high degree of amino acid homology amongst hsp60 of different bacteria; thus, immune responses against hsps show a high degree of cross-reactivity. ***(Van Eden W et al.,2005).***

The most immunogenic bacterial hsps are hsp60 and hsp70, and immune responses against these microbial hsps may initiate cross reactive humoral and cellular immune responses against their human counterparts. When such molecular mimicry mechanisms are in place, myelin-targeted tissue injury leads to the induction of overt clinical disease in a susceptible individual. **(Moudgil KD et al.,2008).**

In the past, high levels IgG antibodies against hsp70 have been reported in the cerebrospinal fluid (CSF) of patients with MS. However, significant difference in the levels of antibodies against hsp27, hsp60 or hsp90 was not observed. ***(Chiba S et al.,2006).***

The presence of cellular and humoral responses against bacterial and mycobacterial hsp70 in CSF and sera from patients with MS, gave rise to the

expectation that immunological cross-reactivity involving infectious/self hsp70 may be involved in the pathogenesis of MS.

The exact prevalence of multiple sclerosis in Egypt can not be given since no organized survey for this purpose was carried out and door to door study was difficult. A community-based survey using McDonald's criteria for diagnosis of MS in Al Quseir, Egypt, has found an MS prevalence of 13.74/100,000. ***(Hamdy N et al,2016).***

There is also data scarcity on the prevalence of H.pylori infection in an Egyptian population truly representative of our nation and the only study found was a study in a rural area of the country. Six hundred and five people were screened for anti-H.pylori antibodies and the overall seropositivity rate of 91.7% has been found in this Egyptian population. The rate of infection was different in different age groups with an increasing trend in older ages. ***(El Dine SS et al.,2008).***

Antibody responses to Hp-specific hsp60 has not been studied in great detail in MS. ***(Gonzalez-Lopez MA et al .,2013).***

In the present study, we assess such antibody reactivities in patients with MS.

Aim of the work

This study aims to prove or disprove the relationship between infection by helicobacter pylori species containing hsp60 and triggering of multiple sclerosis and, if postive relationship is found, to correlate between quantative levels of Hp hsp60 IgG antibodies in sera of MS patients and various epidemiological and clinical data with the goal of better understanding of the pathogenesis of M.S. which may enhance treatment and prognosis in the future.

Chapter 1

Epidemiology

A- epidemiology of multiple sclerosis

Multiple sclerosis (MS) is an immune-mediated inflamatory disease that attacks myelinated axons in the central nervous system, destroying the myelin and the axon in variable degrees. The disease affects nearly 2.3 million people in the world, although the exact number may be much higher as it seems that many people with MS remain undiagnosed in many parts of the world. ***(National Multiple Sclerosis Society,2012).***

There are about 400,000 individuals affected by the disease in the United States, and more than 400 people are newly diagnosed each week. ***(National Multiple Sclerosis Society,2013).***

Comparative studies of different populations have revealed prevalence and incidence rates that vary with geography and ethnicity (figure 1). With a prevalence ranging from 2 per 100,000 in Japan to greater than 100 per 100,000 in Northern Europe and North America. ***(Howard J et al.,2016).***

In Egypt, the exact prevalence of multiple sclerosis can not be detected since no organized survey for this

purpose was carried out and door to door study was difficult. At one community-based survey using McDonald's criteria for diagnosis of MS in Al Quseir, Egypt, the prevalence was 13.74/100,000. Age- and sex-specific prevalence of MS was 27.5/100,000 (for females in the population at or above 17 years old). ***(Hamdy N et al.,2016).***

The results of the this study are in accordance with those of a previous Egyptian retrospective meta-analysis study in different referral centers including five centers in the Cairo metropolitan area and five other centers: one center in each city of Alexandria in north Mediterranean coast, Mansoura, Tanta, and Zagazig in Delta, and Assiut in Upper Egypt which revealed that the prevalence of MS to be 14.1/100,000. ***(Hashem S et al.,2010).***

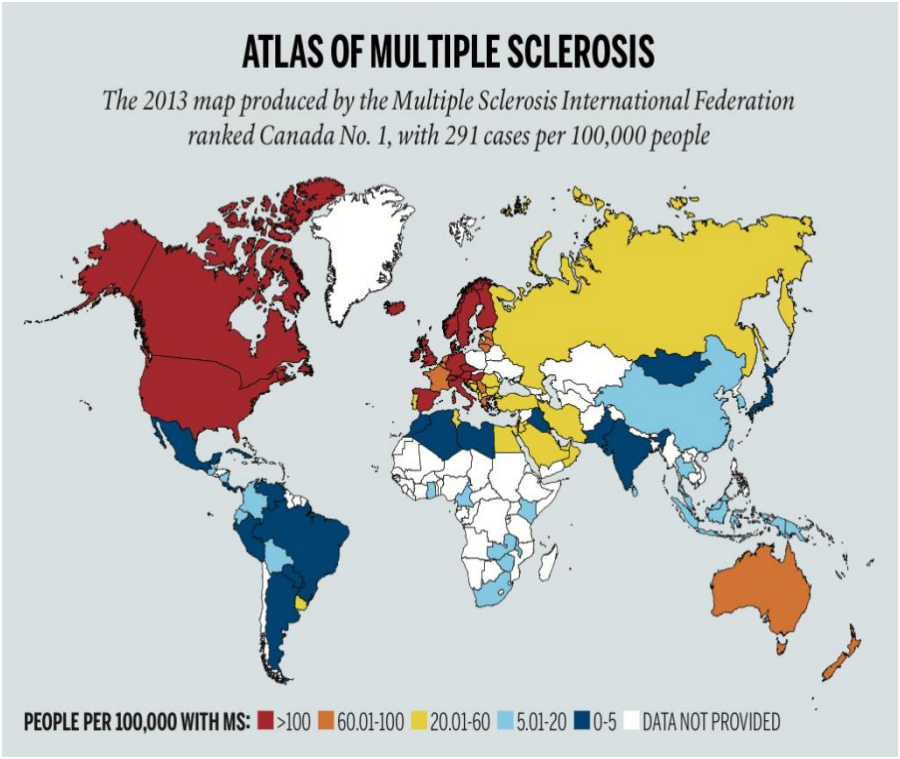

Figure (1): Global prevalence of multiple sclerosis (MS) in 2013 ***(Atlas of MS 2013).***

MS is common in Caucasians of northern European ancestry, but less common where non Caucasians live, in tropical zones and in low-income countries. ***(Kingwell E et al.,2013).***

The most common age of incidence is between 20 and 40 years and it is the leading cause of non-traumatic disability in young adults. MS symptoms rarely occur before age 10 years or after age 60 years. ***(*Milo R, Kahana E et al., 2010*).***

Women are about twice as likely as men to develop MS, except in individuals with the primary-

progressive form of the disease, where there is no gender preference (figure 2). ***(Koch-Henriksen N et al., 2010).***

Decades of birth	Ratio	Type course % RR/RR+PP$^{\Delta}$ by sex		Mean Age at onset by sex°		Mean Time from onset to diagnosis by sex°	
	F/M*	F	M	F	M	F	M
1930–1939	460/196	73.21	52.02§	42.88±13.01	44.64±11.83	8.96±10.62	8.74±9.97
	2.35						
1940–1949	1461/641	79.26	66.84§	39.88±11.28	41.27±10.80 §	7.32±8.77	6.48±7.54
	2.28						
1950–1959	2707/1161	89.52	79.50§	35.87±9.17	36.71±8.97 §	5.57±6.60	5.57±9.11
	2.33						
1960–1969	3340/1391	94.86	89.78§	30.95±7.18	31.54±7.20 §	3.94±4.58	3.61±4.42
	2.40						
1970–1979	2494/1014	97.98	95.18	25.41±5.17	25.96±4.96 §	2.60±3.31	2.42±3.28
	2.46						
1980–1989	828/303	98.95	97.87	19.82±3.68	19.60±4.21	1.69±2.08	1.78±2.23
	2.73						
Total	11290/470606	91.66	84.23§	32.03±10.24	32.97±10.27 §	4.61±6.24	4.39±6.70
	2.41						

**15996 pts; p-value for trend 0.032; $^{\Delta}$14543 pts; °11998 pts; § $p<0.05$ sex M vs. F.*
RR = Relapsing remitting; PP = Primary progressive.
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Figure (2): Sex ratio, percent of relapsing onset, mean age at onset and mean time from onset to diagnosis by birth decades in female (F) and male (M) MS patients. ***(Maria Trojano et al.,2012).***

In Egypt, according to study by Magd et al, the mean age of disease onset was 26.1 ± 7.6 years. Relapsing-remitting MS (RRMS) was the most common presentation (74.6%). Visual or sensory symptoms were the most common at presentation with RRMS, while motor symptoms were the most common presentation in other types of MS. Time to diagnosis was delayed up to 2 years in 27.8% of patients. The mean EDSS score was 3.6 ± 2.1; 55% had EDSS≤3. About half (49%) received a disease-modifying drug. Progressive MS and motor presentation were associated with higher disability. ***(Magd Z et al.,2016).***

Another study by Sherif et al found that the mean age of disease onset was 26.6 ± 7.8 years, with the majority being female (2.11:1). RRMS was the most common type (75.1%). The main presenting symptom was motor weakness (43.9%), which was also the most frequent symptom during the disease course. Family history of MS was found in 2.28%. Higher initial EDSS, black holes, and infratentorial lesions on initial magnetic resonance imaging were independent factors for disease progression by univariate analysis; however, in multivariate analysis, only infratentorial lesions were an independent risk for disease progression. ***(Sherif M et al.,2017).***

Both the prevalence and incidence of MS are increasing over time. Prolonged survival of the patients is thought to be the cause of inreased prevelance, while a number of factors stand behind

increasing MS incidence. In particular, the ratio of disease in women to men has increased over time from less than 1.5 to greater than 2. This seems to be the cause of increasing incidence, and may be due to changes over time in occupation, obesity, birth control, cigarette smoking and later childbirth. ***(Koch-Henriksen N et al.,2010).***

B- EPIDEMIOLOGY OF HELICOBACTER PYLORI INFECTION

Helicobacter pylori, is a gram-negative, urease-producing, spiral-shaped bacterium situated on the epithelial surface of the stomach. It is thought to be the most common chronic bacterial infection affecting human beings. ***(McColl KE et al.,2010).***

Phylogeographic studies support the presence of helicobacter in our early east African ancestors more than 58,000 years ago. *H. pylori* have managed to persist in its only confirmed hosts (humans) since then. ***(Linz B et al.,2007).***

Nearly half of the adult population worldwide are thought to be affected by H. pylori infection , but the prevalence of *H. pylori* infection varies widely by geographic area, race, age, and socioeconomic status, ranging from under 15% to greater than 85% of the population in different countries. ***(Bruce MG et al.,2008).***

The infection rate is especially higher in developing countries (80–90%), where contaminated water, combined with social hardships and poor sanitary conditions, plays a key role. ***(Aziz RK et al.,2015).***

Prevalence rates are less higher in the developed countries, In north European and North American populations, about one-third of adults are still infected, whereas in south and east Europe, South America, and Asia, the prevalence of H. pylori is often higher than 50% (figure 3). H. pylori remains highly prevalent in immigrants coming from countries with high prevalence of H. pylori. ***(Eusebi LH et al.,2014).***

The exact prevelance of H. pylori infection in Egypt cannot be detected accurately due to lack of data. At one study based on rural community, Six hundred and five people were screened for anti-H.pylori antibodies and about 91.7 % of this population was found to be seropositive. The rate of infection was different in different age groups with an increasing prevelance in older ages. ***(El Dine SS et al,2008).***

Table: Population based studies reporting frequency of Helicobacter pylori infection.

Authors (year published)	Location	Age range of subjects (years)	Frequency (%)
Mishra et al (2008)[3]	India	0.67-60	45.7
Mohammad et al (2008)[4]	Egypt	6-15	72.4
Monno et al (2008)[5]	Albania	16-64	70.7
Moujaber et al (2008)[6]	Australia	1-59	15.4
Naito et al (2008)[7]	Japan	4-10	5.3
Zagari et al (2008)[8]	Italy	>32	58
Kori et al (2009)[9]	Israel	0.25-5	24.7
Nouraie et al (2009)[10]	Iran	18-65	68.3
Sasidharan et al (2009)[11]	Malaysia	10-70	14.2
Acosta Garcia et al (2009)[12]	Venezuela	4-14	74
Arslan et al (2009)[13]	Turkey	Mean 25.5	41.5
Breckan et al (2009)[14]	Norway	18-85	33
Cartagenes et al (2009)[15]	Brazil	1-12	50
Chi et al (2009)[16]	Taiwan	Mean 14.3	55
Dube et al (2009)[17]	South Africa	0-60	87
Jackson et al (2009)[18]	United Kingdom	18-70	26
Jafri et al (2010)[19]	Pakistan	1-15	47
Javed et al (2010)[20]	Pakistan	15-65	92
Mansour et al (2010)[21]	Tunisia	25-55	63
Santos et al (2010)[22]	Bolivia	5-8, 6-14, 4-13	74, 48, 78
Shimoyama et al (2010)[23]	Japan	Mean 57.7	61
Sykora et al (2010)[24]	Czech Republic	0-15	7
Yucel et al (2010)[25]	Turkey	2-12	31
Zhang et al (2010)[26]	China	8-15 and 40-79	66

Figure (3): Population based studies reporting frequency of Helicobacter pylori infection. **(Muhammad JS et al.,2012).**

Usually, the prevalence of *H. pylori* increases with age in most countries, however time trend analysis of several large populations showed a decline in prevalence of *H. pylori* infection in recent decades. ***(Peleteiro B et al.,2014).***

The exact mode of transmission is unclear but higher prevelance rates within certain communities suggests person-to-person spread mainly in childhood. Socioeconomic status and living conditions affects

the risk of infection with *H. pylori* in early life. Increased transmission and higher prevalence rates are found in overcrowded conditions associated with childhood poverty. ***(Perez-Perez GI et al.,2004).***

According to Aziz et al., "Over the preceding years and to date, the definitive mode of human infection by *Helicobacter pylori* has remained largely unknown". According to the investigation of many studies, contaminated water was found to be the main causative of transmission and infection. ***(Aziz RK et al.,2015).***

In Egypt, the River Nile is considered the main source of drinking water. It supplies 56.8 billion m^3 of freshwater every year, which represents 97% of all renewable water resources in Egypt. Contamination of drinking water in Egypt was and still a sound problem of a very frightening hygiene risk, threatening the health of the Egyptian community. ***(Agha S et al.,2013).***

It is widely believed that once colonization has happened, it persists throughout life unless otherwise eradicated. ***(Ford AC et al.,2010).*** However, in developing countries, re-infection with *H. pylori* following successful bacterial cure is not uncommon occurring in approximately ~ 12% of individuals shown to have been originally cleared of this bacterium. By contrast, only ~1% of previously infection eradicated individuals are subsequently re-infected in developed countries. Additionally, when

re-infection occurs it is found that it is mostly a recrudescence of the original bacterial strain. ***(Morgan DR et al.,2013).***

Up to 85% of people infected with *H. pylori*, never experience symptoms or problems. ***(Bytzer P et al.,2011)***.

H. pylori infection is a causative agent at peptic ulcer disease, and a risk factor of chronic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and adenocarcinoma of the stomach. ***(Jeong B et al.,2014).***

The World Health Organization classified *H. pylori* as a class I carcinogen because of the epidemiological link of *H. pylori* infection with a higher risk of development of gastric malignancy. ***(Banerjee HN et al.,2011).***

H. pylori infection is responsible for nearly 74.7% of all noncardia gastric cancer cases which is considered to be the third leading cause of cancer-related death worldwide. ***(Fock KM et al.,2014).***

One million deaths per year in the world are owed to Gastric cancer and peptic ulcer together. So, *H. pylori* infection always is thought to be an important health issue. ***(Axon A et al.,2014).***

Helicobacter pylori possibly plays a role at the pathogenesis of Immune thrombocytopenic purpura ***(Stasi R et al., 2009),*** rheumatoid arthritis (RA)

(Meron MK et al.,2010), systemic lupus erythematosus ***(Francis L et al.,2010),*** Sjögren's syndrome ***(Sorrentino D et al.,2004),*** polymyositis (PM)/dermatomyositis (DM) ***(Kalabay L et al.,2002) ,*** systemic sclerosis ***(Radić M et al.,2011),*** Behcet's disease ***(Lidar M et al.,2009)*** and fibromyalgia (FMG) ***(Akkaya N et al.,2011).***