# MOLECULAR DETECTION OF HUMAN RETROVIRUS (HRV-5) IN COMMON RHEUMATIC DISORDERS

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## ABSTRACT

***Objective:*** *HRV-5 has been implicated in the pathogenesis of a number of autoimmune diseases. This study aimed at assessing the role of HRV-5 in the pathogenesis of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and osteoarthritis (OA).*

***Methodology:*** *The study was conducted on 25 female SLE patients with mean age of 35.8±7.4 years and 25 female RA patients with mean age of 48 ± 13.4 years, as well as, 25 female OA patients with mean age of 51 ± 14.5 years, in addition to 25 age-matched apparently healthy female controls. All participants were subjected to detailed clinical assessment and laboratory investigations. HRV-5 proviral DNA was detected with nested polymerase chain reaction (PCR) and positive results were confirmed with DNA sequencing.*

***Results:*** *The frequency of detection of HRV-5 proviral DNA was 8%, 12% and 4% in our RA, SLE and OA patients, respectively. Sequence analysis of the amplified viral segment showed genetic variation between samples with maintenance of the open reading frame, typical of a replicating infectious retrovirus.*

***Conclusion:*** *Our results support the hypothesis that HRV-5 may play a role in the etiopathogenesis of both SLE and RA, but further investigations are needed to confirm its role.*

## INTRODUCTION

The etiology of most autoimmune diseases such as rheumatoid arthritis (RA) has yet to be established, but indirect evidence indicates that a trigger operating on the background of hormonal and genetic predisposing factors may be involved.

RA is one of the most common classical autoimmune diseases *(Silman & Pearson, 2002)*. The contribution of a wide variety of factors such as infectious agents or hormonal factors has been suggested or proven in RA *(Stahl et al., 2000, Dooley & Hogan, 2003 and Mehraein et al., 2003)*. Of the myriad of agents, environmental and genetic, implicated in rheumatoid disease mechanisms and pathology, considerable evidence indicates that viruses may be important environmental

triggers *(Ejtehadi et al., 2006)*.

Systemic Lupus Erythematosus (SLE) is a multisystem inflammatory rheumatic disease, characterized by alternative remissions and exacerbations *(Kammer & Mishra, 2000)*, where auto-antibodies are frequently targeted against intracellular antigens of the cell nucleus *(Arnett & Reueille, 1992*). It is a chronic autoimmune disease, whose etiology is unknown, but is thought to be multifactorial. The prevailing view of autoimmunity entails a causal relationship between genetics and environment, with the immune response to infection playing a prominent role. It is thought that microbial pathogens may initiate autoimmune phenomena in genetically susceptible individuals *(Cikes, 2000*) coincidentally activating immune cells that respond to self. *Kohm et al.(2003) and Soderlin et al. (2003*) highlighted the high prevalence of prior infection in patients newly presenting with all forms of inflammatory arthritis. Retroviruses have been postulated as environmental triggers in the etiopathogenesis of SLE *(Fong et al., 1996)*.

A number of mechanisms by which retroviruses might influence the immune system have been described; molecular mimicry between Ribonucleoprotein (RNP) antigens and retroviral core proteins has been suggested as one possible mechanism for the induction of autoimmunity in SLE *(Oldstone, 1987)*.

Furthermore, infection of genetically susceptible hosts by a potentially large number of commonly occurring viruses may lead to T and B cell dysfunction and autoimmunity *(Dayal & Kammer, 1996)*. Immunoregulatory aberrations are triggered by well-defined viral proteins at the level of antigen presentation, modulation of cytokine activities and disruption of cell death pathway *(Lander et al., 2001)*.

Super antigens are pathogen-derived proteins that provoke a strong T cell immune response. Super antigens are presented to T cells by MHC class II molecules on antigen-presenting cells. They bind outside of the peptide-binding groove of class II, forming a bridge between the MHC molecule and the Vß portion of the T cell receptor (TCRBV). In this way, super antigens are able to activate entire families of T cells bearing particular TCRBV chains *(Papageorgiou & Acharya, 2000)*. Because of this potent immune stimulus, super antigens have been implicated in various autoimmune diseases, providing a possible mechanism for the activation of autoimmune T cells *(Macphail, 1999)*. However, to date studies pointing to their existence in human autoimmune disease have not convincingly resolved the issue.

*Sicat et al. (2005)* reported that the human endogenous retrovirus K18 has super antigen activity that can be induced by infection with the ubiquitous herpes virus Epstein-Barr virus (EBV) and by the cytokine interferon (IFN)-α, which is produced in response to viral activity *(Diebold et al., 2003)*. IFN-α induces MHC class I and class II molecules, augmenting macrophage antiviral function as well as potentiating antibody function and cytotoxic T lymphocyte and natural killer cell activity *(Garcia-Sastre, 2002)*. In SLE patients, it has been shown that serum IFN-α levels correlate with disease activity *(Bennett et al., 2003 and Pascual et al., 2003)*.

Additional evidence implicating retroviruses in rheumatologic disorders is the observation that features of auto-immune diseases are associated with a number of retroviral infections in humans and animals *(Narayan et al., 1993)*. Arthritis, polymyositis, and sialadenitis,

can occur in subsets of individuals infected with Human T Lymphotropic Virus (HTLV I-II) *(Iwakura et al., 1991)*.

Heritable integrations give rise to endogenous retroviral sequences, which comprise up to 1% of human DNA *(Urnovitz & Murphy, 1996)*. The rest are exogenous infections from individual to individual *(Blomberg, 2000)*. HRV-5 is genetically closely related to Simian D-type retroviruses, rodent intracisternal A-type particles, and murine mammary tumor viruses and has little sequence similarity to human immunodeficiency virus (HIV) or HTLV *(Sato et al., 1991)*.

## Aim of Work:

The aim of this study was to assess the role of HRV-5 in the pathogenesis of RA, SLE and OA, which has been previously linked to retrovirus infection. Our approach was based on the hypothesis that in diseases related to HRV-5 infection, proviral DNA would be increased.

# MATERIALS AND METHODS

This study was carried out on 75 female patients. Twenty five patients had SLE with mean age of 35.8 ± 7.4 years and mean disease duration of 5.6 ± 4.5 years. Twenty five patients had RA with mean age of 48 ± 13.4 years and mean disease duration of 11.3 ± 4.7 years. Another 25 patients had OA with mean age of 51 ± 14.5 years and mean disease duration of 12.4 ± 5.3 years. In addition, 25 age-matched apparently healthy female controls were recruited from the department personnel, with no history of autoimmune disorder and with mean age of 36.2±6.9 years.

All patients were recruited from the Outpatient Clinic of the Rheumatology and Rehabilitation Department of Al-Mouwasat Hospital, Dammam, KSA. All SLE patients were diagnosed according to the American College of Rheumatology Criteria *(Tan et al., 1982)*, while RA patients were diagnosed according to the American College of Rheumatology Criteria for RA *(Arnett et al., 1988)*. OA was diagnosed based on clinical and radiographic findings, with no prior history of inflammatory arthritis. 25

Each patient was assessed for concomitant medication and corticosteroid dose was recorded at the time of the study.

OA patients had clinically active disease as defined by diagnostic indices and the absence of serological abnormalities.

Paired serum and EDTA blood were collected using vacutainers (Becton Dickinson U.K). Separated serum was frozen immediately at -20ºc until processed, while the DNA extraction step was carried out within 4 hours using EDTA blood.

## All studied cases and controls were subjected to:

1. **Complete history taking and thorough clinical examination:**

With special emphasis on signs of disease activity and manifestations of internal organ involvement.

2. **Investigations including:**

a) Complete blood picture (CBC) with Coulter counter.

b) Erythrocyte sedimentation rate (ESR) with Westergren method.

c) C-reactive protein (CRP) was measured with the ELISA quantitative method *(Chemicon, USA)* following the manufacturer recommendations.

d) The Rheumatoid factor (RF) was measured with the semi-quantitative latex testing following the manufacturer recommendations *(RF*

*slide, Diesse, Milan, Italy).*

e) Antinuclear antibodies (ANA) with Anafluor-Indirect Fluorescent Antibody Testing- *(DiaSorin-Saluggia- Italy).*

f) Double stranded DNA (ds DNA) antibodies were estimated with Enzyme Immune Assay technique-ETI-dsDNA- *(DiaSorin- Saluggia-Italy).*

g) Complement assay (C) for C3 and C4 with Turbox rate nephelometry machine using Orion Kit (*Espoo, Finland) (Manzi et al., 1996).*

h) X-Rays of the involved joints (for the diagnosis of OA).

**3. PCR Amplification of HRV-5.**

**a) DNA extraction:**

EDTA blood was added to 10 ml tris EDTA (pH 7.0) followed by centrifugation twice at 3000 rpm to obtain a clear pellet. Proteinase K 100µg/mL *(Boehringer Mannheim, France)* digestion of the resultant pellet in the presence of 0.5% sodium dodecyl sulphate (SDS) was carried out by overnight incubation at 55 ℃ followed by proteinase K inactivation for 10 min at 95°C. Finally DNA was obtained by 99% Ethanol precipitation *(Gustincich et al., 1991).*

The suitability of all DNA preparations for PCR together with absence of inhibitors was confirmed by amplification of a 268 bp of beta globin gene, which comprised an internal control, which had been processed with each sample.

**b) PCR amplification:**

Nested PCR was carried out according to *Griffiths et al. (1999)* for the protease region of HRV-5.

Two sets of primers, outer and inner were used for the first and second round PCR, respectively. Their sequences were chosen from published sequences from the Genebank and illustrated as follows:

**The outer set:**

Sense primer 5' TCA GAA GGT GAT TGG CCG AAG TCA 3'

Antisense primer 5' GGT CCT CAT CAT TAG TTA ATG TCA GTC3'

**The inner set:**

Sense primer 5' CCC TTC AGC CAG GAG ATA ATA CT3'

Antisense primer 5' ATG TCT CTT CCC CAT AAT GTG ATG 3'

First round PCR was carried out in 50µL total reaction volume containing 500 ng of DNA template, 200 µM each deoxynucleotide triphosphate (dNTPs) (Boehringer-Mannheim, France), 1 unit of taq polymerase with 1X of its buffer (Promega, U.S.A), 50 pmol each of outer set of primers to be completed to 50µL with sterile nuclease free water. The thermocycler temperature profile *(Perkin Elmar U.S.A)* included initial denaturation step at 95$^{o}$C for 2 minutes followed by 30 amplification cycles each cycle included denaturation at 95$^{o}$C for 30 sec, annealing at 55$^{o}$C for 30 sec, and extension at 72$^{o}$C for 30 sec then after the last cycle, a final extension of 10 minutes at 72$^{o}$C was done.

One microliter of the first PCR was used for second round PCR and further amplification for 30 cycles, using the same protocol with the inner set of primers.

Positive control and water, as negative control were processed in the same manner, both to ensure the fidelity of PCR reaction components and absence of carryover contamination.

The PCR product was analyzed on 2% agarose gel in tris EDTA buffer stained with ethidium bromide (Amersco) to be visualized under ultraviolet transilluminator (Cole palmer) according

to the method of *Sambrook et al. (1989)*.

**c) DNA sequencing:**

One hundred fifty seven bp amplicon from the patients were sequenced in order to confirm the genome as HRV-5 and exclude endogenous retrovirus according to *Griffiths et al. (1997)*.

DNA sequencing and analysis included three steps. Firstly, cycle sequencing reaction "fluorescent dye labeling of PCR products", using ABI prism big Dye Terminator cycle sequencing ready reaction kit, supplied by *PE Applied Biosystem, U.S.A*. Secondly, purification of the labeled extension products from excess unincorporated dyes using Dye Ex spin kit 250, supplied by *Qiagen, Germany*. Finally, loading of purified labeled extension products in both directions with each second-round PCR primer on the ABI Prism 310 Genetic Analyzer for determination of DNA Sequence by capillary electrophoresis.

For data reading and interpretation, base calling view was used which is the nucleotide sequence in the form of letters (A, T, C, and G) with no peaks. Sequences obtained were aligned with the sequences obtained from the GeneBank database with different accession numbers (U46939-U46940).

## Statistical analysis:

The Student t-test was used to compare two means. A p value <0.05 indicated a statistically significant effect. For the statistical analysis the computer program SPSS version 11.0 was used.

# RESULTS

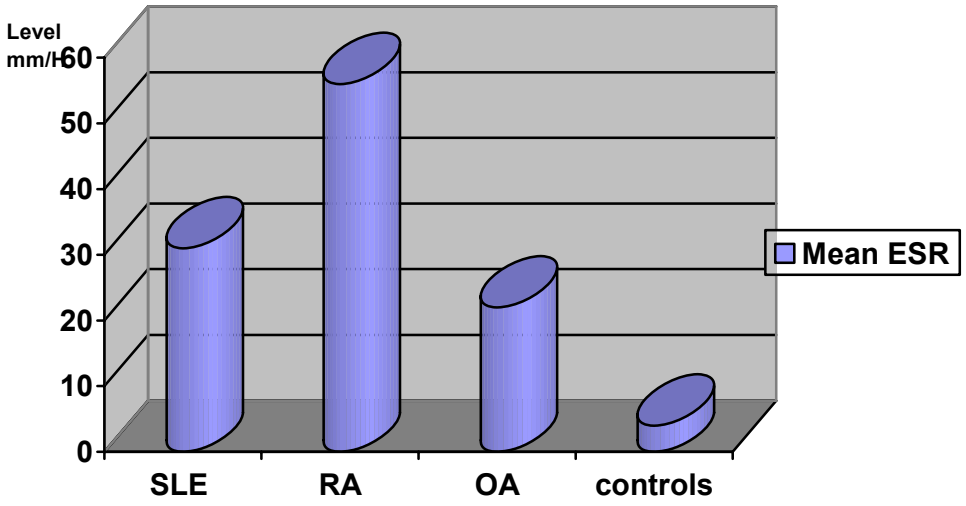
The present study included 25 SLE female patients with mean age of 35.8±7.4 years and mean disease duration of 5.6 ± 4.5 years, 25 female RA patients with mean age of 48± 13.4 years and mean disease duration of 11.3 ± 4.7 years, as well as, 25 female OA patients with mean age of 51± 14.5 years and mean disease duration of 12.4 ± 5.3 years. Twenty five age-matched apparently healthy females with mean age of 36.2 ± 6.9 years were enrolled as a control group.

On comparing the inflammatory markers of SLE, RA and OA patients to that of controls there was a significantly higher level of ESR in the SLE, RA and OA patients compared to controls (graph 1 & 2), while the CRP was significantly higher in both the SLE and RA patients. RF was only significantly higher in RA patients but not in SLE and OA patients when compared to controls (Table 1).

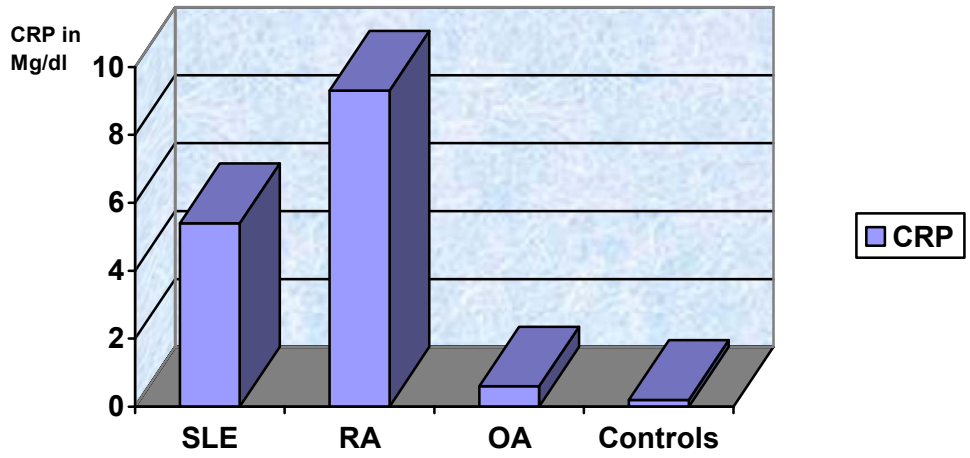
Table (1): Laboratory investigations of the patients and controls.

| | SLE patients | | RA patients | | OA patients | | Controls |
|---|---|---|---|---|---|---|---|
| | Level | SA | Level | SA | Level | SA | Level |
| Mean ESR (mm/h) | 31±11 | r= 15 p<0.05** | 56±13 | r= 24 p<0.05** | 22±12 | r= 18 p<0.05** | 4±2.2 |
| RF | 2/25 | r= 0.4 p>0.5* | 20/25 | r= 49 p<0.05** | 0/25 | r= 0.5 p>0.5* | 0/25 |
| Mean CRP (mg/dl) | 5.4±2.7 | r= 26 p<0.05** | 9.3±2.4 | r= 36 p<0.05** | 0.6±1.1 | r= 2 p>0.05* | 0.2±0.5 |

SA= Statistical analysis **= statistically significant *= statistically insignificant




Graph. (1): Comparative study between patients and control groups regarding the mean ESR levels.

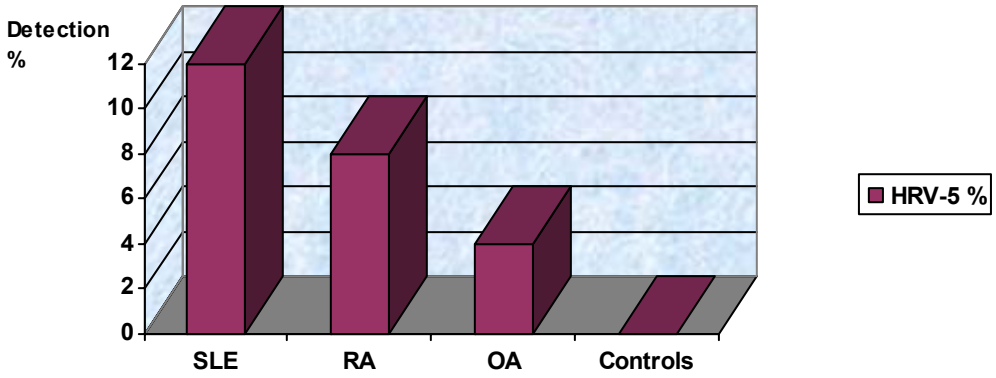



Graph. (2): Comparative study between patients and control groups regarding the mean CRP levels.

Table (2): Showing the detection rate of HRV-5 in patients and controls by PCR.

| | SLE patients | RA patients | OA patients | Controls |
|---|---|---|---|---|
| Number of patients | 3/25 | 2/25 | 1/25 | 0/25 |
| Percentage of patients | 12 % | 8% | 4% | 0% |
| SA | R=0.21<br>p>0.5* | R=0.27<br>p>0.5* | R=0.39<br>p>0.5* | |

SA= Statistical analysis *= Statistically insignificant.




Graph. (3): Comparative study between patients and control groups regarding the detection percentage of HRV-5.

There was no significant difference in the detection rate of HRV-5 DNA between the patients and controls (p>0.5) (Table 2, graph 3). SLE, RA and OA patients showed increased frequency of HRV-5 as compared to the controls (12%, 8% and 4%, respectively, vs. zero %) table (2).

Table (3): Relation between the HRV-5 positive patients and the inflammatory markers within the studied groups.

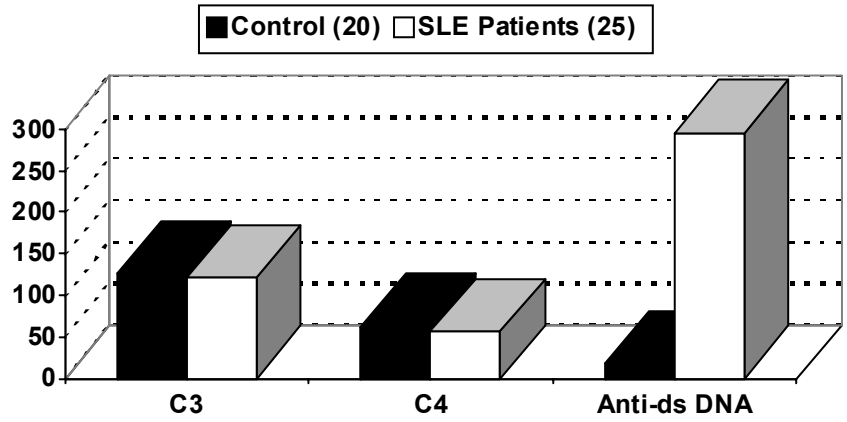
| Parameter | SLE patients | | RA patients | | OA patients | |
|---|---|---|---|---|---|---|
| | HRV-5 Positive | HRV-5 Negative | HRV-5 Positive | HRV-5 Negative | HRV-5 Positive | HRV-5 Negative |
| Mean ESR | 34±2 | 28±10 | 61±3 | 51±12 | 21±1 | 23±13 |
| | r= 28 p>0.5* | | r= 35 p>0.5* | | r= 34 p>0.5* | |
| Mean CRP | 6.7±0.5 | 4.1±2.9 | 9.8±0.2 | 8.8±2.6 | 0.5±0.1 | 0.7 |
| | r= 29 p>0.5* insignificant | | r= 32 p>0.5* insignificant | | r= 36 p>0.5* insignificant | |

As noticed in table (3), the mean ESR and CRP were higher in HRV-5 positive patients than HRV-5 negative patients among both the SLE and RA groups, however the differences were statistically insignificant (p>0.5) (may be due to small sample number).

Table (4): Statistical analysis of the studied parameters in the SLE patient and control groups.

| | $C_3$. (88-201mg/dl) | $C_4$. (10-130 mg/dl) | Anti-ds DNA (>75 Iu/ml) |
|---|---|---|---|
| Control 20 $\bar{X} \pm$ SD | 126 ± 34 | 62 ± 31 | 17 ± 6.7 |
| Patient (25) $\bar{X} \pm$ SD | 121 ± 59 | 56 ± 42 | 296 ± 23.0 |
| t | 0.49 | 0.24 | 2.93 |
| p | >0.05* | >0.05* | <0.005** |

** = Statistically significant * = Non significant.




Graph. (4): Comparative study between SLE and Control groups regarding C3 - C4 and anti-ds DNA.

The complement and anti-ds DNA values of both the controls and SLE patients are summarized in table (4) and graph (4) .The values of anti-ds DNA were significantly higher in the overall SLE patients' group as compared with the control group 296±23.0 vs. 17±6.7 ($p<0.005$). On the other hand, no statistically significant difference was observed in the mean values of the complement C3 and C4, for both the SLE patient and control groups ($p>0.05$).

GGCAGGATCAAGAAGGGAACAAGGGAAATGTGCAACCTTATGTTAGTGCACTCCC

1. ----------A----------A----------
2. ---
3. ----------A----------G----------
4. ----
5. ----------C----------
6. ----

Fig. (1): Nucleotide sequence of HRV-5. The patient's sequence is shown aligned with the prototype HRV-5. A dash indicates identity with the prototype HRV-5 and substitutions indicated that letter primer sequences are omitted.

Schematic presentation of the sequences of isolates of this study was illustrated in Fig (1). It is notable that the open reading frame (ORF) was maintained in all cases and the variation is mostly due to the single base change.

In the photo, the absence of any detectable amplified products in the negative control signifies the absence of carryover contamination. The detection of an amplicom of 157 base pair (bp) in the positive control corresponding to the expected amplified protease gene indicates the fidelity of the PCR reaction components. The samples were considered positive if similar amplicon was detected. The presence of 268-bp amplicon of betaglobin was visualized in all samples, which signifies absence of inhibitors.

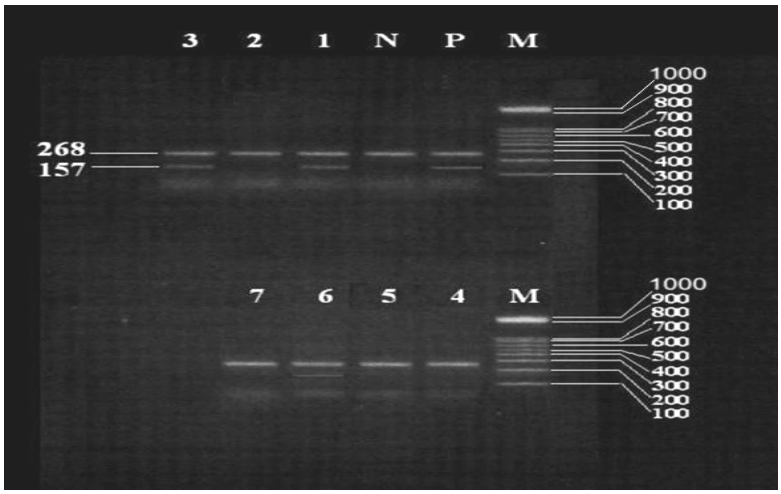



Photo:
PCR products after gel electrophoresis and ethidium bromide staining.
M: Molecular weight marker 100bp ladder ranging in size from 100-1000
P: Positive control, N: Negative control, Lane 1,3 and 6: Positive cases
Lane 2,4,5 and 7: Negative cases

## DISCUSSION

Recent advances have been made with important breakthrough concerning a clearer understanding of the epidemiology and pathogenesis of HRV-5 infection, especially immunopathologic events that result in autoimmune diseases, which will subsequently lead to either novel immune interventions or development of an effective vaccine *(Weiss, 2000)*.

In the current study we tried to demonstrate the association of HRV-5 infection with the occurrence of autoimmune diseases, namely SLE and RA, which highlights the role of HRV-5 as an etiologic agent for both SLE and RA.

The prevalence of the proviral DNA of HRV-5 infection in our SLE and RA patients, with nested PCR, was 12% and 8%, respectively. This is in accordance with the findings of *Griffiths et al. (1999)* who reported that prevalence of HRV-5 in blood samples from SLE patients was 16%, while that from RA patients was only 12 %. In contrast, they did not detect it in patients having other autoimmune diseases. This was in agreement with *Brand et al. (1999)* who demonstrated that proviral DNA, using nested PCR, was present in approximately 50% of synovial samples of arthritic joints and was also found in over 10% of blood samples of RA and SLE patients. *Cooke et al. (1998), Blomberg (2000) and Weiss (2000)* also reported an association between HRV-5 infection and SLE. Two thirds of our positive HRV-5 patients having SLE were in activity, manifested by marked depletion of C3 and C4 accompanied by marked increase of anti-ds DNA, suggesting a possible mechanism for autoimmunity by superantigen stimulation of autoreactive T cells *(Sicat et al., 2005)*.

Although both our SLE and RA patients, in whom HRV-5 was detected, showed high levels of ESR and CRP, yet there wasn't any significant correlation between them, which might be due to the small number of patients in each group.

Sequencing revealed the variation between amplified sequence of our isolates and the prototype sequenced of HRV-5 which was around 4% in the amplified region of the protease gene. These differences indicate that our isolates were

independent isolates and they don't result from contamination of DNA samples with the positive control. These results were in agreement with *Griftiths et al. (1997), Brand et al. (1999) and Griffiths et al. (1999)*. This variation of HRV-5 may be due to the activity of the replicating retrovirus, which may play a role in the pathogenesis of SLE.

In the study done by *Griffiths et al. (1999)*, they detected HRV-5 proviral DNA in 12 of 25 synovial samples from RA patients as well as 3 of 5 synovial samples from OA patients. To the contrary, in a recent study done by *Piper et al. (2006)*, using a real-time (RT) PCR assay, no association was found between HRV-5 and RA or OA. Lack of association of HRV-5 with RA was consistent with the findings of *Gaudin et al. (1999)*.

*Griffiths et al. (1999)* investigated the possibility that up-regulation of HRV-5 expression was simply a byproduct of inflammation. They tested samples from patients with other inflammatory diseases in whom retroviruses have been previously implicated or that showed a similar pattern of inflammation to that seen in arthritis (ie: macrophage-and lymphocyte-rich). HRV-5 was not detected in any of these suggesting some specificity of the virus for rheumatic diseases. They explained the finding of HRV-5 in the synovium of 3 of the 5 OA patients that these patients were selected for arthroscopy because of evidence of inflammatory OA. This would be consistant with the hypothesis that HRV-5 is present in cells that are tropic for the synovium, regardless of the underlying cause of the synovitis. The detection of HRV-5 proviral DNA in inflamed joints but not in normal synovium suggests that the cytokine milieu may have the effect of increasing the number of cells infected with the virus or have a more direct effect on virus replication itself.

In an attempt to detect the precise role of endogenous HRV in RA, *Nelson et al. (2004)* mentioned that the virus could be triggered by the ongoing immune response, or is indeed itself a trigger for RA. Evidently cells within the microenvironment of the joint (for example, B cells) may harbour endogenous HRVs themselves *(Ejtehadi et al., 2005)* and therefore also have the potential to play a role in the disease pathology. It is also possible that external factors may contribute to its activation, leading to changes in endogenous HRV expression through a process of bystander activation. Many exogenous viruses such as EBV and HIV are known to interact, either directly or indirectly, with endogenous retroviruses. Infection with many of these viruses also leads to activation of specific host cells (for example, T cells with reference to HTLV-I) during the course of infection, thus furthering the potential for immune dysregulation *(Sutkowski et al., 2004)*. Numerous cytokines are also known to modulate viral expression. Thus, it is possible that host cells infected and activated by exogenous or endogenous viruses could migrate into the synovium, releasing pro-inflammatory cytokines such as interleukin (IL)-6 *(Yoshihara et al., 2004)* and tumor necrosis factor (TNF) α *(Fu et al., 2002)*. Such events are likely to activate endogenous HRVs within the joint and may explain why levels of endogenous HRV are actively increased in the synovial fluid *(Ejtehadi et al., 2006)*.

It is well known that steroid treatment interferes with the host immune response to viruses. Patients treated chronically with steroids often respond poorly to viral infection. In various animal models, it has been shown that steroid treatment affects the expession of certain mouse endogenous retroviruses *(Tovar et al., 2000)*. However, there have been no studies showing the effect of cytotoxic agents on either human

or murine retroviruses. Because of our small sample numbers, it was not possible to accurately determine whether different drug regimens used to treat SLE and RA patients affected HRV-5 expression.

An important but still unresolved question is whether HRV-5 is of etiologic importance in both SLE and RA patients *(Garry et al., 1995)*. It is certainly unlikely that HRV-5 could cause the whole spectrum of SLE examined in this study. Rather it may be a common infection in which proviral DNA is preferentially detectable in blood from these patients. If this was the cause and HRV-5 was involved in pathogenesis, the most likely hypothesis would be that a specific disease would be an unusual reaction to a common infection *(Griffiths et al., 1999)*. Such phenomenon is well described in other viral infections. For example, lifelong infection with HTLV-1 results in the development of adult T cell leukemia in about 1% of individuals *(Nakagawa & Harrison, 1996)*. Another striking analogy is Parvovirus infection, where serological evidence has implicated B19 infection in about 15% of patients attending an early arthritis clinic *(White et al., 1985)*.

## Conclusions and Recommendations:

Thus, this study supports the hypothesis that SLE and RA could be triggered by a ubiquitous infectious human retrovirus in susceptible individuals.

Further characterization of the role of the virus in both SLE and RA diseases as well as other autoimmune diseases is required.

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# الكشف الجزيئى لفيروس (HRV- 5) فى الأمراض الروماتيزمية الأكثر انتشارا

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**مقدمة البحث:** فى محاولة للكشف عن أسباب الأمراض المزمنة الأكثر انتشارا غير واضحة السبب يحاول الكثيرون الربط بينها وبين بعض الفيروسات ومنها فيروس HRV-5 فى بعض الأمراض المناعية ولذلك تم إجراء هذه الدراسة لمحاولة كشف العلاقة بين هذا الفيروس وبعض الامراض المناعية مثل الذئبة الحمراء و مرض الرثيان المفصلي ومرض إلتهاب المفاصل العظمى المزمن (الفصال العَظْمِي) والذي قد يمثل تفاعل بين العوامل البيئية والوراثية ومن الممكن أن يكون لفيروس HRV-5 علاقة بحدوث بعض هذه الأمراض المناعية.

**أهداف البحث:** وقد هدفت هذه الدراسة إلى تقييم دور HRV-5 فى حدوث مرض الذئبة الحمراء و مرض الرثيان المفصلي ومرض إلتهاب المفاصل العظمى المزمن(الفصالٌ العَظْمِي).

**طرق البحث:** اشتملت هذه الدراسة 25 مريضة بالذئبة الحمراء و 25 مريضا بإلتهاب المفاصل الرثيان المفصليى و 25 مريضا إلتهاب المفاصل العظمى المزمن (الفصال العَظْمِيّ ) بالإضافة إلى 25 من الأصحاء كمجموعة ضابطة وتم الكشف عن الحامض النووى للفيروس بإستخدام سلسلة التفاعل المبلمر (PCR). وقد تم التأكد من التركيب الجينى للحالات الإيجابية بواسطة جهاز تتابع الحامض النووي (دى - إن - إيه) الأوتوماتيكي ثم مقارنته بمثيله عن طريق الاتصال ببنك الجينات بواسطة شبكة الإنترنت.

**نتيجة البحث:** وقد أوضحت النتائج المستوحاة من هذه الدراسة أن الفيروس HRV-5 من الممكن أن يكون له دورً فى تاريخ كل من مرض الذئبة الحمراء ومرض الرثيان المفصلي.