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RESEARCH ARTICLE

Cytotoxicity And Genotoxicity In Rheumatoid Arthritis: A Dual Effect Of Disease And Drugs.

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Abstract

Introduction/ Objectives: to assess cytotoxicity and genotoxicity in RA and the possible cyto and genotoxic effects of some DMARDs used in treatment.
Methods: The study included 30 female RA patients divided according to treatment into three groups and 30 healthy controls. Cytotoxicity was assessed by chromosomal analysis (karyotyping). Genotoxicity by the micronucleus test using cytochalasin–B.
Results: Compared to controls, RA patients had significantly higher CAs in the form of breaks, satellite association, endoreduplication, aneuploidy, double minute, and other aberrations. There was also a statistically significant increase in the markers of genotoxicity in RA patients compared to controls: micronuclei (MN), nucleoplasmic bridges (NPB) and necrotic and/or apoptotic cells. CAs were found in all RA groups, those receiving DMARDs as well as the DMARD naieve group. CAs, the number of binucleated (BN) cells with MN, NPB, and the necrotic and/or apoptotic cells was were significantly higher in MTX and in MTX-SSZ group when compared to RA on NSAIDs only.
Conclusions: RA patients show significantly increased markers of cytotoxicity and genotoxicity, regardless the type of medications received, sugggesting them to represent underestimated disease features which could provide new insights into its pathogenesis.

Introduction:-

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory joint disease worldwide (Liao KP et al, 2009). It affects 1% of the world population and triggers joint inflammations that may worsen patient's quality of life (Collignon O, 2014).

The "Bermuda triangle" of genetics, environment and autoimmunity is involved in the pathogenesis of RA (Júlia K et al,2013). Although the pathogenesis of RA is incompletely understood, genetic factors play a vital role in susceptibility to RA as the heritability of RA is between 50 and 60% (Vasanth KM et al, 2014).

The association of certain chromosome aberrations with rheumatic diseases has been described (Salavoura K et al, 2008). In addition, the possible pathogenic effects of one or more disease-associated chromosomal regions (loci) in RA have been investigated (Czakó M et al, 2002), (Maxwell JR et al, 2012), (Orozco G et al, 2014). These studies often led to controversies as some groups were able to confirm linkage of a certain single nucleotide polymorphism to RA, while others could not (Vasanth KM et al, 2014).

Compared with the general population, patients with RA have a higher risk of developing certain malignancies (Penn I, 1981). A number of studies have found an increased risk of malignancy in RA patients treated with

disease-modifying antirheumatic drugs (DMARDs) (Williams CA et al, 1996). Methotrexate (MTX) is the anchor DMARD in RA treatment, as monotherapy or in combination with other synthetic or biological DMARDs, and is known to have the best cost-effectiveness and efficacy/toxicity ratios. However, toxicity is still a concern. MTX may be associated with hemopoietic malignancies, although the literature consists predominantly of case reports (Woodrick RS et al, 2011).Studies about the cytotoxicity (chromosomal aberrations) and the genotoxicity (DNA damage) in RA and due to the use of DMARDs are scarce in the literature.

Objectives:-

The aim of the present work was to assess chromosomal aberrations(CAs) as a measure of cytotoxicity and micronuclei (MNi)as a measure of genotoxicity in RA patients. Also to assess the possible cytotoxicity and genotoxicity induced by some DMARDs used for treatment.

Patients:-

The study included 30 female RA patients, who fulfilled the ACR/EULAR criteria for RA (Aletaha D et al, 2010) divided according to their treatment into three groups .Two groups received DMARDs for at least 3 months period: MTX only group (n=12 patients), MTX with SSZ (sulphasalazine) group (n=8) and ten DMARDs naieve RA who received only Non Steroidal Anti-Inflammatory Drugs (NSAIDs). The study also included 30 healthy female control subjects matching the same age.

Patients were included from the Rheumatology and immunology unit, Main Alexandria University Hospital.

The study was approved by the ethical committee of Faculty of Medicine according to the Declaration of Helsinki, and informed consent was obtained from all participants included in the study.

All patients were subjected to full history taking including: Drug history: Type, dose, duration of use of the DMARDs and folic acid, pregnancy history including the number of abortions.
Full clinical examination and routine lab investigations were performed for each patient. Assessement of RA disease activity was done by applying DAS28 ESR (Prevoo ML et al, 1995).
Exclusion criteria:

Patients with any disease other than RA (oncologic diseases, diabetes, thyroid, hepatitis C and bilharziazis) were not included in the study, also those receiving DMARDs other than MTX and SSZ.

Assessment of Cytotoxicity and Genotoxicity:-

It was performed by karyotyping (Prevoo ML et al, 1995) (chromosomal analysis) for the study of numerical chromosomal aberrations (as aneuploidy; hypoploidy or hyperploidy) and structural chromosomal aberrations (as breaks, satellite assosciation, double minutes, and endoreduplication). Karyotyping was performed on peripheral blood samples using solid Giemsa stain and GTG- banding technique according to the International System for Human Chromosome Nomenclature (Shaffer LG et al, 2013).
The micronucleus test (Fenech M et al, 2003) to measure genotoxicity and DNA damage was performed using cytochalasin-B.

Statistical analysis:-

Data were fed to the computer using IBM SPSS software statistical package version 20.0. Both statistical analysis and tabulation were done according to Altman (Altman GA,1991). Differences between 2 continuous variables were compared with student unpaired "t" test. Differences between 3 or more continuous variables were compared with one way analysis of variance (ANOVA) using Scheffe's method for multiple comparisons. Differences between proportions were compared with the chi- squared test and Fisher's exact test. Level of significance was set at $p<0.05$.

Results:-

Age of the control group ranged between 39 – 68 yr with a mean of 49.8±9.72. Age of the total RA patients ranged between 30 – 75 yr with a mean of 42.47 ± 11.56 and a disease duration range from 1-30years. All cases were rheumatoid factor positive. All cases were of low socioeconomic status, and 28 of them (93.3 %) were without university degree. 10 RA patients were on NSAIDs only, 12 on MTX and 8 on MTX-SSZ combination. Eleven

cases (39.3%) had abortions, 8 of them (72.7%) were receiving DMARDs. The MTX dose was 15-20 mg/wk, SSZ dose was 1.5-2 gm daily. All RA on DMARDs received concomitant daily folic acid dose of 0.5 mg. All cases were Egyptian non-smoker females.

Results of cytotoxicity:-

Compared to controls, RA patients had significantly higher CAs in the form of satellite association, endoreduplication, aneuploidy, double minute, other aberrations, (Table1, figure1) and had highly significant difference regarding chromosomal breaks ($p<0.001$).

The other chromosomal aberrations encountered in the present study (as loss or gain of some chromosomes) seem to be randomly distributed except in the case of X- chromosome anomalies. There was a strikingly high incidence of X chromosome anomalies in the form of monosomy X, triple X, Xq-and ring X. These were detected in 9/30 RA patients (30%), 6/9 patients (66.6%) were receiving MTX and 3/9 (33.3%) were on NSAIDs.

CAs were found in all RA groups, those receiving DMARDs as well as the DMARD naieve group. (Table 2) CAs were significantly higher in MTX group and in MTX -SSZ group when compared to RA on NSAIDs as regards Sat Ass, endoreduplication and aneuploidy . While the difference in breaks, dmin, and other aberrations were not statistically significant ($p>0.05$). Meanwhile, there was no significant difference in the CAs between the MTX and the MTX- SSZ groups.

There was a highly significant difference ($P\leq0.001$) in all studied markers of cytotoxicity between the control and RA patients irrespective of the disease duration and between RA patients with different disease durations (1-<5 years, 5-<10 years, and 10-15 years). Table 3.

RA patients who were on MTX for a longer duration (> 5 years) showed a statistically significant increase in all the cytogenetic markers, except other chromosomal aberrations, when compared to those who were on DMARDs for less than 5 years. Table 4.

Regarding RA disease activity as measured by DAS28, 53.3% of patients had moderate disease activity and 46.7% had high activity, while no patients were with mild or no activity. There was no significant difference in CAs between RA patients of moderate and high disease activity ($p >0.05$).

Results of genotoxicity (Micronucleus test):-

There was a statistically significant increase in the mean levels of markers of genotoxicity in RA patients compared to controls: the mean micronuclei (MNi) which indicate chromosome breaks or loss ($p<0.01$), the mean nucleoplasmic bridges (NPB) which indicate chromosome rearrangement ($p<0.01$) and necrotic and/or apoptotic cells. (Table5, figure2)

The number of MNi was high (>6 MNi) in 86.67% of RA compared to 0% of the controls, and moderate (3-5 MNi) in 13.33% of RA. Whereas, none of the patients had normal number (0-2) of MNi compared to 80% of the controls. This difference in the MNi number was highly significant (<0.0001).

When comparing RA patients receiving different medications, the number of binucleated cells(BN) with NPB, and the number of necrotic and/or apoptotic cells was statistically higher in RA receiving MTX only and MTX with SSZ when each was compared to the NSAIDs group. While the number of BN cells with MNi was higher but without reaching statistical significance. Whereas, no statistical difference was observed between the two DMARDs groups. (Table 6)

Furthermore, there was no significant difference in markers of genotoxicity between RA with moderate and with high disease activity ($p >0.05$).

Discussion:-

In the present study a significant increase in chromosomal aberrations, in the form of chromosomal breaks, satellite assosciation (sat ass), double minutes (dmin), endoreduplication, aneuploidy and other aberrations, observed in peripheral blood of RA patients compared to controls.

This was in agreement with Vincent et al (Vincent G et al, 1986) who confirmed the significant elevation of the rate of chromosomal abnormalities and the presence of a breaking capacity of the serum in a series of 78 RA patients compared with a control group. On the other hand, Kinne et al. (Kinne RW et al, 2001) detected chromosomal aberrations in synovial tissue and synovial fluid fibroblasts of patients with RA, osteoarthritis, and other inflammatory joint diseases while no aberrations were observed in the peripheral blood of the same patients. This led them to conclude that these findings reflect a common response to inflammatory/microenvironmental stimuli in rheumatic diseases and not specific to RA.

Rheumatoid arthritis patients of the current study had a significantly high frequency of sat ass. Sat ass is known to occur in meiosis as well as in mitosis where the respective chromosomes (meiosis) or chromatids (mitosis) may fail to disjoin so leading to chromosomal aneuploidy (Zellweger H et al,1966). This in turn can explain the high frequency of aneuploidy observed in our RA patients. Lezhava et al. (Lezhava T et al, 2008) have mentioned that the associations of human acrocentric chromosomes account for elevated incidence of chromosome rearrangements and consequently can cause chromosomal disorders.

Furthermore, this high frequency of aneuploidy may account for the increased number of abortions in the present study, which might be due to chromosome non-disjunction occurring in meiosis, as 11/30 RA (39.3%) had abortions. To the best of our knowledge no study on the sat ass in patients with RA was found in the literature.

The finding of higher CAs in RA than healthy controls suggests that cytotoxicity is a significant assosciation with RA whether as an effect of the disease itself or the received medications.

In a trial to accomplish the aims of the current study, we included three RA patient groups, two receiving DMARDs (MTX group and MTX with SSZ group) and the third was on NSAIDs only. This was based on the assumption that NSAIDs do not induce cyto or genotoxicity. CAs were found in all RA groups, those receiving DMARDs as well as the DMARD naieve group.

At the same time, CAs were significantly higher ($p<0.05$) in RA patients on MTX only and in the MTX -SSZ group when compared to group on NSAIDs as regards satellite association, endoreduplication, and aneuploidy which indicates that cytotoxicity is more in those receiving MTX. Actually, 8/11 of RA patients (72.7%) with abortions were using MTX alone or with SSZ. The lack of statistically significant difference between the different RA patients regarding breaks, dmin, and other aberrations, might be explained by the partial protective effect of folic acid used concurrently with MTX. Meanwhile, there was no significant difference between the CAs between MTX only and the MTX- SSZ groups, which suggests that either SSZ is not toxic (as it did not augment the MTX toxicity) or that its toxicity has been masked by the use of folic acid.

Accordingly, as CAs were detected in all RA groups regardless the type of medications received, this suggests that these aberrations are due to the RA disease itself. The assumption that cytotoxicity is a part of the RA disease is worthy as it may raise new insights into the pathogenesis of the disease. Furthermore, as CAs were more significant in those patients receiving MTX with or without SSZ, confirms that DMARD use especially MTX further aggravates cytotoxicity in RA patients, which is in agreement with Cronstein(Cronstein BN, 2005) and Ferraccioli et al. (Ferraccioli GF et al,2002)

The high frequency of endoreduplication observed in the MTX group of the present study can lead to increased risk of malignancy in RA patients, thus explaining the observations made by Woodrick and Ruderman (Woodrick RS et al, 2011) and Williams et al. (Williams CA et al, 1996) who found that RA patients with leukemia or lymphoma showed a trend toward increased prior MTX or azathioprine use compared with matched RA controls without leukemia or lymphoma. To the best of our knowledge the occurrence of dmin and endoreduplication (as a measure of cytotoxicity) in patients with RA were not mentioned in the literature.
In order to assess genotoxicity in RA in the present study, MN test was performed in all patients and healthy controls.

The number of MN detected in any person is classified as normal when lies between 0-2, moderate 3-5, or high if $\geq$ 6 (Ramos-Remus C et al, 2002).

The number of MNi in RA patients in the current study was high (>6 MNi) in 86.67%, and moderate (3-5 MNi) in 13.33%. Whereas, none of the patients had normal number of MNi (0%) compared to 80% of the control group. This difference in the MNi number was highly significant (<0.0001), documenting that genotoxicity is part of RA.

The finding of significantly increased ($p<0.01$) mean NPB (which indicate chromosome rearrangement) and necrotic and apoptotic cells in RA patients compared with controls regardless of their MTX use suggests that RA itself rather than MTX induces genotoxicity which is in accord with the suggestion that the increased risk of leukemia in RA patients may be due to RA itself.

In addition, the mean MNi (which indicate chromosome breaks or loss), are also increased but without reaching statistical significance, this may be a result of the small number of studied patients. Similarly, Karaman et al (Karaman A et al, 2011) found that the MNi frequencies were significantly higher in RA patients -both in the active and the inactive period- than in the controls. Their results suggested that the higher MNi frequency (the DNA damage) in RA can be explained by increased oxidative stress leading to genetic instability. They suggested that increased DNA damage may play an important role in the pathogenesis of RA.

When comparing RA patients receiving different medications, the number of the BN cells with MNi, NPB, and necrotic and/or apoptotic cells were higher in RA receiving MTX only and MTX with SSZ compared to the NSAIDs group. Whereas, no difference between the two MTX groups. This confirms the genotoxicity of MTX despite the concomitant use of folic acid.

Among the various DNA lesions induced by MTX, chromosome breaks are considered the most important because of their potential to cause cell death, mutagenesis, and carcinogenesis (Osipov A.N et al, 2013). Shahin et al (Shahin AA et al, 2001) have observed that MTX produced a significant genetic injury as proved by the increased incidence of CAs and MNi formation in Wistar albino rats and in RA patients. They also reported that folinic acid has a protective effect against MTX genotoxicity in patients with RA. Similar results were reported by Theiss et al. (Theiss JC et al,1989) and Mac Donald et al. (Mac Donald JR et al, 1993) whose studies showed an increase in trimetrexate (TMX) – a drug structurally similar to MTX – genotoxicity, as evidenced by increased CAs in TMX-treated rats.

On the other hand, Cronstein (Cronstein BN, 2005) found that concomitant administration of either folinic acid or folic acid to patients with RA taking MTX demonstrated no difference in the prevention of methotrexate-mediated toxicity.

Ramos-Remus et al (Ramos-Remus C et al, 2002) have performed MN assays on oral mucosal sweeps of 50 MTX treated RA patients, 30 RA patients not receiving MTX and 39 healthy controls. They found that the number of MNi were significantly higher in RA patients as compared with controls. This is in agreement with the results of the current study, but in contradiction they reported no difference in the MNi number between users and non-users of MTX. Accordingly, they concluded that genotoxicity, as assessed by the MN assay, is increased in RA patients and that genotoxicity is associated with RA itself and not with MTX use and folic acid supplementation had no effect on the number of MN.

There was a highly significant difference ($p\leq0.001$) in all the studied markers of cytotoxicity between the control and RA patients even those with disease duration of less than 5 years. Thus, documenting the presence of cytotoxicity early in the disease course, which raises concern about the contribution of the disease process itself to cyto and genotoxicity. The statistical difference was still significant when comparing RA patients with different disease durations, the finding of more markers of toxicity in those with longer disease duration documents the dual effects of disease and treatment in aggravating cell toxicity.

One of the study limitations is that we were not able to delineate the exact relation between RA disease activity and cytot-genotoxicity, as there were no cases enrolled having mild or no disease activity.

Another limitation of the current work, is that its results are largely based on assumption that NSAIDs do not induce cyto/genotoxicity, as actually what is reported is the reduced risk of certain malignancies as colorectal and lung cancers in association with their intake . Studies on RA patients with no treatment at all might be the way to prove this, although it is rare to find such patients as most start receiving NSAIDs before definite diagnosis.

To overcome study limitations we recommend in further studies the inclusion of treatment naieve RA group and a disease control group which would be of value to prove if the findings of cyto and genotoxicity are specific to RA disease itself.

Conclusions:-

RA patients show significantly increased markers of cytotoxicity and genotoxicity, regardless the type of medications received, which sugggest them to represent underestimated disease features which could provide new insights into its pathogenesis. MTX further aggravates the cyto- and genotoxicity, whereas SSZ combination does not seem to play additional role and concomitant folic acid supplementation does not provide the sufficient protection.

Compliance with ethical standards:-

Ethical approval:-

All procedures performed in the study were approved by the ethical committee of Faculty of Medicine and with the 1964 Helsinki declaration and informed consent was obtained from all participants included in the study.

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Conflicts of interest:-

None of the authors has any conflicts of interest to declare.

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Table 1:- Chromosomal aberrations in total RA patients and controls

		Breaks	**Satellite association**	**Double minutes**	**Endo-reduplication**	**Aneuploidy**	**Other aberrations**	**Mitotic index**
Control (n=30)	**Range** **Mean** **± SD**	**0 -3** **0.6** **± 1.07**	**0 – 21** **6.2** **± 6.49**	**0.0 -0.0** **0.0** **± 0.0**	**0.0 – 0.0** **0.0** **± 0.0**	**0.0 – 0.0** **0.0** **± 0.0**	**0.0 – 0.0** **0.0** **± 0.0**	**4.2 – 10.6** **6.8** **± 2.11**
RA patients (n=30)	**Range** **Mean** **± SD**	**0-43** **14.77 ±11.58**	**15 - 68** **38.16** **±14.77**	**0-20** **2.03 ±4.77**	**0-36** **9.7** **± 8.31**	**0-13** **5.3** **± 4.5**	**0-4** **0.70** **±0.51**	**1.5-13.5** **5.76** **± 3.25**
p value		<0.001*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	>0.05

statistically significant at $p \leq 0.05$

Table 2:- Frequency of cells showing chromosomal aberrations in the RA on NSAIDs, MTX and MTX -SSZ (out of 100 cells/ case)

		Breaks	**Satellite association**	**Double minutes**	**Endo-reduplication**	**Aneuploidy**	**Other aberrations**	**Mitotic index**
RA on NSAIDs (n=10)	Range	0 – 36	15 – 48	0 – 15	0 – 16	0 – 8	0 –2	1.5 – 10.1
	Mean ±SD	13.0 ± 12.53	28.7 ± 12.78	3.0 ± 5.37	5.6 ± 5.83	2.1 ± 2.96	0.4 ±0.699	5.72 ± 3.02
RA on MTX (n=12)	Range	0 -43	22 – 68	0 – 20	0 – 28	0 – 13	0 -4	2.4 – 10.1
	Mean ±SD	18.42±12.9	41.33 ±15.92	1.83 ±5.75	11.0 ±7.59	6.92 ±4.13	0.42 ±1.21	5.2 ±2.85
RA on MTX- SSZ (n=8)	Range	4 – 25	33 – 60	0 – 4	4 – 36	0 – 11	0 – 3	3.4 – 13.5
	Mean ±SD	11.5 ±7.23	45.25 ±9.62	1.13 ±1.64	12.88 ±10.63	6.88 ±4.11	1.5 ±0.83	7.38 ±3.02
P_1		>0.05	<0.05*	>0.05	<0.05*	<0.05*	>0.05	>0.05
P_2		>0.05	<0.05*	>0.05	<0.05*	<0.05*	>0.05	>0.05
P_3		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

p1: p value between MTX-RA and NSAIDs group

p2: p value between MTX-SSZ and NSAIDs group

p3: p value between MTX and MTX-SSZ groups

*: statistically significant at $p \leq 0.05$

Table 3:- Frequency of cells showing chromosomal aberrations in control and RA patients with different disease duration (out of 100 cells/ case)

		Breaks	Satellite association	Double minutes	Endo-reduplication	Aneuploidy	Other berrations
Control (n=30)	**Range**	0 – 3	0 – 21.0	0.0 – 0.0	0.0 – 0.0	0.0 – 0.0	0.0 – 0.0
	Mean ±SD	0.6 ± 1.07	6.2 ± 6.49	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RA with disease 1-<5 years (n=13)	**Range**	3-43	17-46	0-2	0-15	0-10	0-4
	Mean ±SD	15.54 ± 11.67	32.38 ±8.97	0.15 ±0.55	6.77 ± 5.07	3.77 ±2.98	0.46 ± 0.98
RA with disease 5-<10 years (n=9)	**Range**	0-36	15-67	0-15	0-28	0-8	0-3
	Mean ±SD	17.11 ±13.66	39.78 ±18.53	3.78 ±5.45	11.78 ± 9.44	3.66± 3.21	0.78 ±0.384
RA with disease 10-30 years (n=8)	**Range**	0-30	25-68	0-20	0-36	1-14	0-3
	Mean ±SD	10.88 ± 9.11	45.75 ±15.54	3.13 ±6.92	12.13 ±10.56	7.70 ±4.47	0.80 ±0.92
P1		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
P_2		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
P_3		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
P4		0.049*	0.042*	0.001*	0.004*	0.014*	0.005*
P5		0.044*	0.002*	0.001*	0.001*	0.006*	0.021*

p1: p value between control and RA patients with disease duration 1-<5 years
p2: p value between control and RA patients with disease duration 5-<10 years
p3: p value between control and RA patients with disease duration 10-30 years
p4: p value between RA patients with disease duration 5-<10 years and 1-<5 years
p5: p value between RA patients with disease duration 10-30 years and 1-<5 years
*: statistically significant at $p \leq 0.05$

Table 4:- Frequency of cells showing chromosomal aberrations in RA patients on DMARDs for different periods (out of 100 cells/ case)

		Breaks	Satellite association	Double minutes	Endo-reduplication	Aneuploidy	Other Aberrations
RA patients on DMARDs for 1-5 years (n=11)	**Range**	4-43	22-60	0-4	0-20	0-10	0-4
	Mean ±SD	17.64 ±11.63	38.64 ±9.85	0.55 ±1.29	10.00 ±6.02	4.67 ±3.50	0.75 ±0.1.22
RA patients on DMARDs for >5 years (n=9)	**Range**	0-32	25-68	0-20	0-36	1-14	0-3
	Mean ±SD	13.22 ± 11.05	48.11 ± 16.17	2.78 ±6.55	13.89 ±11.21	7.45 ±4.32	0.73 ±0.90
P		0.021*	0.013*	0.036*	0.048*	0.036*	0.689

p: p value between RA patients on DMARDs for 1-5 years and RA patients on DMARDs for > 5 years
*: statistically significant at $p \leq 0.05$

Table 5:- results of micronuclei (MN), nucleoplasmic bridges (NPB), and necrotic and/or apoptotic cells in RA patients and control

		BN cells with MN	BN cells with NPB	Necrotic and/or apoptotic cells
Control (n=30)	**Total** **Range** **Mean± SD**	**15** **0 -3** **1.5 ± 1.2**	**8** **0 – 3** **0.8 ± 1.0**	**23** **0 -5** **2.3 ± 1.8**
RA patients (n=30)	**Total** **Range** **Mean± SD**	**652** **4-45** **21.73±12.38**	**398** **3-40** **13.27±9.96**	**288** **4-52** **29.93±12.10**
p value		<0.01*	<0.01*	<0.001**

Table 6:- Frequency of micronuclei, nucleoplasmic bridges (out of 1000 cells/ case), necrotic and/or apoptotic cells (out of 500 cells/ case) in the RA on NSAIDs, MTX and MTX -SSZ

		Total no of BN cells with MNi	**BN cells with NPB**	**Necrotic and/or apoptotic cells/ 500 cells**
RA on NSAIDs	No.	109	92	50
	%	1.09	0.92	1.0
RA on MTX	No.	327	180	142
	%	2.73	1.5	2.37
RA on MTX-SSZ	No.	216	126	96
	%	2.7	1.58	2.4
P1		>0.05	<0.05*	<0.05*
P_2		>0.05	<0.05*	<0.05*
P3		>0.05	>0.05	>0.05

P1: p value between MTX and NSAIDs group
P2: p value between MTX-SSZ group and NSAIDs group
P3: p value between MTX and MTX-SSZ group
p: p value for chi-square test*
Statistically significant at $p \leq 0.05$

Figure 1:- Photographs of: a GTG-banding karyotype of a RA patient showing break in the long arm of chromosome 10 (10q, Upper left), a metaphase spread (Giemsa stained) of a RA patient showing satellite association (sat ass) between 3 chromosomes of the D group and 2 of the G group (Upper right), a karyotype of RA patient (GTG- banding) showing ring X- chromosome (lower left), and metaphase spread of a RA patient stained by Giemsa showing chromosome endoreduplication (Lower right).

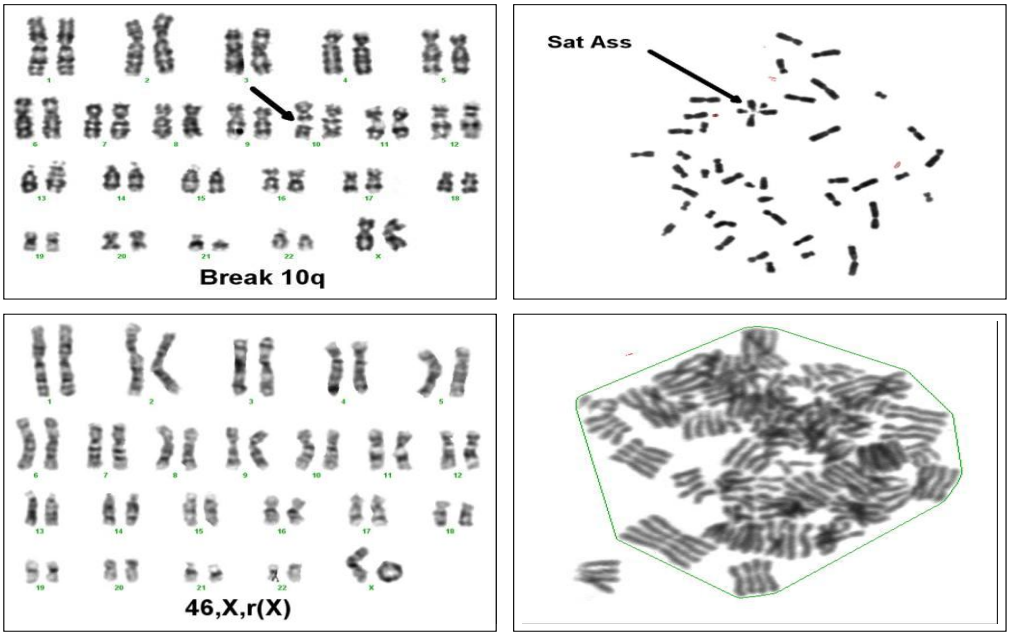
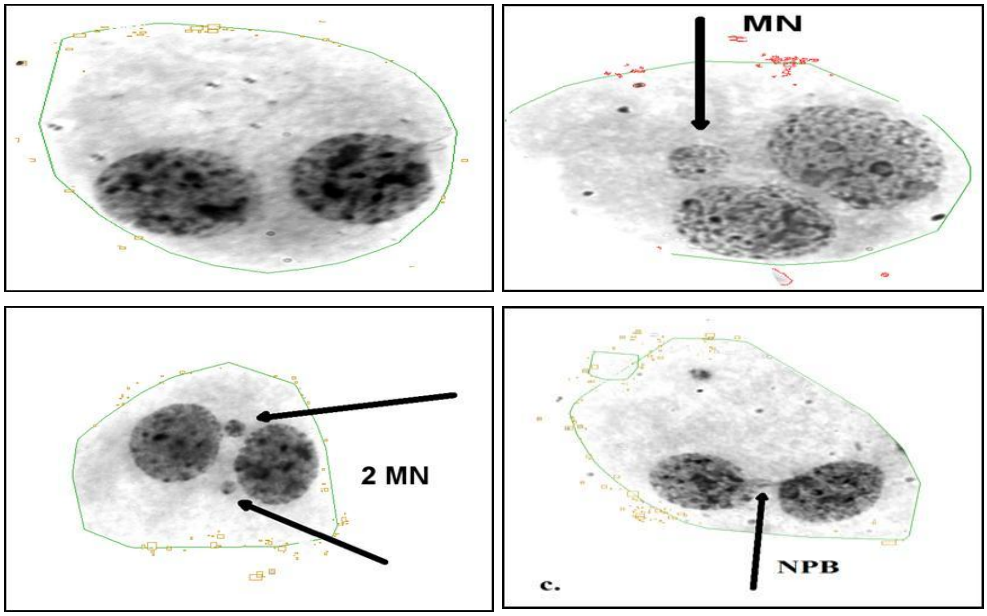


Figure 2:- Photographs of: a normal binucleated (BN) cell (Upper left), a BN cell with one micronucleus (MN) observed in RA patient (Upper right), a BN cell with more than one MN seen in RA patient (Lower left), and a BN with nucleoplasmic bridge (NPB) observed in RA patient (Lower right)



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